Effects of Naloxone on Kidney Weight, Macromolecular Constituents of Kidney and Cortisol Secretion in Fetal and Neonatal Pigs

Dissertation
to obtain the Ph. D. degree
in the Faculty of Agricultural Sciences,
Georg-August-University Göttingen, Germany

presented by
Dingjian Li
born in Guiyang, P. R. China

Göttingen, in July 2007

D 7

1. Name of referee: Prof. Dr. Hans-Jörg Abel

2. Name of co-referee: Prof. Dr. Dr. Nahid Parvizi

Date of dissertation: 19. 07. 2007

DEDICATION

To my beloved parents

and

my beloved Qiannan

CONTENTS

1. Introduction

One of the systems modulating physiological events in the body is the opioid system. According to investigations carried out in the recent decades, opioids are presumed to play a vital role in the control of growth in farm animals and other species (Rahe et al., 1980; Parvizi, 1993). Opioid peptides have been implicated as potential regulators of cell development in nervous and non-nervous tissues, especially concerning cell proliferation and differentiation (Vertes et al., 1982; Kew and Kilpatrick, 1990; Hauser, 1992). The interference in growth and developmental processes by opioids is reversed by antagonists (Crofford and Smith, 1973; Smith et al., 1977; Zagon and McLaughlin, 1981). Blockade of opioid receptors by opioid antagonists enhances in some cases developmental events (McLaughlin, 1994; Zagon et al., 1999). Since no information is available regarding the influence of opioid antagonists on organs in the pig, the present work was undertaken to investigate the effect of naloxone on kidney weight and macromolecular constituents of kidney in fetal and neonatal pigs.

In several species (Buckingham, 1982, 1986), opioid peptides and their receptors play a major role in the regulation of hypothalamo-pituitary-adrenal (HPA) function (Buckingham and Cooper, 1984a; Buckingham, 1986; Lightman and Young, 1988; Rushen et al., 1995; Taylor et al., 1996; Taylor et al., 1997; Zhou et al., 1999). Although opioid antagonists have been found to stimulate the HPA axis (Farren et al., 1999) and increase the release of cortisol (Blankstein et al., 1980; Nikolarakis et al., 1989; Schluger et al., 1998; Williams et al., 2003). There is, however, no data relating to the efficacy of opioid antagonists upon cortisol levels in developing pigs. Thus, we also determined plasma cortisol concentrations in fetal and neonatal pigs treated with naloxone.

2. Literature Review

2.1. Effects of Opioids on Tissue Weights and Macromolecular Constituents of Tissues

2.1.1. Effects of opioids on tissue weights and macromolecular constituents of tissues in the rat

Opioids may affect tissue weights and macromolecular synthesis of tissues in developing and adult rats. Since the rat is very immature at birth, it might be more susceptible to permanent change in growth rate arising from restrictions imposed in early postnatal life than the more mature offspring of farm livestock (Sarkar et al., 1977).

Prenatal morphine exposure induced a severe atrophy of the adrenals in newborn rats at birth and during the early postnatal period. However, morphine did not significantly affect the weight of the liver in rat pups (Lesage et al., 1998). Prenatal morphine treatment did not significantly disturb adrenal weight and weight of the testicles and seminal vesicles in male adult rats. The postnatal body growth up to week 10 after birth was also not disturbed (Dutriez-Casteloot et al., 1999). Unlike prenatal treatment, neonatal morphine exposure caused a significant decrease in body and testes weight in the adult male rat (Hansson et al., 1989). The effects of a single morphine pellet implanted in developing male rats at 27 days of age on reproductive tissues were compared to those found in adult animals after the same treatment (Cicero et al., 1989). In the developing rat, the wet weights of the seminal vesicles and testes were substantially depressed after pellet implantation and these effects persisted for up to 4 weeks when compared to placebo-implanted, age-matched controls. In contrast to these results, there was in adult rats only a transient response (less than 1 week) to morphine on the weights of the seminal vesicles and no effects on testes weights.
Methadone, a long-acting mu-opioid agonist (Wang et al., 2005), was administered either directly to the rat pups from 1 to 19 days after birth, or to the mother from 10

days of gestation to 20 days after birth. Both treatments resulted in significant deficits of body weight and kidney weight of developing rats (Grignolo et al., 1982). In adult rats, repeated methadone administration resulted in deficits in heart weight (Bareis et al., 1978).

Investigations have documented that opioid antagonists also influence normal organ growth. Offspring delivered by female rats received daily injections of 50 mg/kg naltrexone (NTX) throughout pregnancy had larger organ weights (McLaughlin et al., 1997). A survey of six major organs (i.e., brain, heart, kidney, liver, muscle, lung) showed that at birth, postnatal day 10, and at weaning (i.e., postnatal day 21) these organs (with the exception of the lungs at birth) weighed substantially more than their control counterparts. Measures of the dry weights of these organs showed parallel increases, suggesting that total cellular content was elevated. Calculation of relative organ weights revealed that at birth, brain, kidney, and muscle of NTX-exposed rats were increased 26% to 32% above control levels, whereas relative lung weights of the NTX group were subnormal by 20%.

Chronic administration of naltrexone to newborn rats significantly stimulates organ weights and enhances tissue maturation (Zagon and McLaughlin, 1983a; Bartolome et al., 1986; Zagon et al., 1987; McLaughlin, 1994). Naltrexone's effects on growth depend not only on dosage, but also on sex and the organ system examined. In the Zagon and McLaughlin (1985) study, 10 organ systems (i.e., adrenal, brain, gonads, heart, kidneys, liver, lungs, m. triceps surae, spleen, and thymus) from 21-day old rats were observed. In male rats the most marked decreases from control levels occurred in the weights of thymus, spleen, liver, testes, triceps surae and heart. In the females, the ovary, spleen, liver, kidneys, and thymus were subnormal by 22-29%. Naltrexone did not appear to affect wet weights of the adrenals or lungs of either sex. Increases of kidney weight were observed in both sexes. The changes in wet weight were similar to those for body weight, suggesting that a proportional increase or decrease in animal growth took place.

Zagon et al. (1989b) reported that daily injections of 40 mg/kg (-) naloxone throughout the preweaning period depressed body weight of 21-day-old rats, and the wet weights

of the liver, spleen, thymus, heart, and triceps surae muscle from these animals were subnormal (reduced 19-33% from control values).

Zagon et al. (1999) provide evidence for the first time that native opioid peptides serve to regulate DNA synthesis in a wide variety of organ systems during prenatal life. A strong case can be made that a principal endogenous opioid peptide modulating prenatal organ growth is the pentapeptide, [Met^5]enkephalin. In view of its growth properties, [Met^5]enkephalin has been termed the opioid growth factor (OGF) (Zagon et al., 1993; Zagon, Wu and McLaughlin, 1996; McLaughlin, 2002). OGF exerts a direct and tonic inhibitory influence on embryogenic events (Zagon et al., 1987; Zagon et al., 1991b; Zagon et al., 1999). Acute exposure of the pregnant rat to OGF resulted in a decrease in DNA synthesis in cells of organs representing all three germ layers. The Preproenkephalin A gene (PEA) that encodes for the endogenous opioid pentapeptides methionine- and leucine enkephalin, was also implicated in proliferation of cultured rat embryonic fibroblasts (Weisinger et al., 1995). The findings of the mechanism by which OGF functions in prenatal life with respect to DNA synthesis are in concert with, and extent, those in earlier reports that have documented the direct and receptor-mediated modulation by OGF on DNA synthesis in homeostatic situations in the postnatal organism (Zagon et al., 1999). The receptor responsible for OGF activity is the zeta (ζ) opioid receptor. In fetal rats, blockade of endogenous opioid-opioid receptor interaction using naltrexone produced an increase in DNA synthesis. NTX elevated the labeling indexes (LI) of the cerebrum, skin, spinal cord, cerebellum, rib, adrenal cortex, heart, liver, tongue, and intestine by 16 to 63% above control values, although vertebra and lung did not have an increase in the LI after exposure to NTX (Zagon et al., 1999).

In neonatal rats, further studies with [Met^5]enkephalin revealed that the effects on cell replication were dependent on dosage (Zagon et al., 1991b; McLaughlin, 1996; Zagon, Wu and McLaughlin, 1996). Concomitant administration of naloxone blocked the inhibitory effects of [Met^5]-enkephalin on cell division (Isayama et al., 1991; Zagon et al., 1991b; McLaughlin, 1996). Naloxone alone had no effect on DNA synthesis (McLaughlin, 1996). Blockade of endogenous opioid interaction by NTX was

accompanied by a significant increase in the labeling index (LI) relative to control levels (Isayama et al., 1991; McLaughlin, 1994; McLaughlin, 1996). The effects of NTX on the developing aorta recorded in the Zagon et al. (1996) study corroborate and extend similar reports in developing brain, heart, and retina, neoplastic cells and tissues, epithelium of the tongue and cornea, and microorganisms (Isayama et al., 1991; Zagon et al., 1991a; Zagon et al., 1992; Sassani et al., 1993; Zagon et al., 1993; McLaughlin, 1994; Zagon et al., 1995).

Subcutaneous (s.c.) injection of morphine to rat pups profoundly inhibited brain DNA synthesis in animals 1 and 4 days of age but not in older animals. Moreover, morphine was shown to inhibit [^{3}H]thymidine incorporation into brain DNA during the first postnatal week. The effect was observed in defined brain regions, dose-dependent and reversed by the opioid antagonist naloxone (Kornblum et al., 1987).

Similarly, administration of ß-endorphin to preweaning rats intracisternal (i.c.), but not s.c., markedly inhibited brain DNA synthesis (Kosterlitz et al., 1985; Bartolome et al., 1991) in a manner dependent upon the course of development of specific brain regions (Lorber et al., 1990). ß-endorphin's effect was restricted to the first three postnatal weeks (Lorber et al., 1990; Bartolome et al., 1991). Coinjection of naloxone plus ß-endorphin i.c. completely antagonized the response (Lorber et al., 1990; Bartolome et al., 1991; Bartolome and Bartolome, 1994). Naloxone alone caused small but significant increases in brain DNA synthesis, suggesting a tonic influence on tissue DNA by endogenous opioids in the CNS (Bartolome et al., 1991).

Cholecystokinin (CCK) occurs predominantly as CCK_8, which is believed to act as a neuromodulator or neurotransmitter (Rehfeld, 1985). Although the precise role of CCK_8 in brain function is currently unknown, increasing evidence suggests that it may act physiologically as an opioid antagonist. Bartolome and Lorber et al. (1994) reported that CCK_8 injected i.c. together with ß-endorphin effectively prevented ß-endorphin from inhibiting brain DNA synthesis in 10-day-old rats. However, CCK_8 given alone i.c. did not alter basal levels of DNA synthesis in the brain.

The neurochemical effect of postnatal exposure to methadone (METH) on the whole brain and cerebellum were examined in 21- and 60-day-old rats. At 21 days, METH

rats had severe deficits in the weight and total DNA content of the brain and cerebellum, as well as in brain DNA concentration. At 60 days, total brain DNA content also was reduced in METH animals (Zagon et al., 1982).

In 6-day-old rat cerebellum, acute administration of a wide variety of opioids (synthetic and natural, exogenous and endogenous), some extremely selective for certain opioid receptors, had no effect on cell proliferation at concentrations as high as 10 mg/kg (Zagon et al., 1991b). In 11-day-old rats, acute administration of D-Met^2-Pro^5-enkephalinamide, a long acting synthetic enkephalin analog (Bajusz et al., 1977; Szekely et al., 1977), resulted in a marked reduction in the labeling of cerebral and hypothalamic DNA between 1 to 12 h (Vertes et al., 1982). Except a decrease in DNA at 1 h no effect was observed in the cerebellum. The decrease in DNA synthesis found at 1 h was prevented by naloxone injected 5 min prior to enkephalinamide.

In developing rats, inhibition of DNA synthesis and cell proliferation by μ- or δ-opioid agonists was observed in brain cell cultures (Barg et al., 1992; Barg and Nah et al., 1993; Leslie et al., 1998). Moreover, in vitro studies have provided considerable evidence that κ receptors regulate cell division, with complex effects that depend on the age of the target cell (Barg and Belcheva et al., 1993; Gorodinsky et al., 1995). Tencheva et al. (1995) investigated the changes in the levels of in vitro RNA synthesis in developing rat brain after continuous block of opioid receptors. Repeated naloxone treatment induced increased levels (27-48%) of RNA synthesis during the early postnatal period.

Administration of opioid antagonists has been reported to increase cell proliferation and modulate brain development (Zagon et al., 1987; Schmahl et al., 1989; Lorber et al., 1990; Barg et al., 1992). In neonatal rats, when opiate receptors were continuously blocked by naltrexone, large animals with correspondingly bigger brains developed. This effect occurred in both sexes (Zagon and McLaughlin, 1983b; Zagon et al., 1984). Increased brain weight was accompanied by an increase in the number of neurons and glia (Zagon and McLaughlin, 1983b; Hauser et al., 1987; Kornblum et al., 1987; Zagon et al., 1987; Lorber et al., 1990).

In addition, Schmahl et al. (1989) examined the effect of naltrexone on the proliferation of the 4-12-week-old rat forebrain subependymal layer (SEL).

Naltrexone, when given daily throughout the preweaning period, evoked a long-lasting increase of the mitotic rate and the [^{3}H]thymidine labeling index at all ages with no sex-preference. This effect was most significant about 8-10 weeks after ending the naltrexone treatment.
Although the fundamental mechanisms which control cellular proliferation in the developing brain are largely unknown (Bartolome et al., 1997), differing opioid mechanisms may regulate cell division in different brain regions (Leslie et al., 1998).

Several investigators have examined the effect of narcotic drugs on adult rat brain protein synthesis with conflicting results (Hitzemann et al., 1976b). In vivo and in vitro, single or repeated doses of morphine inhibited protein synthesis in brain (Clouet et al., 1970; Kuschinsky, 1971; Datta et al., 1972; Hitzemann et al., 1976b; Lee et al., 1979; Rönnbäck et al., 1986). In vivo, both acute and chronic morphine treatments were found to increase (Clouet, 1971; Stolman et al., 1971; Loh et al., 1974; Lang et al., 1975; Hitzemann et al., 1976b; Loh et al., 1977; Sushkova et al., 1992), decrease (Clouet and Ratner, 1967, 1968; Clouet, 1971; Loh et al., 1974; Loh et al., 1977) or have no effect (Cox et al., 1970; Castles et al., 1972; Tulunay et al., 1975; Retz et al., 1982) on brain protein synthesis. A number of reports have dealt with the effects of opiate treatment on the in vivo incorporation of amino acids into protein, and the lack of agreement among these reports suggests that the results are affected by a number of variables including the brain area and subcellular fraction examined, the time course chosen, the route of administration of the radioactive precursor, the dose of opiate administered, and the nature of the opiate treatment. In vitro, acute morphine treatment decreased the ability of brain ribosomes to incorporate amino acids into proteins (Clouet and Ratner, 1968). Inhibition of protein synthesis was attributed to a decreased polysome stability (Clouet, 1970). Repeated injections of morphine were shown to increase brain protein synthesis in vitro (Clouet et al., 1968). This increased activity appeared to be due to alterations in both the ribosomes and the pH 5 enzyme complex (Stolman et al., 1971; Loh et al., 1977).

The majority of work in developing rats has been concentrated on the central nervous system (CNS). However, some studies have been carried out in the peripheral tissues.

Endogenous opioids affect gonadal cell proliferation (Orth, 1986; O, 1990). Exposure of fetal rat testes to naloxone for a total of 51 h significantly increased Sertoli cell proliferation. Blockade of opiate action in testes, maintained in vitro, enhanced the stimulation of Sertoli cell proliferation by FSH. Moreover, when antiserum to endorphin was administered intratesticularly, the level of Sertoli cell division in testes of perinatal rats was increased (Orth, 1986). These findings suggest that ß-endorphin produced within the testis is a paracrine modifier of the proliferative response of Sertoli cells to FSH, and endogenous ß-endorphin depresses the effect of FSH on Sertoli cells during perinatal development.

In preweaning rats, intracisternal (i.c.), but not subcutaneous (s.c.) or intrathecal (i.t.), administration of ß-endorphin produced a marked and long-lasting inhibition of liver DNA synthesis (Lorber et al., 1990; Bartolome et al., 1991; Bartolome and Bartolome, 1994). This effect was restricted to the first three postnatal weeks (Lorber et al., 1990; Bartolome et al., 1991). Coinjection of naloxone plus ß-endorphin i.c. completely antagonized the response. Naloxone alone caused significant increases in liver DNA synthesis (Bartolome et al., 1991). Similarly, CCK_8 blocked the liver DNA effect of ß-endorphin via actions within the brain, as s.c. administration of CCK_8 was ineffective. However, CCK_8 given alone i.c. decreased DNA synthesis in the liver (Bartolome and Lorber et al., 1994).

DNA synthesis in the developing rat uterus can be affected by the non-selective opioid peptide [D-Met^2,Pro^5]enkephalinamide (ENK) (Vertes et al., 1995). After acute ENK treatment, an decrease in in vitro [^{3}H]thymidine incorporation into uterine DNA was observed in 7-, 14- and 21-day-old rats. This effect was mainly limited to the first 12 h after ENK injection. The rate of DNA synthesis in rat uterus of 21-32 days of age was also inhibited by this opioid peptide (Vertes et al., 1996). However, the opioid action was more marked in younger animals. The decrease in DNA synthesis found after ENK treatment was completely prevented by naloxone. Similar results were obtained with treatment of [Met^5]enkephalin. Naloxone alone increased the DNA synthesis in the developing uterus. The effect was also age-dependent. The result suggests that

endogenous opioid peptide(s) exerts a tonic inhibitory effect on the rate of cell proliferation in developing rat uterus (Vertes et al., 1995).

Oestradiol (OE)-induced DNA synthesis in the adult, ovariectomized rat uterus could be inhibited by a single dose of [D-Met^2, Pro^5]enkephalinamide (ENK). The rate of inhibition was strictly time dependent (Ordog et al., 1992). Morphine and [D-Ala^2, D-Leu^5]-enkephalin administered 12 h, as well as dynorphin A fragment 1-13 given 2 h before decapitation also inhibited OE-induced uterine DNA synthesis. The ineffectivity of [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAGO), being one of the most selective ligand to μ sites (Leslie, 1987), suggests that receptors other than μ might be involved in the action of morphine injected 12 h before killing. The inhibition of OE-induced DNA synthesis by ENK administered 12, 2 or 1 h before killing was completely prevented by naloxone (NAL) injected 30 min prior to the corresponding ENK treatment. However, NAL treatment alone did not influence the OE-induced [6-^{3}H]-thymidine ([^{3}H]TdR) incorporation, suggesting that endogenous opioid peptides might not exert a tonic effect on OE-induced DNA synthesis. Interestingly, Ordog et al. (1992) could not demonstrate any significant effect of [Met^5]-enkephalin on OE-induced [^{3}H]TdR incorporation, although the presence of [Met^5]-enkephalin and of the transcripts of its precursor in the uterus (Kilpatrick et al., 1986; Petraglia et al., 1986; Jin et al., 1988; Muffly et al., 1988). They proposed the involvement of endogenous opioid peptides as autocrine/paracrine factors in the progesterone antagonism on OE-induced epithelial DNA synthesis.

The potential influence of opioid peptides on heart development have been reported earlier (Zagon et al., 1984; Zagon et al., 1985). Opioid peptides appear to have profound regulatory control of events related to DNA synthesis in cardiac morphogenesis (McLaughlin, 1994). Morphine exerts direct effects on cultured cardiac myocytes from neonatal rats. These effects are mediated via the delta and the kappa opioid receptors, as mu opioid receptors are not present in neonatal cardiomyocyte cultures (Ela et al., 1997).

In neonatal rats, the inhibitory effect of morphine on DNA synthesis was tissue-specific. While morphine significantly inhibited [^{3}H]thymidine incorporation into

brain DNA, it had no significant effect on DNA synthesis in the spleens of the same animals (Kornblum et al., 1987).

McLaughlin (2002) studied the influence of endogenous opioid blockade by naltrexone during prenatal life on postnatal heart development. Pregnant rats received daily injections of 50 mg/kg NTX throughout gestation; offspring were cross-fostered at birth to mothers not receiving NTX. In general, NTX-treated offspring weighed more than controls at birth and throughout preweaning life. At weaning (day 21), weights of experimental animals did not differ significantly from controls. At birth, the wet weights of the total heart were comparable between experimental and control groups. However, at 10 and 21 days of age, NTX-exposed rats had weights of the heart that were markedly elevated from control subjects. At 35 days of age, male and female rats in the NTX treatment group had heart weights that were increased 12% from their control counterparts; these differences were only statistically significant for the females. In the adult rat, DNA content was increased 53 and 63% in NTX-treated male and female rats, respectively, relative to controls. RNA concentration and content values were comparable between NTX and control groups at all ages except day 21, when a 12% increase in RNA content was recorded for the NTX-treated pups relative to controls. At 10 and 21 days, protein concentrations of the total heart were increased by as much as 75 and 51%, respectively, in NTX-treated offspring relative to control rat pups. In the adult, protein concentration was comparable between NTX and saline-treated rats. The data suggest that endogenous opioids function to regulate cardiac growth during the prenatal period, and that disruption of this process has long-term implication for cardiac biology.

In adult rats, opioids may influence macromolecular synthesis of peripheral tissues.

D-methadone, which is without CNS effects, is known to have inhibitory effects on cellular biosynthetic processes in spermatogenic cells, and atrophic effects on the prostate, seminal vesicles and testes of animals (Thomas et al., 1975). In vitro, racemic methadone inhibited in a dose-related fashion the decarboxylation of L-[1-^{14}C]-leucine and its incorporation into the protein of adult rat testicular cells (Jakubovic et al., 1978). The inhibition was highly significant even at 10 μM methadone. Similar dose-

related inhibitory effects of racemic methadone were apparent on both RNA and DNA synthesis, although the inhibition of DNA synthesis seemed to be less than that of RNA synthesis.

Morphine may induce renal injury in multiple ways. One of them is to induce mesangial cell (MC) proliferation (Singhal et al., 1992). Singhal et al. (1992) evaluated the effect of opiates on rat MC proliferation and matrix synthesis. The results suggest that in short term cultures, morphine in higher concentration suppresses cell growth but this effect disappears in prolonged cultures, whereas morphine in lower concentrations enhances cell growth even in short term cultures. Naloxone was able to inhibit the effect of morphine (in higher concentrations) in short term cultures. The authors concluded that prolonged exposure to morphine enhances MC proliferation and matrix synthesis. Since PGE_2 has been reported to attenuate the growth of MC as well as production of matrix by MC (Homma et al., 1988; Ardaillou et al., 1990), it seems a lack of PGE_2 in morphine treated cells may only be partly responsible for enhanced growth and increased matrix production by MC. Similarly, morphine directly modulated the proliferation of rat kidney fibroblasts and had a bimodal action (Di Francesco et al., 1994; Singhal et al., 1998). In vitro, morphine enhanced kidney fibroblast proliferation at lower concentrations. At higher concentrations morphine suppressed the proliferation of fibroblasts. Since morphine promotes apoptosis of fibroblasts at higher concentrations, morphine-induced cell suppression may partly be attributed to the occurrence apoptosis. Furthermore, morphine enhanced the proliferation of cultured rat renal medullary interstitial cell (RMIC) (Singhal et al., 1997). This effect was dose and time dependent.

In rodent, acute administration of opiates has been reported to decrease the in vivo incorporation of radioactive nucleotides into liver RNA (Loh et al., 1977). Levorphanol, a synthetic analogue of morphine, inhibited RNA synthesis in regenerating rat liver (Sakiyama et al., 1969; Becker et al., 1972). In female rats, levorphanol specifically inhibited the nucleolar ribosomal RNA synthesis in liver cells (Sakiyama et al., 1969). It did not show any effect on messenger and transfer RNA nor on other nucleolar RNA synthesis. In males, the results suggest that levorphanol inhibits the synthesis of all classes of hepatocyte RNA, though the effect is somewhat

more pronounced on the synthesis of ribosomal RNA components (Becker et al., 1972). Several batches of levorphanol also produced severe inhibition of RNA synthesis in rat liver (Rossman et al., 1971).

Morphine administration to rats for five weeks intensified protein biosynthesis in the kidneys, skeletal muscles; specific radioactivity of blood serum proteins increased (Sushkova et al., 1992). Whereas Hashiguchi et al. (1996) noted that central morphine administration resulted in marked decreases in liver, spleen, and muscle protein synthetic rates of adult rats. In addition, a central injection of [D-Ala^2, N-Me-Phe^4,Gly^5-ol]enkephalin (DAGO, a specific μ-receptor agonist) to adult rats produced a significant decrease in tissue protein synthesis in liver, spleen, gut mucosa, gut serosa, kidney, gastrocnemius and plantaris muscle, but did not alter rates of protein synthesis in the brain, heart and soleus muscle (Hashiguchi et al., 1997). The findings that no changes were detected in cardiac protein synthesis and ATP content (Hashiguchi et al., 1997) are in contrast with those previously reported in the isolated perfused rat heart, in which strong correlations among the rates of cardiac protein synthesis, intracellular pH, and tissue concentrations of creatine metabolites (Sugden et al., 1991), ATP, and GTP (Jefferson et al., 1971) were demonstrated and could very well be due to differences in the experimental model.

2.1.2. Effects of opioids on macromolecular constituents of tissues in the human

Clinical observations on infants and children exposed in early life to opiates reveal a retardation in somatic and neurobiological development (Wilson, 1975; Wilson et al., 1979; Lifschitz et al., 1983; Hayford et al., 1988; Bartolome et al., 1997), since the drug can easily cross the placental barrier into the fetal body and to the maternal milk and affects both pre- and postnatal development of the child (Slamberova, 2003). The retarded effects of narcotics on development persist long after birth (Haynes, 1984; Slamberova, 2003). Morphine depresses human brain development (Hutchings, 1982). Children delivered by methadone-exposed mothers often have subnormal head circumference measurements (Wilson, 1975). Kaltenbach et al. (1987) found that the difference was not clinically significant but rather reflects the relationship between

birth weight and head circumference. Moreover, diffuse, reactive astrocytosis, with regressive astrocytic changes, has been noted in the postmortem brains of chronic (non-HIV-infected) i.v. heroin abusers (Gosztonyi et al., 1993).

Naeye et al. (1973) reported that heart, liver, pancreas, adrenal glands, spleen, thymus and kidneys of newborn infants whose mothers used heroin up until delivery were affected. Growth retardation was mainly due to a subnormal number of cells in the various organs. The nuclear and cytoplasmic volumes of most cells were approximately normal. A direct effect of heroin on antenatal growth seems likely. In addition, some heroin addicts have low serum concentrations of growth hormone, and it is known that human fetuses who are deficient in growth hormone have a subnormal number of cells in their organs at birth (Naeye et al., 1971; Cushman, 1972). The high incidence of meconium histiocytosis in the placentas of heroin-exposed infants suggests that they experienced episodes of hypoxia or some other form of distress during fetal life, perhaps related to episodes of heroin withdrawal (Naeye et al., 1973).

In vitro, alteration of DNA synthesis by opioids is not restricted to neural systems, as it occurs in nonneural cells and in the immune system as well (Maneckjee and Minna, 1990; Maneckjee and Biswas et al., 1990; Taub et al., 1991; Barg and Belcheva et al., 1993). Tissue culture studies show that [Met^5]enkephalin can depress the growth of a variety of neural and non-neural cell types of human origin (Zagon et al., 1989c), and that enkephalin immunoreactivity is associated with proliferating cells of the developing human cerebellum (Zagon et al., 1991b). Using radiolabeled [Met^5]enkephalin, Zagon and Gibo et al. (1990) have discovered that the ζ-receptor is in abundance in the human cerebellum during the first postnatal weeks, but is not detected in adulthood. As discussed earlier this opioid-receptor interaction has been made responsible for the opioid activities as OGF (see page 10).

2.2. Effects of Opioids on Cortisol Secretion

2.2.1. Effects of opioids on cortisol secretion in the rat

In the rat hypothalamo-pituitary-adrenal (HPA) activity is influenced markedly by opioids (Lotti et al., 1969; Buckingham, 1982; Buckingham and Cooper, 1984a, b). Morphine given to pregnant rats induced inhibition of the HPA axis in newborns (Lesage et al., 1998; Dutriez-Casteloot et al., 1999). However, this inhibition was lost when the animals became 80-90 day-old (Dutriez-Casteloot et al., 1999).

Activation of the HPA axis by opioids and its dependence on the subtype of opioid receptor is present during the early neonatal period. Intravenously, the highly selective κ-agonist, U50,488H, increased plasma adrenocorticotrophin (ACTH) and corticosterone by postnatal day 2 in the rat, whereas a stimulation by morphine was not observed until postnatal day 5 (Adamson et al., 1991). Similar to the adult, the δ receptor agonist DPDPE was without effect (Buckingham and Cooper, 1986; Bero et al., 1987; Tsagarakis et al., 1990). Since both neonatal and adult studies have shown that kappa agonists are more potent than mu agonists in stimulating the HPA axis (Pfeiffer et al., 1985; Iyengar et al., 1986; Adamson et al., 1991; Pechnick, 1993), the κ receptor is implicated in early control and maturation of the HPA axis as well as HPA neuroendocrine regulation and the stress response.

In adult rats, acute morphine administration induced an increase in plasma levels of cortisol, an effect that was prevented by naloxone (Martinez et al., 1990). The chronic administration of morphine suppressed the HPA axis (Buckingham, 1982; Buckingham and Cooper, 1984a). The morphine response could be blocked by the μ antagonist ß-funaltrexamine (Buckingham and Cooper, 1986). However, in rats made tolerant to morphine by implantation of morphine-containing pellets, no significant changes in cortisol levels were observed (Martínez et al., 1990). Naloxone treatment induced an increase in plasma cortisol levels in these animals.

In summary, in the adult rat, acute administration of opioid agonists produces an increased HPA activity (Nikolarakis et al., 1987, 1989; Ignar and Kuhn, 1990; Martínez et al., 1990; Alcaraz et al., 1993; Pechnick, 1993; Kokka et al., 1994; Zhou et al., 1999), which has been assumed to be CRF-dependent (Wang et al., 1996; Taylor et al., 1997b). In contrast, chronic exposure to these agonists results in the development of tolerance to and dependence on opioid-induced endocrine secretion (Martínez et al., 1990; Gonzálvez et al., 1991; Vargas et al., 1997). Long-term treatment with opiates suppresses the HPA axis (Buckingham, 1982; Tsagarakis et al., 1989) and reduces adrenal cortisol steroid production and ACTH levels (Munson, 1973; Ho et al., 1978; Buckingham and Cooper, 1984a). Opioid withdrawal produces complex endocrine alterations in rats (Maldonado et al., 1992), including an activation of the HPA axis activity (Martínez et al., 1990; Gonzálvez et al., 1994; Vargas et al., 1997), possibly because of the overproduction of CRF.

2.2.2. Effects of opioids on cortisol secretion in the sheep

A stimulatory action of opiates on the fetal HPA axis is suggested by reports of increased fetal cortisol levels after the administration of opioid peptides to fetal sheep (Bousquet et al., 1984; Brooks and Challis, 1988).

Brooks and Challis (1988) demonstrated that endogenous opioid peptides were capable of stimulating the HPA axis in fetal sheep during late gestation. Bolus injection of equimolar amounts of ACTH or Leu-enkephalin both provoked a significant increase in the plasma cortisol concentration in sheep fetuses between days 120 and term (Bousquet et al., 1984). A site of action of opioids at the level of the hypothalamus or pituitary is supported by the finding that Leu-enkephalin had no effect on cortisol output from dispersed fetal adrenal cells. However, in adult sheep, ACTH, but not Leu-enkephalin, provoked a rise in the plasma cortisol concentration, suggesting that the Leu-enkephalin response is peculiar to fetuses and neonatal sheep (Carson and Challis, 1982), but is lost some time thereafter.

U50,488H, the kappa agonist, administered directly to the sheep fetus, resulted in a significant increase in ir-ACTH and ir-cortisol (Taylor et al., 1996). This stimulation

was completely blocked by concurrent naloxone administration. However, the molar dose of naloxone used was seven times that of U50,488H, and was two times the dose used to block the increase in ir-cortisol seen with i.v. administration of morphine (Taylor et al., 1994). The authors suggest that the stimulatory action of U50,488H on the pituitary-adrenal axis is mediated, at least in part, by the release of CRF and arginine vasopressin (AVP) via hypothalamic κ-opioid receptors, although an enhanced sensitivity of pituitary corticotrophs to these secretagogues is also a possibility (Taylor et al., 1996).

In late-term fetal sheep, intravenous administration of the mu selective agonist DAMGO resulted in a 92% increase in plasma ir-ACTH and ir-cortisol. DPDPE, the delta selective agonist, elicited a much smaller increase (52%) in ir-ACTH but did not result in a significant increase in plasma ir-cortisol. In contrast, there was a 7-fold increase in ir-ACTH and a significant increase in ir-cortisol with the kappa selective agonist U50,488H (Taylor et al., 1997b). When the same agonists were administered intracerebroventricularly, there was no change in ir-ACTH or ir-cortisol. The results show that the primary effect of these opioid agonists appears to be on the stimulation of ACTH release. In addition, it is likely that the kappa opioid system plays a more prominent role in modulation of the ovine fetal HPA axis than the mu or delta systems (Taylor et al., 1996; Taylor et al., 1997b).

Studies demonstrated a stimulation of the fetal sheep HPA axis by i.v. the Met-enkephalin analogue FK33-824 (Wang et al., 1986) and an inhibition after intracerebroventricular (i.c.v.) administration of the same compound (Wang et al., 1988). Results obtained in late-term sheep fetuses suggest that μ-preferring opioid agonists such as FK33-824, increase fetal plasma levels of ir-ACTH and ir-cortisol (Brooks and Challis, 1988).

The action of morphine on the fetal HPA axis was observed in fetuses older than 125 days. In fetal sheep, plasma cortisol levels were inhibited by low doses of morphine and increased by high doses of morphine, such that moderate doses appeared to have no effect. The increase in plasma ir-cortisol was associated with a significant increase in ir-ACTH that was naloxone-reversible (Taylor et al., 1997).

Carson and Challis (1982) have shown that Leu-enkephalin, injected into newborn lambs, provoked a rise in the plasma cortisol concentration in a dose-dependent fashion. It is likely that such an action is secondary to effects on brain or pituitary hormone release. The failure of Met-enkephalin to produce a response which achieved statistical significance may be due to the increased stability of the Leu-enkephalin compared with Met-enkephalin.

ß-endorphin infusion into the third ventricle of the adult sheep enhanced the secretion of cortisol in the peripheral blood under non-stressed conditions (Przekop et al., 1990; Domanski et al., 1993). Pretreatment with naloxone suppressed the increase in plasma cortisol induced by ß-endorphin (Przekop et al., 1990). Naloxone infusion did not alter the secretion of cortisol.

The synthetic opioid DAMME reduced basal ACTH and cortisol concentrations (Redekopp et al., 1985). Inhibition of cortisol secretion was reported in adult sheep given DAMME by slow i.c.v. infusion (Wang et al., 1988).

Morphine decreased plasma ACTH and cortisol (Redekopp et al., 1985; Parrott and Thornton, 1988; Parrott and Goode, 1993). Peripherally-administered morphine inhibited cortisol release stimulated by stress (Parrott and Thornton, 1989) or CRH (Parrott and Goode, 1992). Peripheral infusion of naloxone enhanced basal and stress-induced cortisol release in sheep (Parrott and Thornton, 1989; Thornton and Parrott, 1989; Matthews and Parrott, 1990; Parrott and Goode, 1993). Parrott and Goode (1993), however, found that i.c.v. injections of morphine, dynorphin and DADLE did not affect cortisol secretion. In the same study, i.c.v. naloxone administration was ineffective in blocking the effects of exogenously administered opioids, whereas i.v. naloxone abolished the response, suggesting an action at the pituitary or adrenal level.

2.2.3. Effects of opioids on cortisol secretion in the human

It is well established that opioid peptides have an inhibitory effect on the HPA axis in the human (Delitala et al., 1981a, b; Demura et al., 1981; Grossman, 1983; Taylor et al., 1983; Grossman and Delitala et al., 1986; Wang et al., 1996). Unlike many mammalian species, including the rat, mouse, sheep, guinea pig, cat and dog

(Buckingham, 1982; Nanda et al., 1992; Pechnick, 1993), the acute administration of opiate alkaloids and opioid peptides have been shown to reduce the secretion of ACTH and cortisol in human subjects (McDonald et al., 1959; Stubbs et al., 1978; Zanoboni et al., 1987). The reason behind this difference in HPA response to opioids between humans and most experimental animal species is not known yet.

In man, the administration of enkephalin decreased the secretion of ACTH and cortisol (Lamberts et al., 1983; Steven et al., 1983). Basal plasma concentrations of ACTH and cortisol were lowered by Met-enkepahlin (Delitala et al., 1981b) and Leu-enkephalin was reported to decrease cortisol (Smythe et al., 1980).

mu-Opiate agonists suppress cortisol and ACTH secretion in man (del Pozo and Martin-Perez, 1980; Pfeiffer and Herz, 1984; Pfeiffer et al., 1986). Acute administration of morphine lowered circulating cortisol (Delitala et al., 1983; Zis et al., 1984), and similar responses were seen following methadone, pentazocine, nalorphine and buprenorphine (Delitala et al., 1983; Rolandi et al., 1983). Serum cortisol decreased after i. m. administration of a partial opiate antagonist nalorphine (Rolandi et al., 1981). The i.m. administration of buprenorphine, a derivative of the morphine alkaloid thebaine with agonist-antagonist properties, resulted in a significant fall in serum cortisol values (Rolandi et al., 1983). Morphine, methadone, pentazocine and nalorphine, administered intravenously, all caused falls in serum cortisol (Delitala et al., 1983). Since the effects were not antagonized by low dose naloxone, changes in cortisol are probably mediated via κ- or δ-receptors.

Enkephalin analogues induce a decrease in plasma levels of ACTH and cortisol (George et al., 1974; Hellman et al., 1975; Stubbs et al., 1978; Howlett and Rees, 1986). The effects seem to be mediated via δ-receptors (Gaillard et al., 1981; Allolio et al., 1982).

kappa-opiate receptors represent an important fraction of the total opiate receptor capacity in human brain. i.m. administration of the κ-opioid receptor agonist spiradoline (U-62066E) caused a dose-dependent stimulation of cortisol release in man (Ur et al., 1997). However, the data (Pfeiffer et al., 1986) showed that a kappa-agonist inhibited secretion of cortisol and ACTH by acting at stereospecific, but naloxone-

insensitive opioid receptors. It seems that κ-receptor agonists suppress the HPA axis by a mechanism differing from the one of mu-agonists.

Endocrine consequences of opioid blockade in man are well recognized. In humans, opioid antagonists stimulate the release of ACTH and cortisol (Morley, 1981; Pechnick, 1993; Martin del Campo et al., 1994; Williams et al., 2003). However, the type of opioid receptor that may be related to these endocrine effects is unknown (Williams et al., 2003).

A stimulatory response of the pituitary-adrenal axis induced by naloxone was observed in man (Blankstein et al., 1980; Morley et al., 1980). Naloxone significantly increased the secretion of ACTH and/or cortisol in normal subjects (Lamberts et al., 1983; Steven et al., 1983; Taylor et al., 1983; Coiro et al., 1984; Cohen et al., 1985; Leslie et al., 1985; Wang et al., 1986; Ehrenreich et al., 1987; Jackson et al., 1989; Al-Damluji et al., 1990; Jackson et al., 1990; Martin del Campo et al., 1994; Torpy et al., 1997). Significant dose-dependent hormonal effects were also found (Cohen et al., 1983; Grossman and Clement-Jones, 1983b; Martin del Campo et al., 1994). However, it has been noted that naloxone only stimulated a rise in ACTH and/or cortisol levels at high dose (Grossman and Clement-Jones, 1983a; Grossman and Moult et al., 1986; Pfeiffer et al., 1986). The failure of some groups to observe an effect was related to the use of low doses of naloxone (Spiler and Molitch, 1980; Wakabayashi et al., 1980; Delitala et al., 1982; Pontiroli et al., 1982). Similarly, small doses of naloxone did not induce the changes in serum cortisol caused by larger doses of the drug (Volavka and Cho et al., 1979; Morley et al., 1980; Grossman and Moult et al., 1981), and, higher doses of naloxone were required to block the effects of exogenous opioids and opiates on serum cortisol (del Pozo and von Graffenried et al., 1980; Delitala et al., 1983). Furthermore, the stress induced increase in ACTH (Serri et al., 1981) or cortisol (Morley et al., 1980) was enhanced after high doses of naloxone while lower doses had no effect (Spiler and Molitch, 1980; Wakabayashi et al., 1980; Engquist et al., 1981; Pontiroli et al., 1982). The naloxone insensitivity of the pituitary-adrenal axis suggests that these responses are modulated by κ- or δ-receptors (Grossman and Moult et al., 1986).

Michelson et al. (1996) and Vythilingam et al. (2000) assume that naloxone does affect the HPA axis via a central effect.

Naltrexone significantly increased levels of both ACTH and cortisol in humans (Volavka and Mallya et al., 1979; Mendelson et al., 1986; Martin del Campo et al., 1994; King et al., 2002). The results of the King et al. (2002) study showed reliable and steady increases in ACTH and cortisol levels within two hours after a single oral dose of 50 mg naltrexone. Heroin addicts also showed continued elevated HPA hormone levels during intermediate-term treatment with daily oral naltrexone (Kosten et al., 1986a, b). The data indicate that orally administered naltrexone acutely disinhibits the HPA axis and suggest that heightened HPA response to naltrexone might be a direct effect of opioid antagonism on hypothalamic-pituitary hormone release and not due to a nonspecific effect (King et al., 2002).

2.2.4. Effects of opioids on cortisol secretion in the pig

There are very few works concerning the effect of opioids on HPA axis. In the prepubertal pig, the κ-opiate agonist PD 117302 partially antagonized CCK-8 stimulated cortisol secretion (Parrott et al., 1991).

Naloxone administration increased serum cortisol concentrations in gilts (Barb et al., 1986). Naloxone also increased plasma cortisol and ACTH concentrations in response to nose restraint in young non-pregnant pigs (Rushen and Ladewig, 1991; Rushen et al., 1993). Estienne et al. (1988) observed that serum cortisol concentrations increased significantly in intact but not hypophysial stalk-transected gilts following naloxone injection and suggested that the opioid antagonist enhances cortisol secretion primarily by a central action in pigs. On the other hand, in gilts received naloxone at 7.5 min after the birth of the first piglet, plasma cortisol continued to increase over the 4 h following the birth of the first piglet (Jarvis et al., 1998). The result was similar to the control group. But this experiment is not exclusive. Thus it can not be concluded that opioids do not affect cortisol secretion during parturition in the pig.

3. Aims and Objectives

As the literature review indicates that opioids can influence organ growth and development as well as the HPA axis, the purpose of the present experiments was to investigate whether naloxone, an opioid antagonist affects kidney weight, macromolecular constituents of kidney and cortisol secretion in fetal and neonatal pigs. To this end the following points were studied:

- The effect of chronic naloxone treatment on kidney weight as well as DNA, RNA and protein contents of kidney in male and female fetal and neonatal pigs.
- The effects of acute and chronic treatments with naloxone on cortisol secretion in male and female fetal and neonatal pigs.

4. Materials and Methods

4.1. Fetuses

Experiments were carried out on a total number of 46 pregnant German Landrace sows (parity range of 1 – 2). They were kept in individual crates under controlled environmental conditions (temperature 20° to 22°C; lights on from 0600 to 1800 h), receiving a standard diet twice daily (at 0630 and 1130 h) and water ad libitum. Food was withheld from 12 hours before to 12 hours after surgery. On day 100 (range 97 to 103) post conception (p.c.; first day after mating = day 0, term = 113 ± 1 days), one fetus per sow was chronically catheterized. Twenty-four fetuses were treated with naloxone and 22 fetuses were treated with saline.

4.1.1. Surgical procedures

A chronic vascular catheter was placed into the fetal jugular vein as described previously (Bauer and Parvizi, 1996). Briefly, sows were sedated with azaperone (2.5 mg/kg, Stresnil®; Janssen, Neuss) 30 minutes before the induction of general i.v. anesthesia with ketamine-hydrochloride (10 mg/kg of ketamine via an ear vein, Ketamine®; Atarost, Twistringen). A silicon catheter was placed into the maternal external jugular vein and was used for maintenance of anesthesia with ketamine-hydrochloride. Lateral abdominal laparotomy was performed and one fetal compartment of the uterus was exteriorized and opened. A silicon catheter was placed into the fetal jugular vein and tunneled to the neck. Following intra-amniotic application of 500 mg kanamycin (Albrecht, Aulendorf) the fetal membranes and uterine wall were closed with one suture and the uterus was placed back into the abdominal cavity. The fetal catheter was tunneled subcutaneously and exteriorized at the back of the sow. On the day of surgery and on the two following days sows received 625000 IU penicillin and 625 mg dihydrostreptomycin (Tardomyocel comp. III, Bayer, Leverkusen) i.m. Fetuses and dams were allowed to recover for 48 hours before the experiments were started. Fetal catheters were flushed daily with 1 ml of

saline and filled with 0.6 ml of saline containing 50 IU heparin to keep them patent. The sex of fetuses was determined at surgery and was confirmed after birth.

4.1.2. Experimental protocol

4.1.2.1. Treatments and blood sampling

Male (22) and female (24) fetuses (only animals with two or more blood sampling days were included) were randomly assigned to one of the two treatment groups, naloxone or control. In order to eliminate possible diurnal variations, all experiments started at the same time of the day (0830 to 0930 h). Blood sampling (1 ml per sample) was started 30 minutes before i.v. administration of naloxone or saline and lasted for 120 minutes with a sampling interval of 15 minutes (Table 3). Volume was restored with warm (38°C) physiological saline after each sample. Naloxone hydrochloride (1 mg/kg estimated fetal weight; Sigma, Munich, FRG) was dissolved in 0.5 ml of saline, controls received vehicle only. To assess the physiological condition of the fetus, blood samples (0.5 ml) were withdrawn before the first sample and immediately after the withdrawal of the last sample. Blood pH and hematocrit (Ht) were analyzed using blood gas system 280 (Bayer Vital GmbH & Co., Geschaeftsbereich Diagnostics, Fernwald, FRG; calibrated on rectal temperature of the sow on the day of experiment). Only healthy fetuses were included in the study ($7.5 > pH > 7.3$ and hematocrit > 25%). Treatments were administered every day and the blood sampling procedure was carried out repeatedly at 48 h intervals up to term or as long as catheters were patent. Heparinized samples were kept on ice and centrifuged immediately after the last sample was obtained. Plasma was stored at –20°C until analysis.

4.1.2.2. Tissue sampling

At day 1 after birth, renal tissues were taken from the catheterized fetus (only animals born alive were used) and from two other littermates, one male and one female with a

birth weight similar to the operated fetus. Kidneys were harvested, decapsulated, rinsed with physiological saline solution to remove excessive blood, weighed, sealed in aluminum bags and frozen in liquid nitrogen and stored at −80°C until analyzed. Ten treated animals (4 males and 6 females) and twenty-one untreated controls (9 males and 12 females) were used for assaying kidney DNA, RNA and protein levels.

Table 3: Experimental Schedule

Group[a]	Number of fetuses[b]		Treatment and blood sampling schedule of fetuses on days 102, 104, 106, 108 and 110 of pregnancy
	Male	Female	
			Period 1 (-30* -15 0) ↑ Period 2 (15 30) Period 3 (45 60) Period 4 (75 90*min)
1	10	14	1 mg naloxone ↑
2	12	10	saline (control)

[a] Experiment was started on day 102 and repeated on days 104, 106, 108 and 110 of gestation.
[b] Number of fetuses at the beginning of the experiment.
*Blood pH and hematocrit were measured at times indicated.

4.2. Neonates

Seventeen German Landrace sows (parity range from 1 – 3) were kept in individual pens under controlled environmental conditions (temperature 20° to 22° C; lights on from 0600 to 1800 h). They received a standard diet twice daily (at 0630 and 1130 h) and had access to water ad libitum. Sixty-eight neonates (2 males and 2 females per sow) of similar birth weight were randomly assigned to three groups. In treated group, twenty-eight animals were provided with subcutaneous pumps (naloxone) at day 1 after birth. In control group 1, six animals were implanted with osmotic pumps (saline). In control group 2, thirty-four animals were subjected to a sham operation.

4.2.1. Implantation of osmotic pumps

At day 1 after birth, placement of pumps was performed immediately following blood collection (see experimental protocol) under general i.m. anesthesia (Hostacain®, Hoechst, FRG).

The skin of left or right flank was opened and an Alzet osmotic pump (model 2ml4, Alza Corporation, Palo Alto, California, USA), containing either saline (3 males and 3 females) or naloxone (14 males and 14 females), was implanted under the skin of the flank. Naloxone hydrochloride (Sigma, Munich, FRG) was dissolved in saline. The Alzet osmotic pumps are guaranteed by the manufacturer to produce a constant pumping rate of 2.42 ± 0.09 µl/h (mean ± SEM) over a period of 28 days. The concentration of naloxone in the pump was such that with a constant pumping rate of 2.5 µl/h a quantity of 0.167 mg of naloxone was delivered each hour. Thirty-four neonates (17 males and 17 females) were sham operated. In all three groups, the skin incision was closed with one suture.

4.2.2. Experimental protocol

Blood samples (3 to 5 ml) were collected from each neonate via jugular venipuncture at days 1, 7 and 14 after birth to assess plasma cortisol. In order to eliminate possible diurnal variations, venipuncture was performed at the same time of each sampling day (0830 to 0930 h). Plasma was stored at –20°C until analysis.

At 14 (range 13-15) days of age, animals were weighed before blood sampling and exsanguination. Kidneys were collected, weighed and stored at –80°C until assayed. Eight naloxone treated neonates (4 males and 4 females), two control neonates implanted with pumps (1 male and 1 female) and six control neonates sham operated (3 males and 3 females) were used for renal DNA, RNA and protein analysis.

4.3. DNA, RNA and Protein Analysis

4.3.1. DNA and RNA determination

The frozen kidney samples (500 mg) were homogenized using Potter-Elvehjem-homogenizer (Braun Melsungen) in denaturing solution GTC (1 ml GTC per 100 mg tissue) and were taken into 2 ml microcentrifuge tubes for determinations of DNA and RNA. The homogenates were stored at –20°C (for DNA measurement) and –80°C (for RNA measurement) respectively until analyzed.

4.3.1.1. DNA analysis

The procedure for extraction of DNA in the tissue was similar to that described by Powell and Gannon (2002) with some modifications.
DNA was determined, in triplicate, in 100 µl of each of the homogenates. To 100 µl of homogenate, 100 µl of phenol and 100 µl of 24: 1 chloroform/isoamyl alcohol were added, mixed and incubated for 5 min at room temperature and then centrifuged at 10,000 ɡ for 5 min. The upper aqueous phase was transferred to a clean tube and 0.1 vol of 3 M sodium acetate (pH 5.5) and 2.5 vol of 100% ethanol were added to the supernate. The suspension was incubated at –20°C for at least 1 h. DNA was recovered by centrifugation at 10,000 ɡ for 15 min. The supernatant was discarded and the pellet was washed twice with 70% ethanol. DNA was resuspended in TE buffer (pH 7.5) until dissolved and quantitated by measuring absorbance at 260 and 280 nm.

Aliquots of DNA samples (1 µl) were separated in an agarose gel containing 0.7% agarose in 1X TAE buffer and 0.3 µg/ml EtBr. The gel was run at 4.1 V/cm for 1 h 35 min using orange G as front-marker (orange mix). The DNA was recorded using a CCD camera (Kappa CF 8/1 FMC) and saved by Movie Machine II (Fast Multimedia) program.

4.3.1.2. RNA analysis

The procedure for extraction of total RNA in the tissue was similar to those previously described by Chomczynski et al. (1987) and Chirgwin et al. (1979) with some modifications.

RNA was determined, in duplicate, in 300 µl of each of the homogenates. To 300 µl of homogenate, 300 µl of phenol, 240 µl of 49: 1 chloroform/isoamyl alcohol and 60 µl of 2 M sodium acetate (pH 4.0) were added in succession and the mixture was vortexed after each addition. Samples were centrifuged for 20 min at 10,000 ɡ and the aqueous phase RNA was precipitated with an equal volume of 100% isopropanol at –20°C for at least 1 h. Precipitated RNA was pelleted by centrifugation, resuspended in 30 µl of GTC and 3 µl of sodium acetate, reprecipitated with isopropanol, rinsed twice with 70% ethanol to remove residual amounts of guanidine contaminating the pellet, and dissolved in DEPC-treated water. Total RNA was quantitated by reading the A_{260} and A_{280} (Mielenz, 2002).

RNA integrity was checked in a denaturing formaldehyde-MOPS buffer system (Sambrook et al., 1989).

5 µg-10 µg of total RNA was used for RNA gel electrophoresis. RNA Samples were denatured in a 5X RNA loading buffer (Quiagen, RNeasy Mini Handbook 1997, modifies) by heating at 65°C for 10 min and subsequently placed on ice. To prepare 1.4% formaldehyde–MOPS gel, agarose was dissolved in DEPC-H_2O. Then 10X MOPS buffer and 12.3 M formaldehyde were added to the melted agarose cooled to 70°C. The gel was poured onto a gel casting platform and the gel platform was placed in an electrophoresis tank containing 1X MOPS running buffer. The samples were separated in the gel run at a voltage of 3.5 V/cm for 2 h. The RNA was visualized directly upon illumination with UV light and photographed under UV light.

4.3.2. Protein measurement

0.2-0.3 g of renal tissue was homogenized using an Ultra-Turrax (Janke and Künkel) in Tris buffer (vide supra) to a final concentration of 2-4%. Protein was determined, in triplicate, in 5 µl of each of the homogenates by the Bradford method (Bradford, 1976), using BSA (Bovine Serum Albumin) as a standard.

4.4. Cortisol Analysis

Plasma cortisol levels were determined in duplicate in 20 µl aliquots by a rapid direct enzyme immunoassay (EIA) as described (Marc et al., 2000). The method had been evaluated for direct determination in 20 µl plasma diluted 1: 20 without prior extraction. The antibody (code no. 602) was raised in a rabbit against cortisol-21-hemisuccinate-BSA. Its crossreactivities were as follows: cortisol, 100%; cortisone, 45%; corticosterone, 15%; desoxycorticosterone, 8%; progesterone, 8%; and testosterone, 3%. The intra- and inter-assay coefficient of variation was 8.9% and 12.6%, respectively.

4.5. Statistical Analysis

Because there were no differences between neonatal control groups 1 (saline pump) and 2 (sham operated), the results from the two groups were combined. In the following, the combined group of neonates implanted with saline pump and sham-operated neonates is referred to as neonatal control group.

To compare body and kidney weights and macromolecular content of kidney between birth and day 14 after birth, data obtained from fetal and neonatal control groups (the figures for males and females were pooled) were subjected to the Mann-Whitney rank sum test. Naloxone effects during late gestation and at the first 14 days of life (the data for 14-day-old male and female neonates were pooled because of no sex differences) were evaluated by Mann-Whitney rank sum test and Students' t-test, respectively.

In both fetuses and neonates, since no sex differences were found, the data of cortisol for males and females were combined.

Average pre-treatment levels of cortisol in each fetus were determined as the mean value of the first three samples (-30, -15 and 0 min); post-treatment cortisol levels were evaluated for three post-treatment periods of 30 min each, representing the first, second and third 30 min after treatment, respectively (Table 3). In the following, these 4 periods will be referred to as blood sampling periods 1 to 4. For detection of acute effects on each experimental day, differences between plasma concentrations of cortisol during pre- and the three post-treatment periods were calculated for all fetuses and tested for statistical significance in each group by one-way repeated measures ANOVA. To evaluate long-term effects, differences between basal cortisol levels (levels before naloxone or saline injections on each experimental day) were analyzed by two-way repeated measures ANOVA. The course of basal cortisol levels throughout the whole experimental period was determined in both control and treated groups of fetuses, using linear regression.

The Mann-Whitney rank sum test was used to compare basal cortisol levels between fetal control group on the first day of treatment (day 102) and neonatal control group.

Differences between cortisol levels were tested for statistical significance in each neonatal group by one-way repeated measures ANOVA.

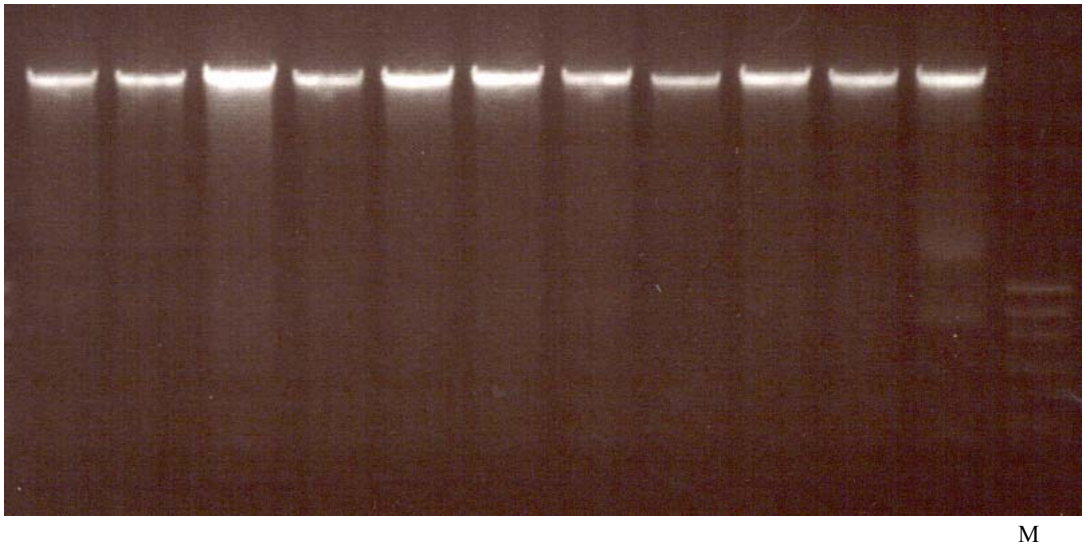
Statistically significant differences were assumed at the level of $p < 0.05$. Data are expressed as mean ± SEM.

5. Results

5.1. Qualitative Evaluation of DNA and RNA

Integrity and purity conditions of DNA and total RNA extracts as shown in Fig. 1 were fulfilled. 28S and 18S ribosomal RNA bands are indicators of quality of extracted RNA.

A



B

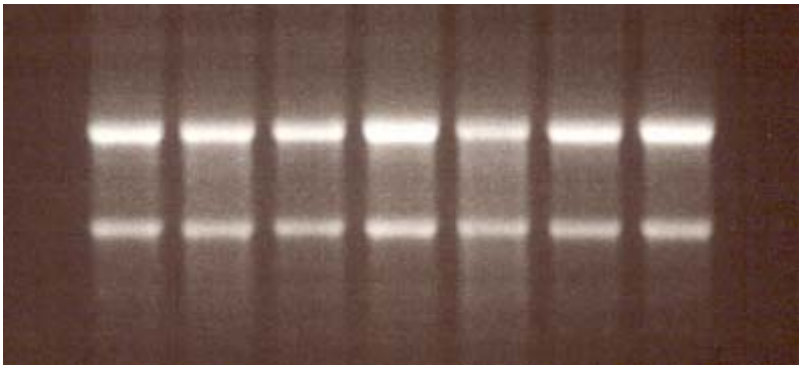


Figure 1: DNA bands after DNA gel electrophoresis in TAE buffer (A). Lane M: marker. (B) 28 S rRNA and 18 S rRNA bands after denaturing RNA gel electrophoresis in formaldehyde-MOPS buffer system.

5.2. Kidney Weight and Macromolecular Constituents of Kidney at Birth and at Neonatal Day 14 in Control Pigs

5.2.1. Developmental changes in the kidney weight

The variations in body weight, kidney weight and relative kidney weight expressed as per cent of body weight during development are given in Fig. 2. The results showed, as expected, that both body weight (D 1 = 1.27 ± 0.04 kg vs D 14 = 4.15 ± 0.37 kg) and kidney weight (D 1 = 4.95 ± 0.33 g vs D 14 = 12.23 ± 1.20 g) in pigs increased significantly ($P \leq 0.001$) from birth to day 14 after birth, whereas the relative weight of the kidney (D 1 = 0.39 ± 0.02% vs D 14 = 0.30 ± 0.01%) decreased significantly ($p < 0.01$).

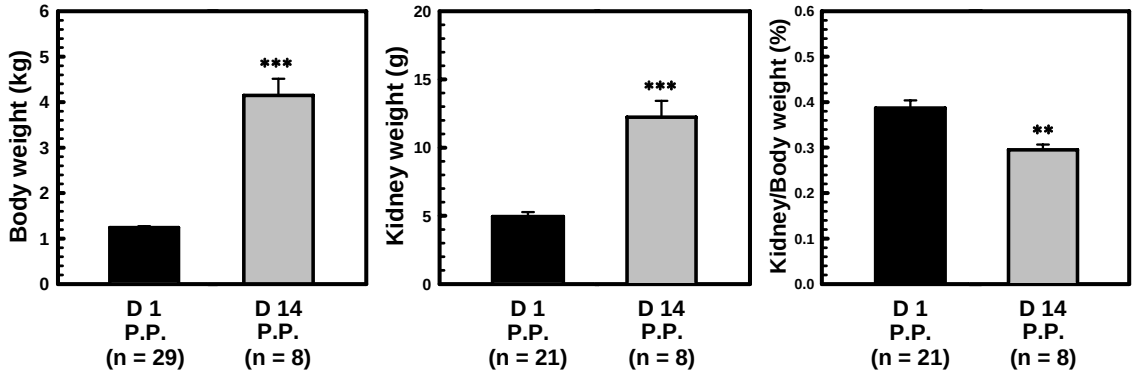


Figure 2: Body and kidney weights and relative kidney weight at day 1 (D 1) and at day 14 (D 14) postpartum (p.p.) in pigs. ** ($p < 0.01$) and *** ($p \leq 0.001$) show statistically significant differences between the two age groups.

5.2.2. Developmental changes in amount of macromolecular kidney constituents

From birth to day 14 p.p., there was no change in DNA concentration of kidney, whereas concentrations of RNA (D 1 = 1.68 ± 0.13 µg/mg vs D 14 = 2.51 ± 0.17 µg/mg; $p < 0.01$) and protein (D 1 = 55.72 ± 2.38 mg/g vs D 14 = 84.64 ± 3.97 mg/g; $p \leq 0.001$) increased significantly (Fig. 3). The total content of DNA (D 1 = 19.18 ± 1.03 mg vs D 14 = 46.57 ± 4.69 mg), RNA (D 1 = 8.36 ± 0.89 mg vs D 14 = 30.85 ± 3.79 mg) and protein (D 1 = 274.47 ± 20.75 mg vs D 14 = 1027.21 ± 98.25 mg) also increased significantly ($p \leq 0.001$) during this period (Fig. 4). Although significant ($p \leq 0.001$) increases in ratios of RNA to DNA (D 1 = 0.43 ± 0.03 vs D 14 = 0.68 ± 0.08) and protein to DNA (D 1 = 15.26 ± 0.88 vs D 14 = 22.28 ± 0.90) were observed in the present study, no changes in the ratio of kidney weight to its DNA content could be determined (Fig. 5).

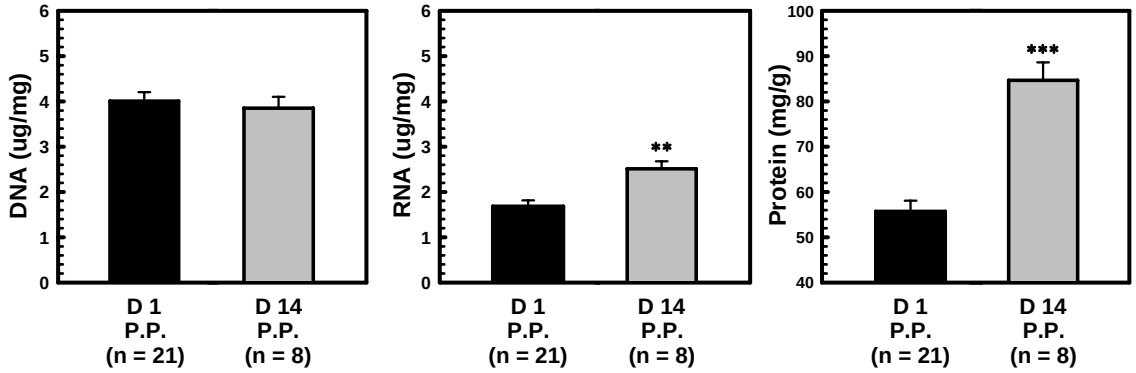


Figure 3: DNA, RNA and protein concentrations in kidney at day 1 (D 1) and at day 14 (D 14) postpartum (p.p.) in pigs. ** ($p < 0.01$) and *** ($p \leq 0.001$) show statistically significant differences between the two age groups.

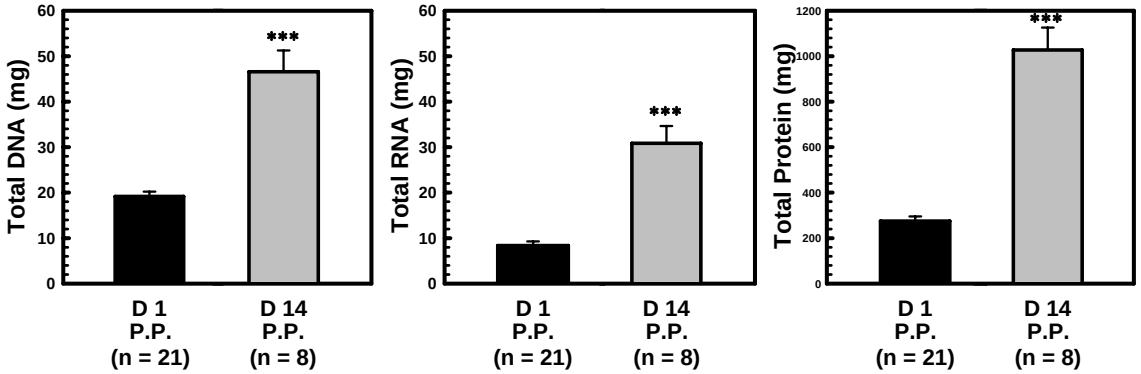


Figure 4: Total DNA, RNA and protein content in kidney at day 1 (D 1) and at day 14 (D 14) postpartum (p.p.) in pigs. *** ($p \leq 0.001$) shows a statistically significant difference between the two age groups.

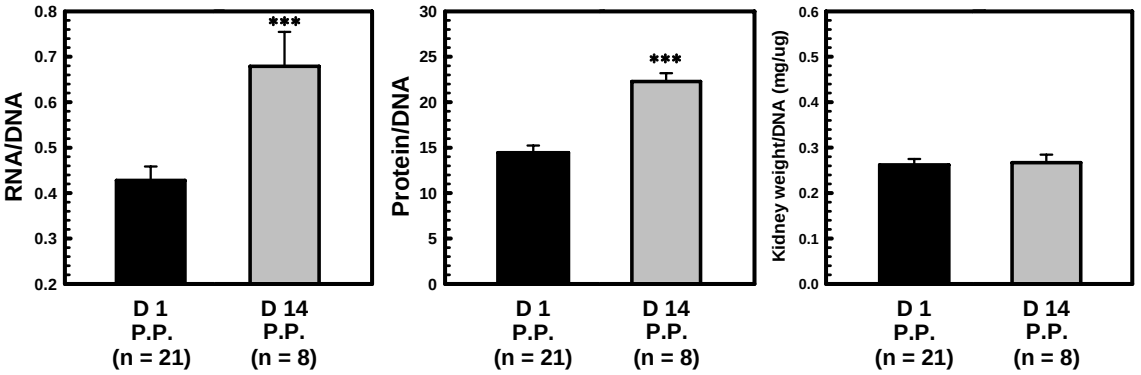


Figure 5: Ratios of RNA to DNA, protein to DNA and kidney weight to DNA at day 1 (D 1) and at day 14 (D 14) postpartum (p.p.) in pigs. *** ($p \leq 0.001$) shows a statistically significant difference between the two age groups.

5.3. Effects of Naloxone on Kidney Weight and Macromolecular Constituents of Kidney in Fetal Pigs

Since there was a sex difference in the effect of naloxone, we describe the results of females and males separately.

5.3.1. Kidney weight and macromolecular constituents of kidney in female and male fetal pigs treated with saline during late gestation

At day 1 after birth, there were no statistically significant differences in any parameter analysed between female and male pigs treated with saline during late gestation (Table 4). However, it is worth mentioning that the protein concentration of kidney in males was slightly but not significantly higher than in females (60,79 ± 4,86 mg/g vs 51,93 ± 1,46 mg/g).

Table 4: Body weight, kidney weight and macromolecular content of kidney at day 1 after birth in control female (n = 12) and male (n = 9) pigs[a]

Parameter	Control Females	Control Males
Birth weight (kg)	1.29 ± 0.06	1.24 ± 0.07
Kidney weight (g)	5.05 ± 0.48	4.81 ± 0.47
Kidney/birth weight (%)	0.39 ± 0.02	0.39 ± 0.03
DNA (µg/mg)	3.93 ± 0.18	4.11 ± 0.41
RNA (µg/mg)	1.65 ± 0.15	1.72 ± 0.25
Protein (mg/g)	51.93 ± 1.46	60.79 ± 4.86
Total DNA (mg)	19.27 ± 1.31	19.05 ± 1.74
Total RNA (mg)	8.55 ± 1.32	8.14 ± 1.21
Total protein (mg)	262.40 ± 26.12	290.56 ± 34.72
RNA/DNA	0.44 ± 0.05	0.42 ± 0.04
Protein/DNA	13.57 ± 0.79	15.62 ± 1.46
Kidney weight/DNA (mg/µg)	0.26 ± 0.01	0.26 ± 0.03

[a] Each value represents the mean ± SEM.

5.3.2. Kidney weight and macromolecular constituents of kidney in female fetal pigs treated with naloxone during late gestation

In female pigs, naloxone did not show any effects on birth weight, kidney weight and kidney macromolecular content at late gestational period (Table 5). It is, however, noteworthy that the concentration of protein (59.55 ± 5.02 mg/g vs 51.93 ± 1.46 mg/g) in the treated group was higher, though not significantly, than in the controls ($p < 0.1$).

Table 5: Body weight, kidney weight and macromolecular content of kidney at day 1 after birth in control (n = 12) and naloxone treated (n = 6) female pigs[a]

Parameter	Control Females	Treated Females
Birth weight (kg)	1.29 ± 0.06	1.17 ± 0.08
Kidney weight (g)	5.05 ± 0.48	5.25 ± 0.60
Kidney/birth weight (%)	0.39 ± 0.02	0.45 ± 0.03
DNA (μg/mg)	3.93 ± 0.18	4.49 ± 0.54
RNA (μg/mg)	1.65 ± 0.15	1.49 ± 0.14
Protein (mg/g)	51.93 ± 1.46	59.55 ± 5.02
Total DNA (mg)	19.27 ± 1.31	22.62 ± 1.94
Total RNA (mg)	8.55 ± 1.32	7.95 ± 0.86
Total protein (mg)	262.40 ± 26.12	310.63 ± 36.10
RNA/DNA	0.44 ± 0.05	0.39 ± 0.06
Protein/DNA	13.57 ± 0.79	14.46 ± 2.12
Kidney weight/DNA (mg/μg)	0.26 ± 0.01	0.24 ± 0.03

[a] Each value represents the mean ± SEM.

5.3.3. Kidney weight and macromolecular constituents of kidney in male fetal pigs treated with naloxone during late gestation

In male pigs naloxone did not exhibit any effect on body weight at late gestational period. However, both kidney weight (Nal = 7.12 ± 0.62 g vs Con = 4.81 ± 0.47 g; $p < 0.05$) and relative kidney weight (Nal = 0.60 ± 0.04% vs Con =0.39 ± 0.03%; $p = 0.001$) were significantly higher in treated males than in controls (Fig. 6).

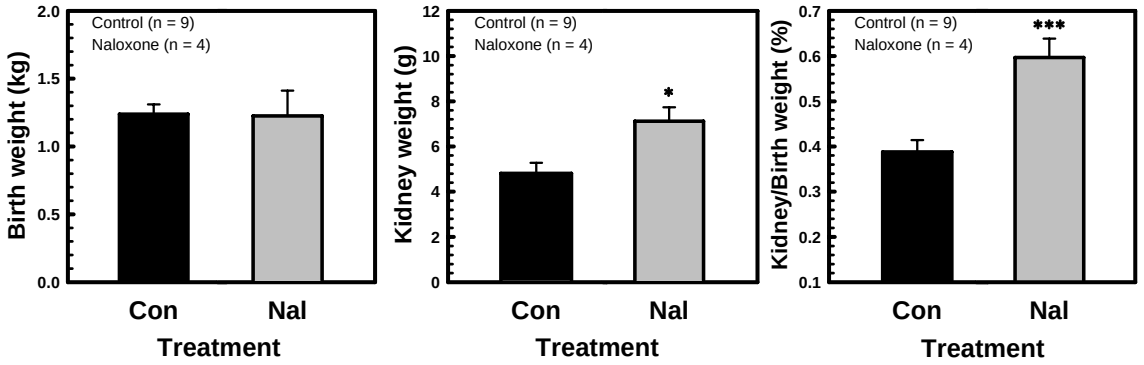


Figure 6: Body and kidney weights and relative kidney weight at day 1 after birth in control (Con) and naloxone (Nal) treated male fetal pigs. * ($p < 0.05$) and *** ($p = 0.001$) show statistically significant differences between the two groups.

The results of analysis of macromolecular composition of kidney demonstrated that although naloxone had no effects on the concentrations of DNA and RNA (Fig. 7), total DNA (Nal = 26.94 ± 1.82 mg vs Con = 19.05 ± 1.74 mg) and RNA (Nal = 14.12 ± 1.38 mg vs Con = 8.14 ± 1.21 mg) content in treated males was significantly higher ($p < 0.05$) than in the control group (Fig. 8).

Interestingly, naloxone also affected protein concentration. In treated males the protein concentration was significantly lower ($p < 0.05$) than in controls (Fig. 7). No significant difference in total protein content between the two groups was observed (Fig. 8).

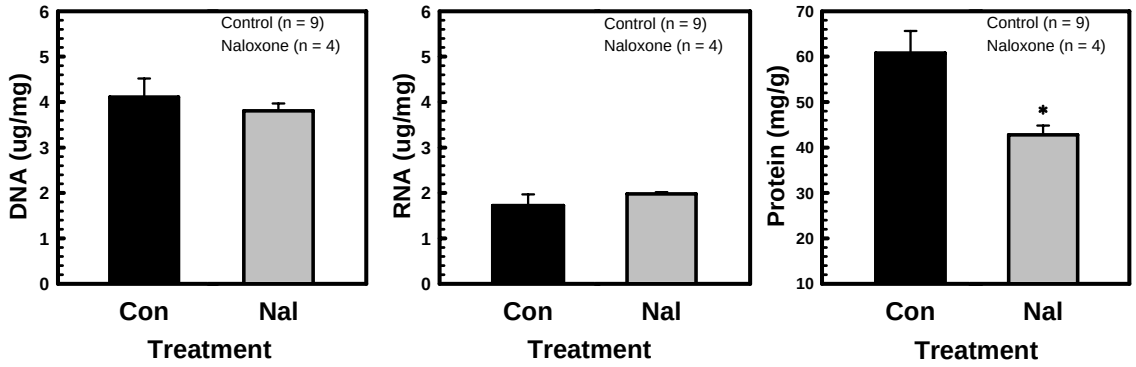


Figure 7: DNA, RNA and protein concentrations in kidney at day 1 after birth in control (Con) and naloxone (Nal) treated male fetal pigs. * ($p < 0.05$) shows a statistically significant difference between the two groups.

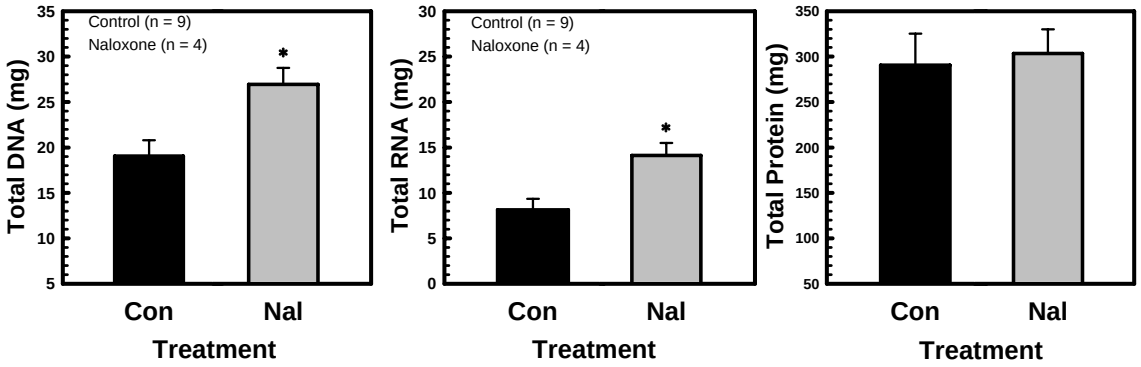


Figure 8: Total DNA, RNA and protein content in kidney at day 1 after birth in control (Con) and naloxone (Nal) treated male fetal pigs. * ($p < 0.05$) shows a statistically significant difference between the two groups.

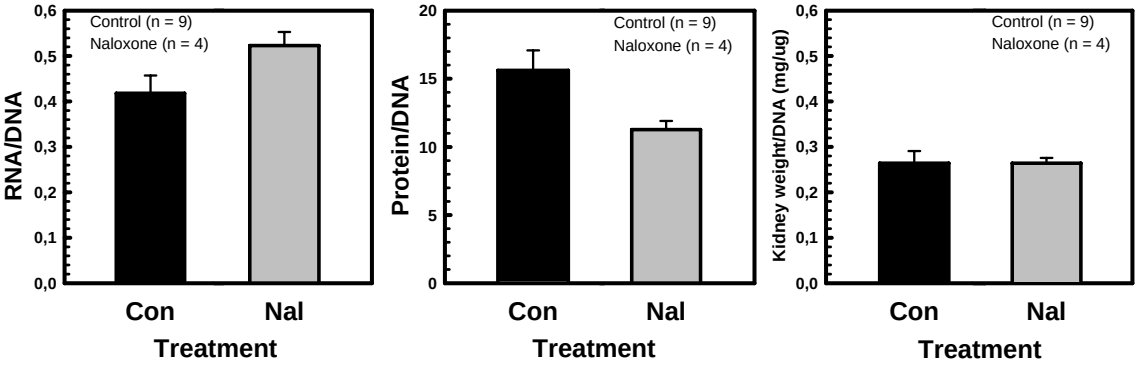


Figure 9: Ratios of RNA to DNA, protein to DNA and kidney weight to DNA at day 1 after birth in control (Con) and naloxone (Nal) treated male fetal pigs. Note: treated males have a lower ratio of protein to DNA than controls ($p < 0.1$).

5.3.4. Comparison of kidney weight and macromolecular constituents of kidney in female and male fetal pigs treated with naloxone during late gestation

There was no difference in body weight between females and males treated with naloxone during late gestation. In the male, kidney weight was higher in comparison to the female, although this was not statistically significant ($p < 0.1$). Whereas relative kidney weight in male pigs was significantly higher ($p < 0.05$) than in females (Fig. 10).

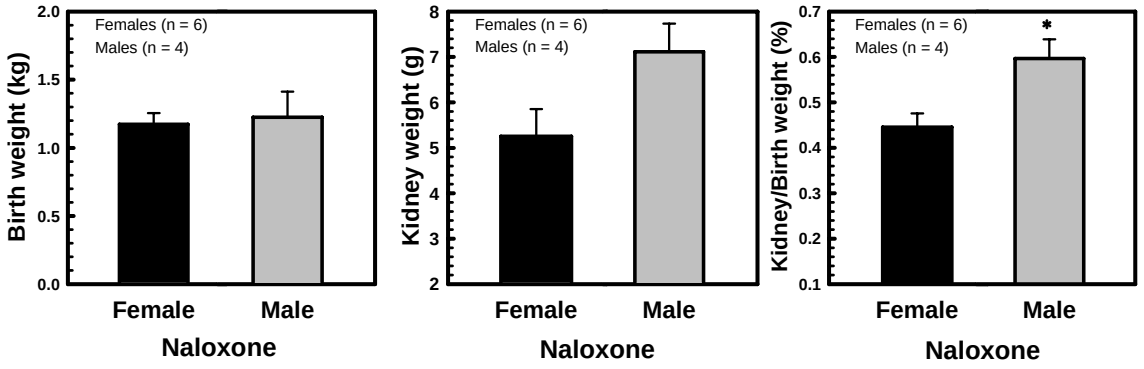


Figure 10: Body and kidney weights and relative kidney weight at day 1 after birth in naloxone treated female and male fetal pigs. * ($p < 0.05$) shows a statistically significant difference between the two groups. Note: $p < 0.1$ in kidney weight.

Interestingly, the concentration of RNA was increased by naloxone ($p < 0.05$) in males compared with females (Fig. 11). Total RNA content of kidney in the male was also significantly higher ($p < 0.01$) than in the female (Fig. 12), apparently due to the effects of naloxone on kidney weight and RNA concentration mentioned above. The ratio of RNA to DNA in males was higher than in females, although this was not significant (Fig. 13). No differences were found in DNA concentration and total DNA content.

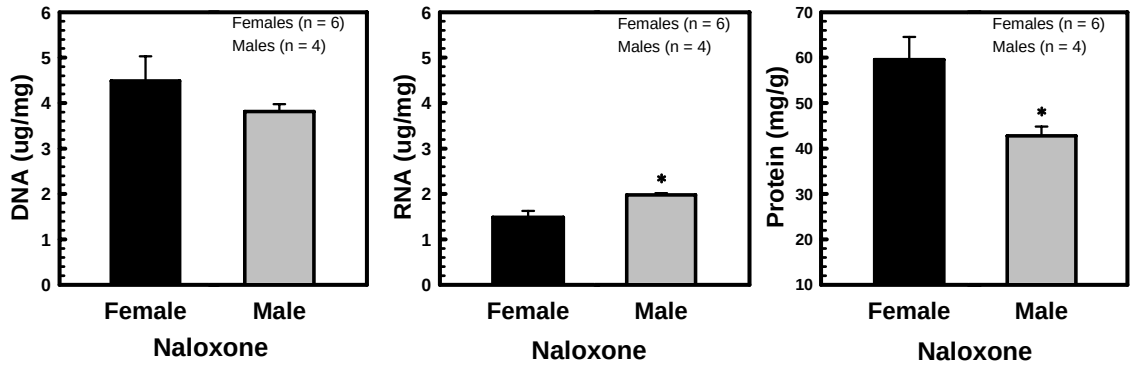


Figure 11: DNA, RNA and protein concentrations in kidney at day 1 after birth in naloxone treated female and male fetal pigs. * ($p < 0.05$) shows a statistically significant difference between the two groups.

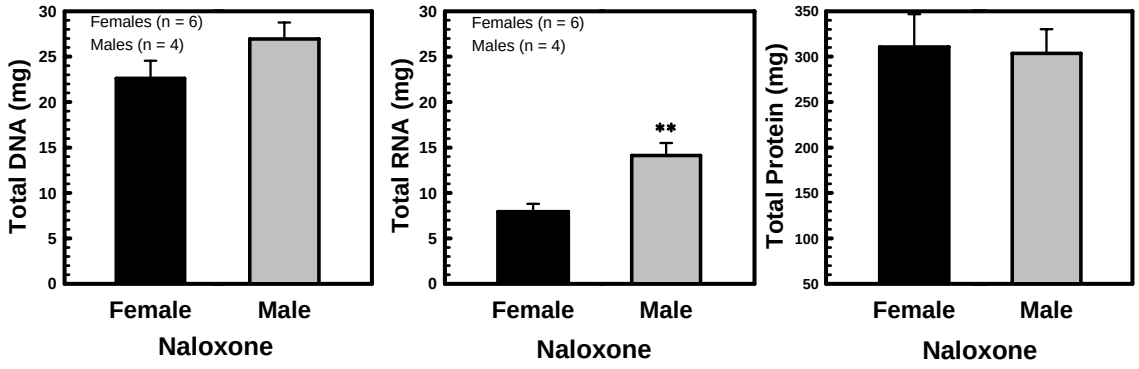


Figure 12: Total DNA, RNA and protein content in kidney at day 1 after birth in naloxone treated female and male fetal pigs. ** ($p < 0.01$) shows a statistically significant difference between the two groups.

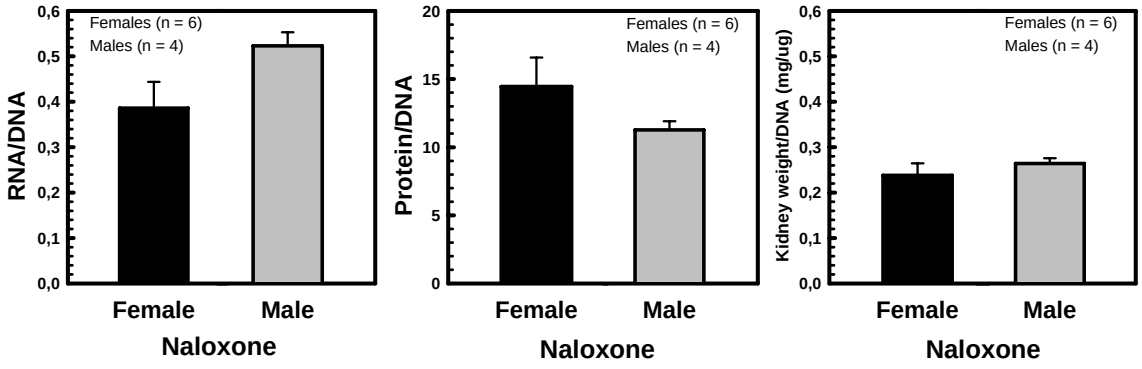


Figure 13: Ratios of RNA to DNA, protein to DNA and kidney weight to DNA at day 1 after birth in naloxone treated female and male fetal pigs. Note: treated males have a higher ratio of RNA to DNA than treated females ($p < 0.1$).

Moreover, the result showed that in males protein concentration was significantly lower ($p < 0.05$) than in females (Fig. 11). No significant differences were evident between the treated animals in total protein content and the ratios of protein to DNA and kidney weight to DNA (Fig. 12 and 13).

5.4. Kidney Weight and Macromolecular Constituents of Kidney in Neonatal Pigs Treated With Naloxone After Birth

As shown in Table 6, naloxone exerted no significant actions on carcass weight, kidney weight, relative kidney weight as well as cellular parameters outlined in the study within the first 14 days of life.

Table 6: Birth weight, carcass weight, kidney weight and macromolecular content of kidney at day 14 after birth in control (n = 8) and naloxone treated (n = 8) pigs[a]

Parameter	Control Neonates	Treated Neonates
Birth weight (kg)	1.16 ± 0.03	1.23 ± 0.07
Carcass weight (kg)	4.15 ± 0.37	4.15 ± 0.43
Kidney weight (g)	12.23 ± 1.20	11.98 ± 1.14
Kidney/carcass weight (%)	0.30 ± 0.01	0.29 ± 0.01
DNA (µg/mg)	3.85 ± 0.25	3.61 ± 0.22
RNA (µg/mg)	2.51 ± 0.17	2.22 ± 0.15
Protein (mg/g)	84.64 ± 3.97	83.13 ± 3.46
Total DNA (mg)	46.57 ± 4.69	44.08 ± 5.77
Total RNA (mg)	30.85 ± 3.79	27.11 ± 3.73
Total protein (mg)	1027.21 ± 98.25	979.93 ± 82.92
RNA/DNA	0.68 ± 0.08	0.63 ± 0.05
Protein/DNA	22.28 ± 0.90	23.70 ± 1.83
Kidney weight/DNA (mg/µg)	0.27 ± 0.02	0.28 ± 0.02

[a] Each value represents the mean ± SEM.

5.5. Comparison of Macromolecular Constituents of Kidney in Male Pigs Treated With Naloxone During Late Gestation and 14-day-old treated Male Pigs

Some developmental changes in the amount of macromolecular kidney constituents from birth to day 14 after birth had been verified and different effects of naloxone on cellular parameters in fetal and neonatal kidneys had been found. Thus we compared the results obtained from male pigs treated with naloxone during late pregnancy with those from 14-day-old treated male pigs in order to investigate whether naloxone affects developmental events.

Four interesting effects of naloxone were noticed. Firstly, there was no difference in kidney weight between the two groups (Fig. 14), although a trend toward an increase in kidneys of 14-day-old experimental male neonates was observed (11.72 ± 1.81 g (4 treated male neonates) vs 7.12 ± 0.62 g (4 treated male newborns), $p < 0.1$; however, 12.71 ± 1.61 g (4 control neonates) vs 4.81 ± 0.47 g (9 control newborns), $p \leq 0.001$).

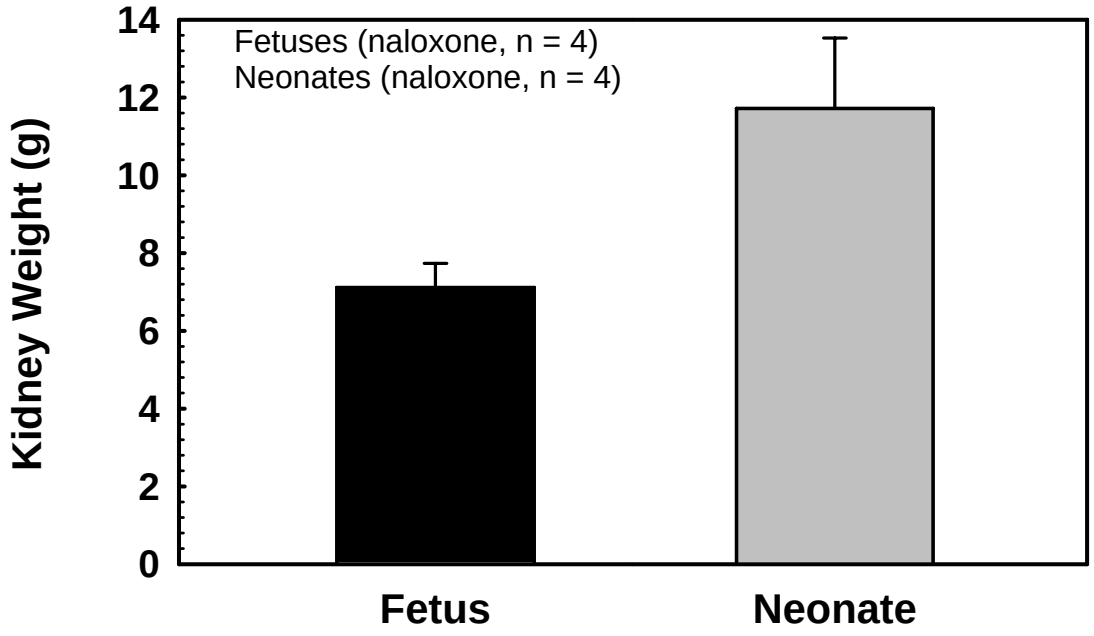


Figure 14: Kidney weights in male pigs treated with naloxone during the fetal period and 14-day-old treated male neonates. Note: $p < 0.1$.

Secondly, no significant difference in total DNA content of kidney was found (Fig. 15). It is also interesting to note that the ratio of kidney weight to DNA in 14-day-old treated male neonates was higher than in male pigs treated with naloxone during late pregnancy (Fig. 17B), although this was not significant ($p < 0.1$).

Thirdly, naloxone treatment attenuated the significant difference seen in total RNA content of kidney between newborn and neonatal male pigs (Fig. 16).

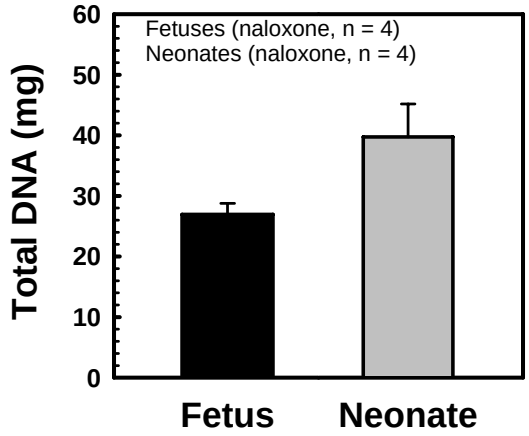


Figure 15: Total DNA content in male pigs treated with naloxone during the fetal period and 14-day-old treated male neonates.

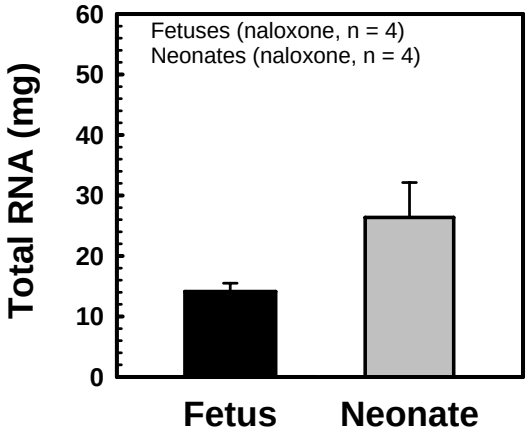


Figure 16: Total RNA content in male pigs treated with naloxone during the fetal period and 14-day-old treated male neonates.

Fourthly, the ratio of RNA to DNA was significantly higher in 14-day-old control male piglets (0.70 ± 0.12 (4 control male neonates) vs 0.42 ± 0.04 (9 control newborns), $p < 0.05$). This difference was not any more evident when the piglets were treated with naloxone during fetal period (Fig. 17A).

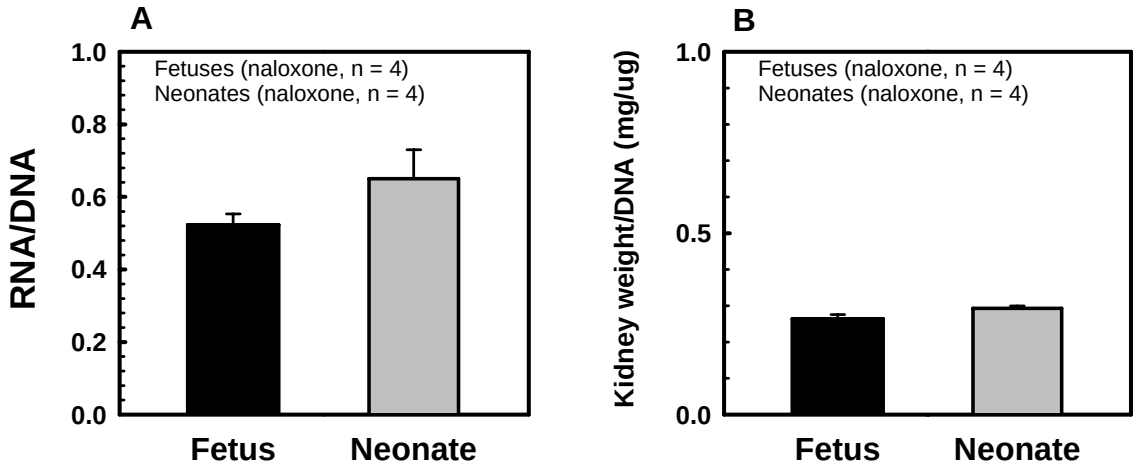


Figure 17: Ratios of RNA to DNA and kidney weight to DNA in male pigs treated with naloxone during the fetal period and 14-day-old treated male neonates. Note: $p < 0.1$ (B).

5.6. Effects of Naloxone on Plasma Cortisol Levels in Fetal and Neonatal Pigs

5.6.1. Changes in plasma cortisol levels from gestational day 102 to day 14 after birth in control pigs

The result showed that from day 102 to day 106 of gestation, plasma cortisol maintained at a constant level (45.64 ± 3.25 ng/ml), which then began to rise until birth (Fig. 18). By day 110 of gestation and at day 1 after birth, cortisol concentrations increased to 83.94 ± 9.07 ng/ml and 92.66 ± 12.17 ng/ml respectively, both of which

were significantly higher compared to day 102 of gestation (49.45 ± 5.73 ng/ml). Thereafter, cortisol decreased to reach a value of 59.76 ± 6.24 ng/ml by day 14 postpartum.

Additionally, this study showed that there was a statistically significant positive correlation between cortisol levels and the gestational period examined ($r = 0.391$, $p < 0.01$; Fig. 19A), whereas after birth cortisol levels decreased significantly with neonatal age ($r = –0.248$, $p < 0.05$; Fig. 19B).

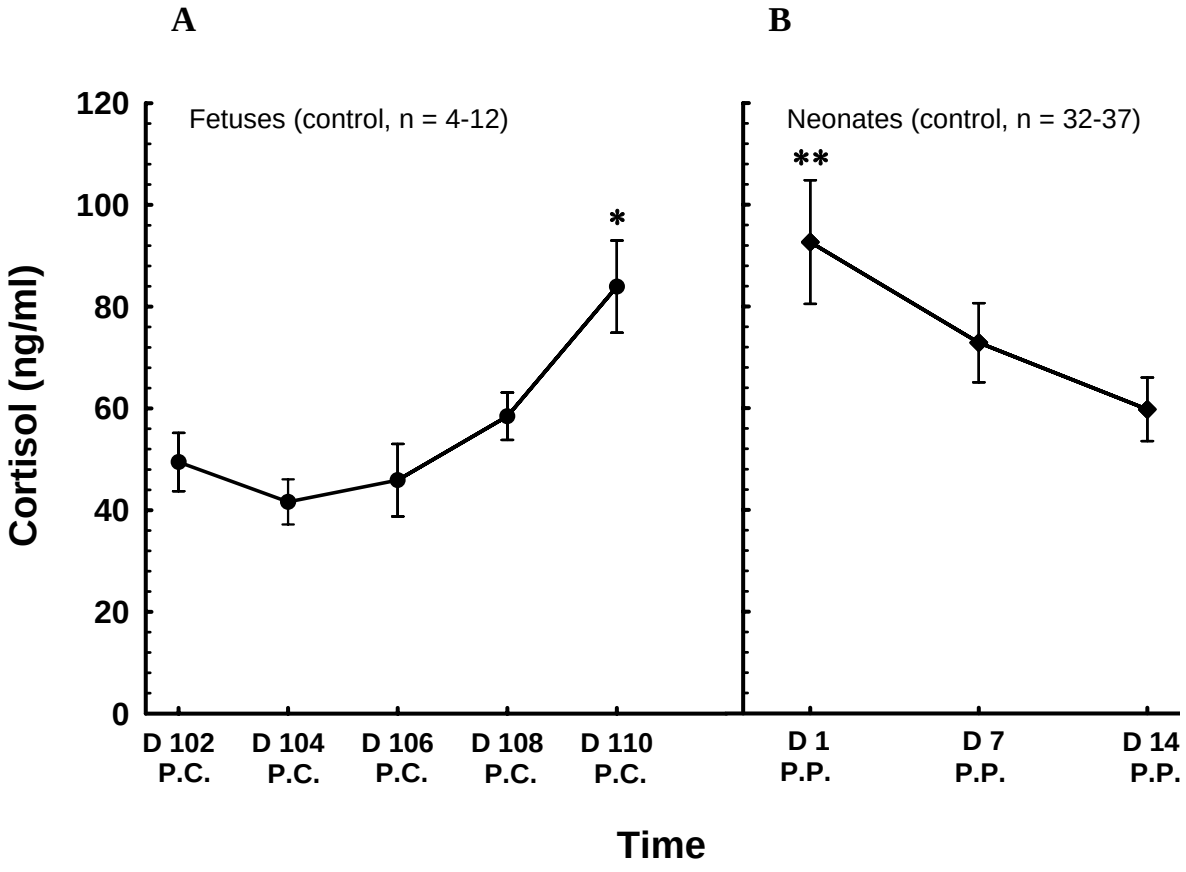


Figure 18: Changes in plasma cortisol levels from day 102 post coitus (p.c.) to day 14 postpartum (p.p.) in control pigs. Each point represents 4 to 37 animals. * ($p < 0.05$) shows statistically significant differences from days 102, 104 and 106 p.c. ** ($p < 0.01$) shows statistically significant difference from day 102 p.c.

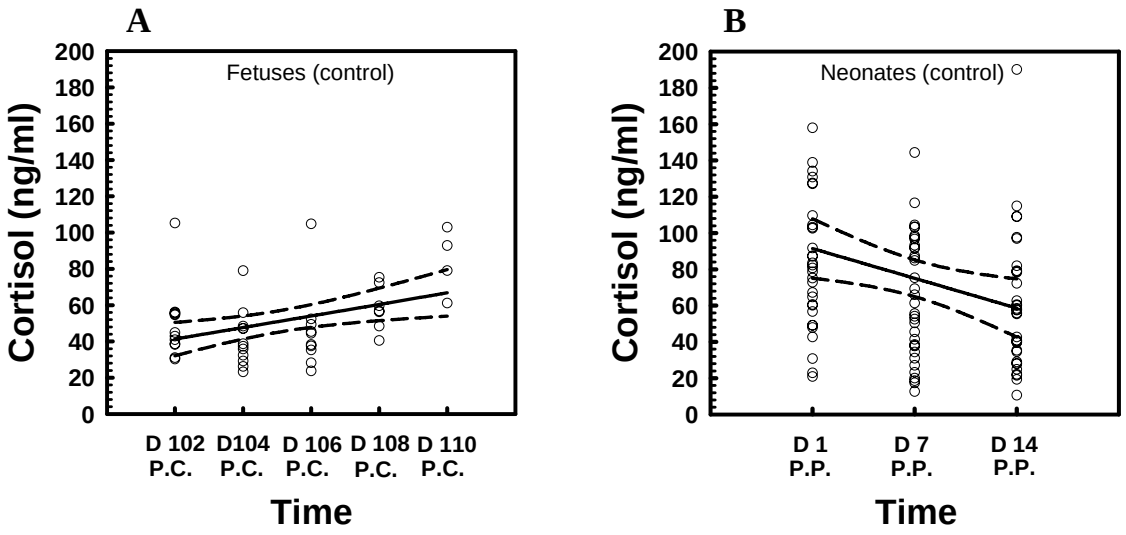


Figure 19: Relationship of plasma cortisol concentrations in control fetuses to fetal gestational age (A) and in control neonates to neonatal age (B). p.c. = post coitus; p.p. = postpartum.

5.6.2. Effects of naloxone on plasma cortisol levels in fetal pigs

On day 102 of gestation, the first day of blood sampling, basal cortisol levels (levels before injections; first sampling period) did not differ significantly between the control and treated groups (Fig. 20).

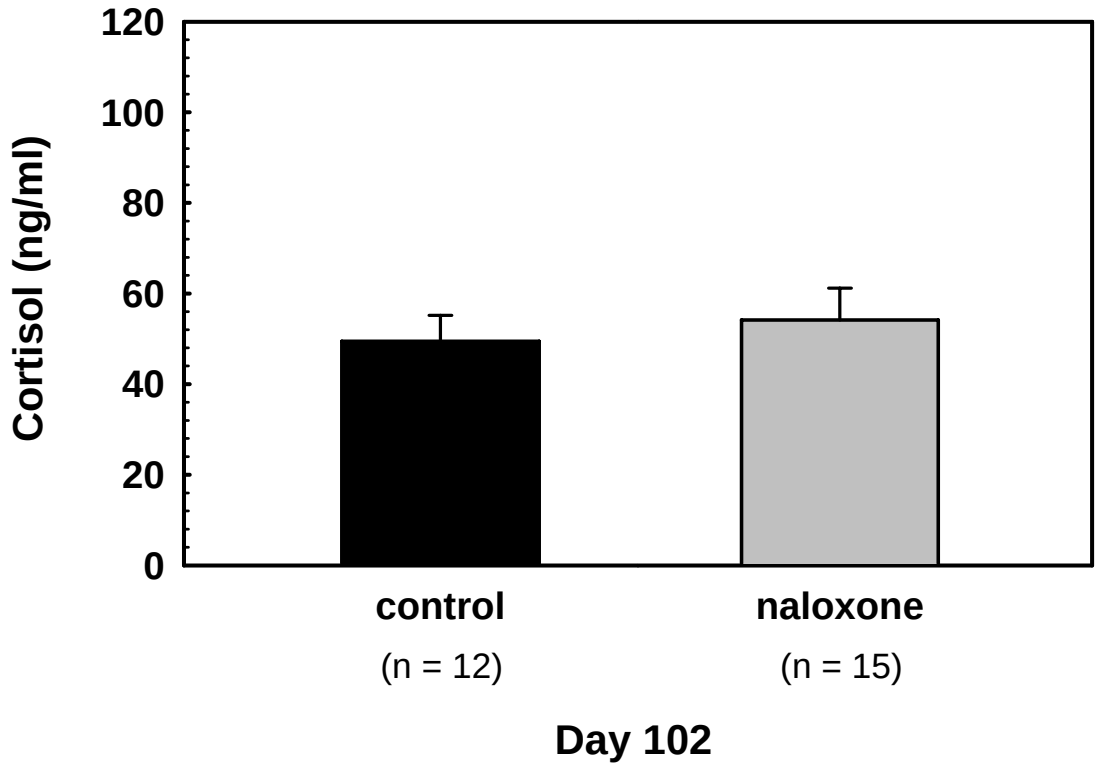


Figure 20: Basal cortisol levels in two fetal groups on day 102 of gestation (first experimental day).

5.6.2.1. Short-term effects of naloxone on plasma cortisol levels in fetal pigs

In control fetuses no changes in plasma cortisol levels were observed during the four blood sampling periods on days 102, 104, 106, 108 and 110 of gestation (Fig. 21). However, application of naloxone caused a significant increase in plasma cortisol levels, except on day 110 (Fig. 22). Naloxone elicited a statistically significant increment in cortisol levels on day 102 (54.14 ± 7.03 ng/ml; 72.43 ± 5.45 ng/ml; 76.83 ± 6.86 ng/ml and 73.33 ± 7.44 ng/ml for blood sampling periods 1 to 4, respectively), day 104 (66.19 ± 6.45 ng/ml; 90.84 ± 6.34 ng/ml; 100.54 ± 6.36 ng/ml and 100.65 ± 8.19 ng/ml for blood sampling periods 1 to 4, respectively), day 106 (76.26 ± 11.03 ng/ml; 107.30 ± 12.80 ng/ml; 102.95 ± 10.24 ng/ml and 98.25 ± 9.87 ng/ml for blood sampling periods 1 to 4, respectively) and day 108 (89.75 ± 12.02 ng/ml; 119.36 ±

15.58 ng/ml; 139.53 ± 20.12 ng/ml and 148.02 ± 34.99 ng/ml for blood sampling periods 1 to 4, respectively).

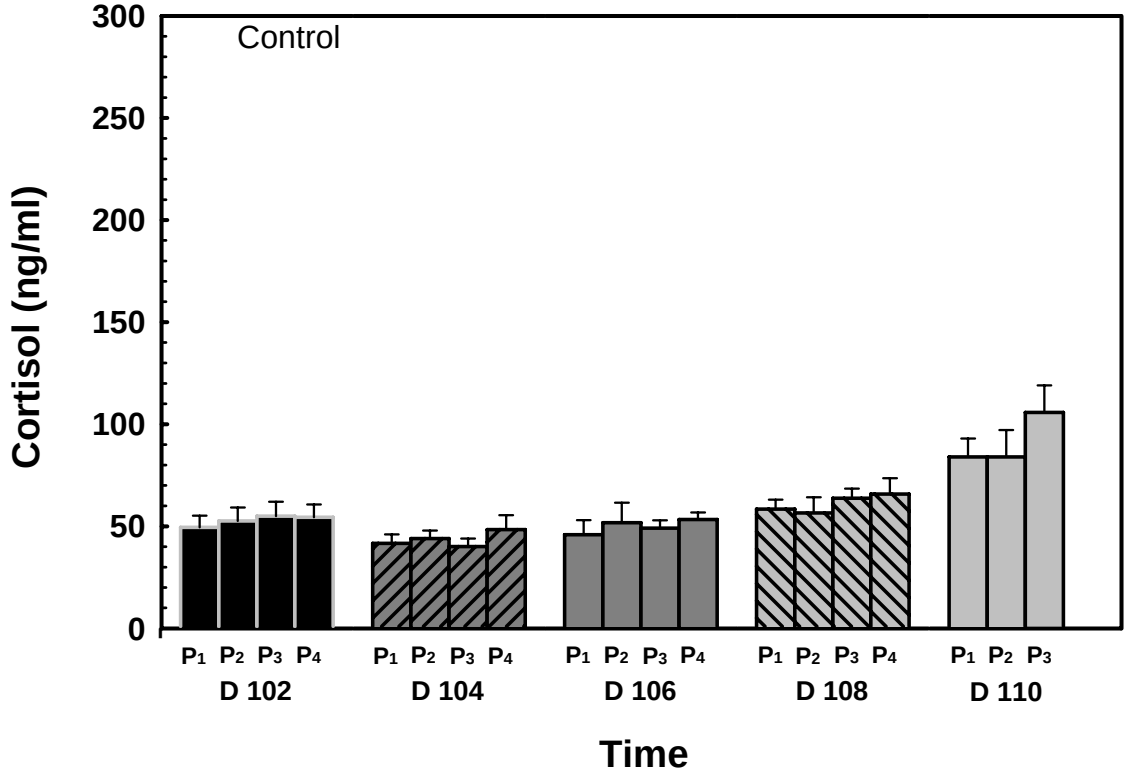


Figure 21: Average plasma cortisol levels from –30 to 0 min before (P 1), to 15 to 30 (P 2), 45 to 60 (P 3) and 75 to 90 (P 4) min after iv injection of 0.5 ml saline in pig fetuses on gestational days 102, 104, 106, 108 and 110. P = period.

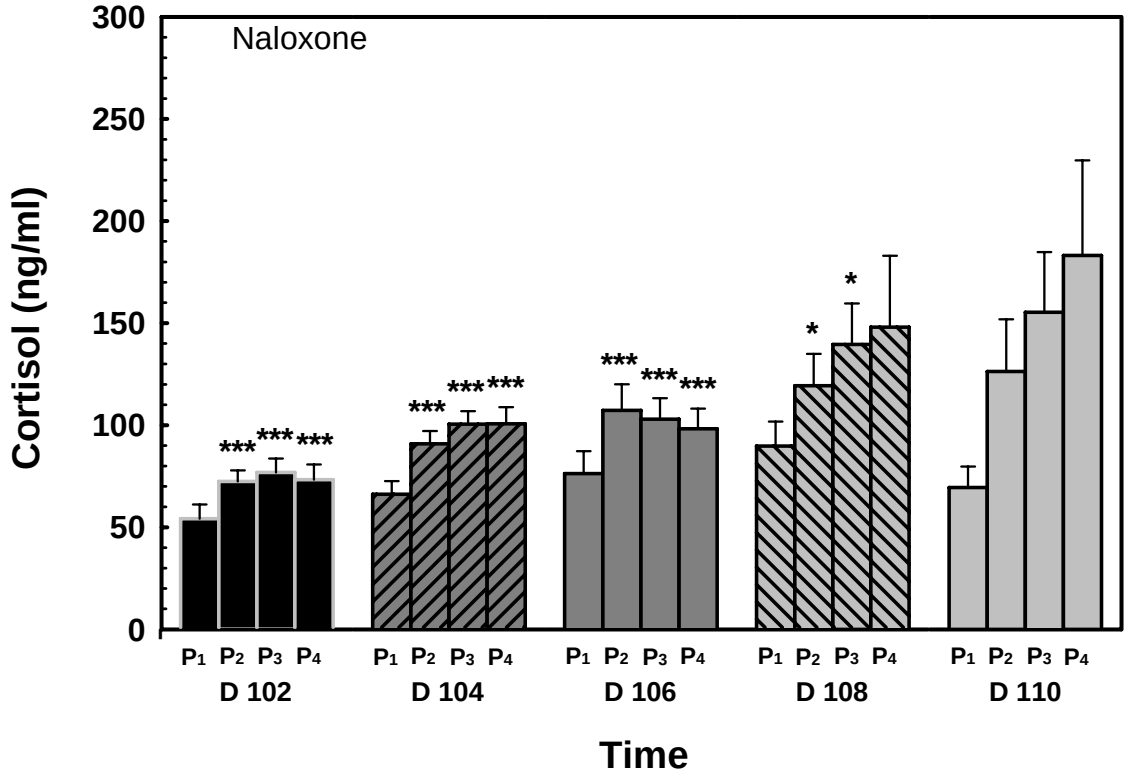


Figure 22: Average plasma cortisol levels from –30 to 0 min before (P 1), to 15 to 30 (P 2), 45 to 60 (P 3) and 75 to 90 (P 4) min after iv injection of 1 mg/fetus naloxone in pig fetuses on gestational days 102, 104, 106, 108 and 110. P = period. * ($p < 0.05$) and *** ($p < 0.001$) show statistically significant differences vs P 1 on the same day.

5.6.2.2. Long-term effects of naloxone on plasma cortisol levels in fetal pigs

On days 104 and 106 of gestation, basal cortisol levels (levels before injections) in naloxone-treated fetuses were 67.49 ± 6.66 ng/ml and 77.10 ± 11.88 ng/ml, respectively (Fig. 23), both of which were significantly higher than in the control group (D 104 = 41.63 ± 4.42 ng/ml and D 106 = 45.88 ± 7.13 ng/ml; Fig. 18A). At day 108, naloxone also induced an increment ($P < 0.1$) in basal cortisol levels when compared with controls. Naloxone had a chronic stimulatory effect on cortisol release over the treatment period, when the levels prior to the treatment (levels before naloxone injection on each day) were compared (days 102 to 110 of gestation; Fig. 23). In fetuses treated with naloxone, basal cortisol levels increased significantly from 54.14 ± 7.03 ng/ml on day 102 to 89.75 ± 12.02 ng/ml on day 108. Analysis also showed that there was a significant positive correlation between basal cortisol levels and the period of naloxone treatments ($r = 0.296$, $p < 0.05$; Fig. 25A).

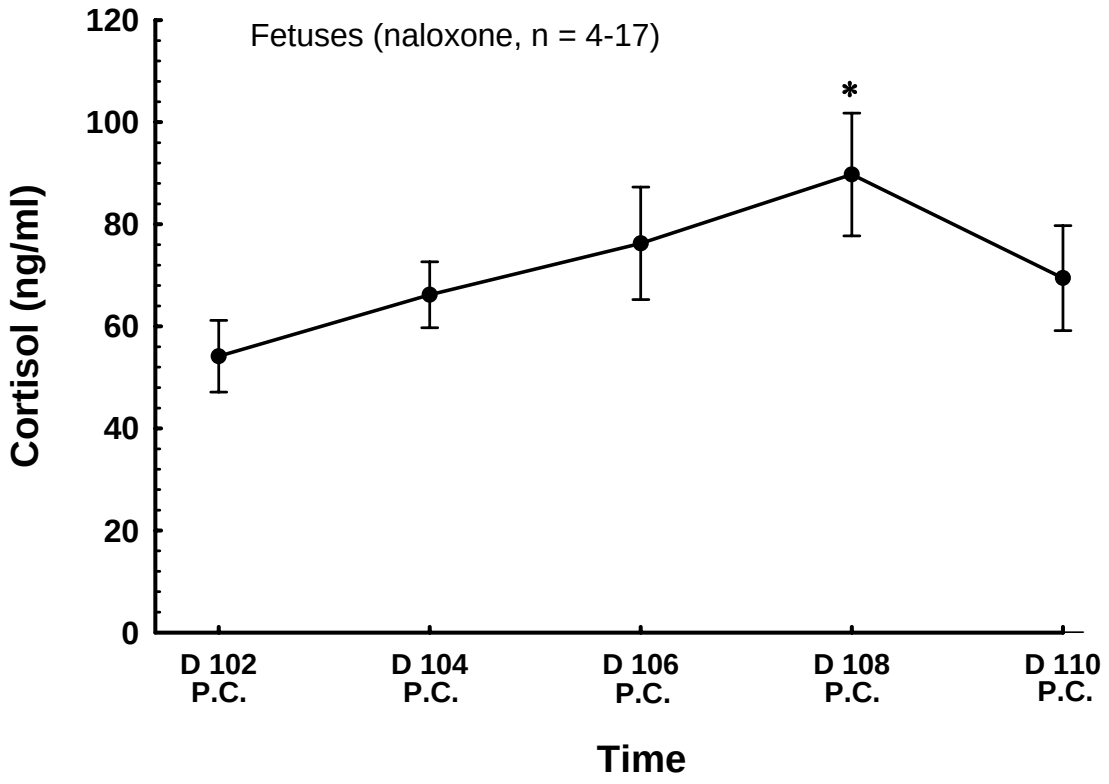


Figure 23: Long-term effects of repeated treatments with 1 mg naloxone/fetus on plasma cortisol levels in pig fetuses. The treatments (iv injections) were repeated every day. Each point represents 4 to 17 animals. * ($p < 0.05$) shows statistically significant difference from day 102 post coitus (p.c.).

5.6.3. Effects of naloxone on plasma cortisol levels in neonatal pigs

No changes in the plasma level of cortisol were observed in neonates exposed to naloxone (Fig. 24). Plasma cortisol level was 78.95 ± 8.38 ng/ml at 1 day after birth, and it was 71.2 ± 8.78 ng/ml and 79.18 ± 15.29 ng/ml at days 7 and 14 postpartum respectively. However, unlike controls, cortisol levels in treated neonates did not decrease significantly with age ($r = 0.002$, $p > 0.05$; Fig. 25B). These results indicate that naloxone has a chronic stimulatory effect on cortisol secretion in neonates.

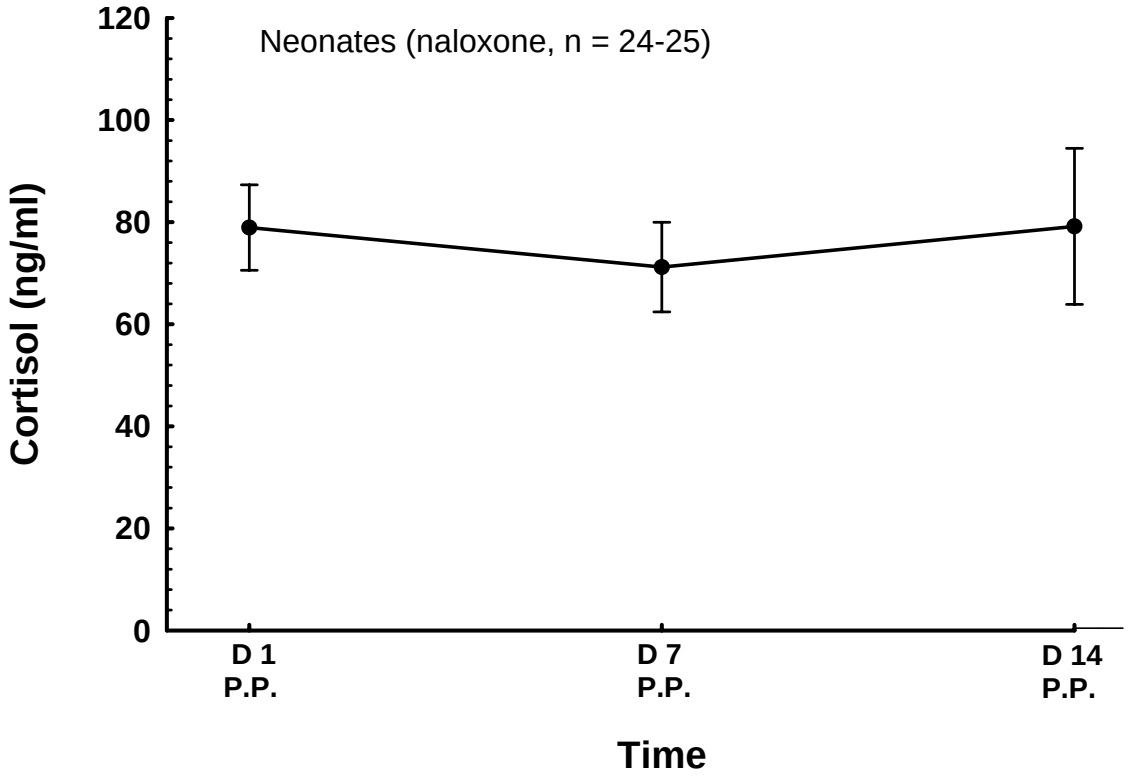


Figure 24: Plasma cortisol levels in pig neonates treated with naloxone after birth. Each point represents 24 to 25 animals. p.p. = postpartum.

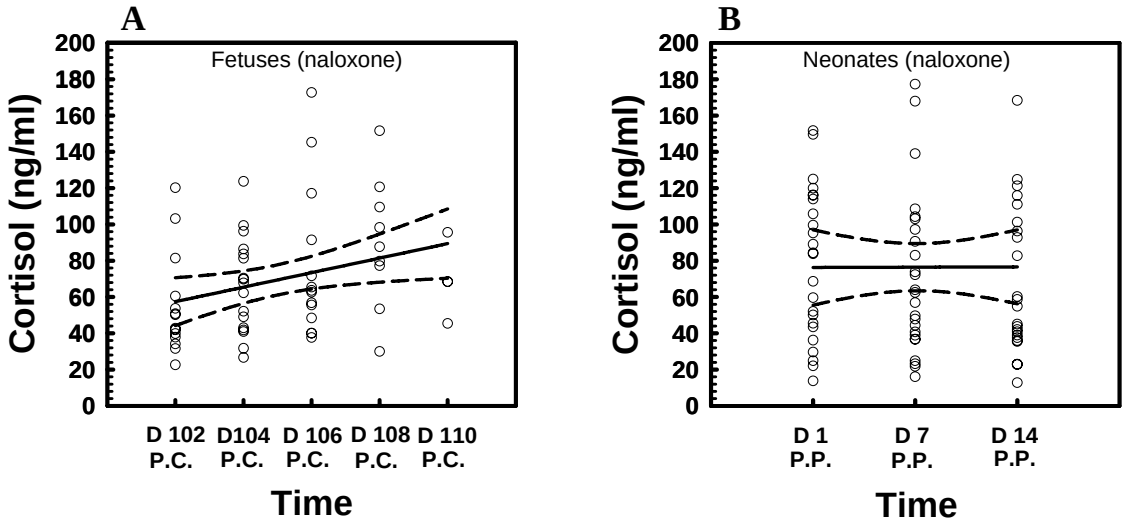


Figure 25: Relationship of plasma cortisol concentrations in naloxone treated fetuses to fetal gestational age (A) and in naloxone treated neonates to neonatal age (B). p.c. = post coitus; p.p. = postpartum.

6. Discussion

We found no sex-differences in body and kidney weights of 1-day-old pigs treated with saline during late gestation or in 14-day-old neonates implanted with saline pumps after birth. This is in agreement with findings published by Thomas et al. (1971). In pigs, the kidneys gain little or no weight during the last 16 days before birth. In the miniature pig, the early phase of kidney growth (1-28 days) mirrors the approximately linear growth of the whole pig and little change in the ratio of kidney weight to body weight has been reported from day one to day 196 after birth (Friedman et al., 1994). However, we observed that the relative weight of the kidney decreased significantly from birth to day 14 postnatally. Similar results were also obtained in bovine (Gore et al., 1994) and in the rat (Solomon et al., 1973).

We observed no change in DNA concentration of pig kidney from birth to 14 days of age. However, concentrations of RNA and protein increased significantly. The results corroborate earlier findings (Friedman et al., 1995) and suggest that the increment in RNA concentration associated with age is correlated to the increase in protein synthesis in this organ. Nevertheless, our data are not in accord with those of Marx et al. (1992) who reported that DNA, RNA and protein concentrations increased significantly from day 1 after birth to the age of 7 days, afterwards, these parameters decreased. In the present study, the total content of DNA, RNA and protein increased significantly between birth and postnatal day 14. Similar results were obtained by Sarkar et al. (1977) and Friedman et al. (1995). The increase in total DNA content may be taken as an index of increase in cell numbers or hyperplasia (Friedman et al., 1995). Thus, the present data may reflect a continuous growth by cell proliferation in kidney from birth to 14 days. In consonance with earlier reports (Sarkar et al., 1977), we observed that the ratio of RNA to DNA was low (less than 1.0) at birth and on day 14. In addition, during this period, a significant increase in the ratio of RNA to DNA was noted. The same was found for organs of miniature pigs (Friedman et al., 1995) and of the Yorkshire pig (Sarkar et al., 1977), although Marx et al. (1992) noted that from day 1 to day 60 changes in RNA to DNA ratio were not significant. Moreover, in the current study, the ratio of protein to DNA increased significantly whereas the ratio of

kidney weight to DNA did not change. Similar results were reported by Sarkar et al. (1977). Elevations in the ratios of RNA and protein to DNA suggest increases in transcriptional and translational activity (Godfredson et al., 1991) and increases in the ratios of RNA and protein to DNA may be taken as indices of increases in cell size or hypertrophy (Sands et al., 1979; Friedman et al., 1995). It is, however, worth mentioning that protein to DNA ratio overestimates the increase in cell size and that a better index of this might be the ratio between weight of the organ and its DNA content.

In rodents, opioids can influence organ growth and development. However, to our knowledge no data for the pig have previously been published.

Pregnant rats receiving twice-daily injections of naloxone had heavier offspring than controls (Nakamoto et al., 1988). Chronic administration of naltrexone to newborn rats also significantly stimulated body weight (Zagon et al., 1984; Bartolome et al., 1986; McLaughlin, 1994). The effect was dependent on dosage (Zagon and McLaughlin, 1983a, 1985; Zagon et al., 1984). The doses chosen in our experiment had no effect on overall body weight.

Daily intravenous (i.v.) injections of 1 mg/kg naloxone significantly increased kidney weight of male fetuses. However, naloxone did not influence DNA or RNA concentrations and total amounts of DNA and RNA to a large extent followed the increase in kidney weight. The higher DNA content may indicate that naloxone affects renal cell number and leads to active hyperplasia of the kidney in males during late gestation. The amount of RNA is considered to be associated with rapid protein synthesis (Zeman, 1983; Zeman et al., 1986). Interestingly, in the present study, the protein concentration in treated males was significantly lowered by naloxone. This may be indicative for a naloxone inhibition of translation or protein synthesis in the male kidney at late gestational period. However, because of increased kidney weight, the total content of protein was not significantly different from that in controls. In female fetuses, although naloxone had no effects on kidney development or macromolecular constituents of kidney, it is noteworthy that the concentration of protein in the treated group was higher ($p < 0.1$) than in the controls.

Interestingly, the concentration of RNA was increased by naloxone ($p < 0.05$) in males compared with females. Total RNA content of kidney in the male was also significantly higher than in the female, apparently due to the effects of naloxone on kidney weight and RNA concentration mentioned above. High RNA concentrations provide the capacity for high rates of protein synthesis (Skjaerlund et al., 1994). Taken together, what we can conclude from these observations is that in fetal pigs naloxone may exert different actions on RNA and protein synthesis depending on the gender of the individual.

During development, there were significant differences between 1-day-old control male newborns and 14-day-old control male neonates in (1) kidney weight; (2) total DNA content of kidney; (3) total RNA content of kidney and (4) the ratio of RNA to DNA. Interestingly, these differences were not observed when 1-day-old naloxone treated male newborns were compared with 14-day-old naloxone treated male neonates. As mentioned above, our results illustrate that naloxone increased male fetal kidney weight and point to a major role of hyperplasia in renal weight gain of treated male fetuses. Naloxone attenuated the significant differences seen in the total amount of RNA and in the ratio of RNA to DNA between control male newborns and control male neonates, since no differences existed in kidney weight as well as DNA and RNA concentrations between the two treated male groups. In 1-day-old treated males the DNA concentration was relatively high whereas kidney weight was relatively low when compared with 14-day-old treated males, thus the ratio of kidney weight to DNA in treated male newborns was lower ($p < 0.1$) than in treated male neonates. These results indicate that naloxone affects the kidney development and interferes with DNA and RNA synthesis in male kidney during prenatal life.

A second major question in this study was related to whether blockade of opioid receptors in neonatal life has repercussions on kidney development. Using the pump implantation method, we found that naloxone did not result in any changes in carcass weight, kidney weight, relative kidney weight as well as cellular parameters outlined in the study within the first 14 days of life. These results differ from the reports in the rat (Zagon and McLaughlin, 1983a, b; Bartolome et al., 1986; Zagon et al., 1987; McLaughlin, 1994; Vertes et al., 1995). The discrepancy may be explained by

methodological differences (implantation vs. injection). In adult mice, morphine pellet implantation did not influence the brain and liver RNA concentration and the RNA content of whole brain (Harris et al., 1975). Moreover, chronic implantation of morphine pellets did not alter the incorporation of (^{3}H)-lysine into brain or liver protein (Harris et al., 1974).

The results presented in this paper illustrate that naloxone increased fetal but not neonatal kidney weight in a sex-dependent manner, and point to a role of hyperplasia in renal weight gain of treated-fetal males. Opioid antagonists such as naltrexone and the less potent and short-acting compound naloxone have been long regarded as pure antagonists of many biological actions of opioid substances and devoid of intrinsic activity (Zagon et al., 1997). Previous experiments have shown that administration of opioid antagonists exerts effects on normal growth (Bartolome et al., 1991; Isayama et al., 1991; Sassani et al., 1993; McLaughlin et al., 1997; Zagon et al., 1999). Exposure of fetal rat testes to naloxone significantly increased Sertoli cell proliferation (Orth, 1986; O, 1990). The effects of prenatal naloxone treatment on brain development were demonstrated by Zagon and McLaughlin (1987). In developing rat pups treated peripherally with naloxone, increases in DNA synthesis in different populations of brain cells were reported by other investigators (Vertes et al., 1982; Zagon and McLaughlin, 1987; Schmahl et al., 1989). Intracerebral administration of naloxone caused significant increases in brain and liver DNA synthesis of preweaning rats (Bartolome et al., 1991). Naloxone alone increased the DNA synthesis in the rat developing uterus (Vertes et al., 1995). Moreover, in the present study, the interruption of opioid-receptor interactions by naloxone showed that DNA synthesis could be increased in male fetal kidney, suggesting that endogenous opioids in the male fetus play a regulatory role in DNA synthesis of the kidney, with the increases associated with opioid antagonist treatment indicative that opioids serve as inhibitory growth factors. In addition, the endogenous opioid related to growth must be tonically active and in a delicate equilibrium, because disturbances of peptide-receptor interaction by an antagonist resulted in notable changes in DNA synthesis. The opioid peptide that participates in renal embryogenesis is presently unclear. However, extensive research of the role of opioid peptides and development of the nervous system (Zagon and

McLaughlin, 1983b, 1987, 1991b; Isayama et al., 1991) and other systems (Vertes et al., 1995; McLaughlin, 1996; Zagon et al., 1999; McLaughlin, 2002) has identified [Met^5]-enkephalin as a tonically active element that serves as an negative growth factor. This pentapeptide, derived from proenkephalin A (PEA), has been termed opioid growth factor (OGF), and appears to play a major role in growth (Zagon and McLaughlin, 1991a, 1993; Zagon, Wu and McLaughlin, 1996) via the zeta (ζ) opioid receptor (Zagon and Goodman et al., 1990; McLaughlin et al., 1997; Zagon et al., 1999). Opioid growth factor also modulates cell proliferation and tissue organization during cancer (Zagon and McLaughlin, 1987, 1989a, b; Sassani et al., 1993; Zagon et al., 1994a; Zagon et al., 1996; Zagon et al., 1997; Bisignani et al., 1999), cellular renewal, wound healing, and angiogenesis (Zagon and McLaughlin, 1983a, b, 1992; Isayama et al., 1991; Zagon et al., 1999). Both OGF and the ζ receptor have been localized in a variety of developing and renewing cells and tissues (McLaughlin et al., 1997). In rodents, the expression of PEA mRNA and of its encoded peptide Met-enkephalin was transient, being found only during development and absent in adults (Keshet et al., 1989; Kew et al., 1990; Isayama et al., 1991). Consistent with classical opioid action, the effects of OGF are blocked by concomitant exposure to naloxone (Zagon and McLaughlin, 1987, 1989b, 1991b; Isayama et al., 1991; Zagon et al., 1994b; McLaughlin, 1996). Opioid growth factor appears to be autocrine produced and secreted by both neuronal and non-neuronal cells in biological systems that are undergoing development, cellular renewal, or repair (McLaughlin et al., 1997), and is constitutively expressed (Bisignani et al., 1999). Blockade of OGF-ζ receptor interaction would permit cells to escape the regulating nature of OGF and accelerate replication. Nevertheless, these observations do not rule out the possibility of the involvement of other receptor types in the modulation of cell proliferation (Leslie, 1987; Leslie et al., 1998; Zhu and Pintar, 1998; Zagon et al., 1999). A study by Hatzoglou et al. (1996) showed that ethylketocyclazocine, a general opioid agonist (Kampa et al., 2004), inhibited in a dose-dependent manner the proliferation of opossum kidney cells. This effect was antagonized by the general opioid receptor antagonist diprenorphine. Similar results were obtained by the specific kappa$_1$ opioid receptor agonist U69593. [D-Ser^2, Leu^5]enkephalin, Thr^6 (DSLET, a delta agonist)

produced minimal effects at much higher concentrations equally antagonized by diprenorphine. Various groups also demonstrated the presence of classical opioid receptors (Simantov et al., 1978; Neidle et al., 1979; Quirion et al., 1983; Slizgi and Ludens, 1985; Hatzoglou et al., 1996; Wittert et al., 1996) and opioid peptides (such as ß-endorphin, dynorphin, morphine) in kidney (Neidle et al., 1979; Bhargava et al., 1988). On the other hand, although opioid antagonist actions on DNA synthesis are mediated predominantly by opioid receptors, the modulation of growth by narcotic antagonists such as naloxone (Zagon and McLaughlin, 1989b) could be envisioned to take place by mechanisms that are partially or wholly unrelated to opioid systems (Geber et al., 1976; Sawynok et al., 1979; Brown et al., 1980; Vertes et al., 1982; Zagon and McLaughlin, 1983a, b).

In summary, the evidence that naloxone can markedly alter the course of development (i.e. naloxone can stimulate kidney growth in male fetuses) supports the intriguing possibility that endogenous opioid peptides and opiate receptors play a modulatory role in cell proliferation, migration, and differentiation. Although, the reason for the different effects of naloxone on male and female fetal kidneys remains unknown. Whether administration of naloxone late in gestation influences biosynthesis of macromolecules in the kidney of males well into the postnatal period warrants further study. Moreover, whether defects in opioid peptide regulation are involved in renal dysfunction occurring during embryogenesis remains to be elucidated. Opioid antagonists may prove to be of therapeutic value in rescuing developing kidneys that are deficient in cells. Such intriguing hypotheses also need to be addressed in future studies.

The present result showed that fetal plasma cortisol levels increased toward the end of gestation. Then the concentrations further increased to a maximum at day 1 after birth. Thereafter, cortisol levels decreased by 14 days of age. Other studies showed that in the last week or ten days of gestation the pituitary-adrenal axis of the fetal pig was mature (Silver et al., 1983). Current data are in agreement with the findings that the gradual rise in fetal pig plasma cortisol began 4-6 days before term (Silver et al., 1979; Wise et al., 1991) and the prepartum rise in fetal plasma cortisol reached a maximum at birth (Fevre, 1975; Silver et al., 1979). In several animal species (Avery, 1975; Liggins, 1976; Challis and Sloboda et al., 2000; Schmidt et al., 2004), fetal adrenal cortisol plays an important role in the onset of parturition as well as the maturation of a number of major organ systems, including the lung, kidney, heart, liver and gut, which are essential for extrauterine survival (Magyar et al., 1980; Challis and Brooks, 1989; Castro et al., 1992; Broughton-Pipkin et al., 1994; Mesiano and Jaffe, 1997; Taylor et al., 1997b; Fowden et al., 1998; Nathanielsz, 1998; Gluckman et al., 1999; Challis and Matthews et al., 2000; Bolt et al., 2002; Yeung and Smyth, 2002). However, in pigs, the role of the fetal HPA axis in the initiation of labor is not clear (Randall et al., 1990). High plasma cortisol levels in the neonatal pig may well account for their viability (Silver et al., 1983).

In the pig fetus, naloxone acutely increased plasma cortisol levels on days 102, 104, 106 and 108 of gestation. However, although naloxone resulted in an elevation of cortisol at 110 days, this increase was not statistically significant due to large variations between animals. Naloxone stimulated cortisol secretion in a number of species (Blankstein et al., 1980; Morley et al., 1980; Haracz et al., 1981; Gosselin et al., 1983; Barb et al., 1986), presumably by specific blockage of opioid receptors (Estienne et al., 1988). Numerous studies demonstrated acute increases in cortisol levels in man after intravenous infusion of naloxone (Cohen et al., 1983; Conaglen et al., 1985; Ehrenreich et al., 1987; Delitala et al., 1994; Martin del Campo et al., 1994; Schluger et al., 1998), and cortisol levels rose significantly above basal from 20 to 90 min after naloxone (Torpy et al., 1994). In dairy cows, intravenous naloxone application induced a significant increase in plasma cortisol, reaching peak value at 30

to 60 min after injection (Nanda et al., 1992). Likewise, in gilts (Barb et al., 1986), chimpanzees (Gosselin et al., 1983), horses (Alexander and Irvine, 1995), sheep (Parrott and Thornton, 1989; Thornton and Parrott, 1989) and rats (Martínez et al., 1990), naloxone enhanced cortisol secretion. Moreover, in the present study, chronic naloxone administration also produced a significant rise in plasma cortisol concentrations in both fetal and neonatal pigs. On days 104 and 106 of gestation, basal cortisol levels in the treated fetus were significantly higher when compared with controls. At day 108, basal cortisol levels were also increased by naloxone, whereas this increase was not significant due to the elevation of basal cortisol levels in control fetuses. Nevertheless, it is found that there are no data available to date concerning the chronic effect of naloxone on the secretion of cortisol. Thus it is possible that this study is the first to illustrate that chronic naloxone treatment elicits a significant increment in basal cortisol levels.

In fetal and neonatal pigs, inhibition of the endogenous opioid action by competitive blockade of the receptors with naloxone induced elevations of plasma cortisol. It is indicated that there is a tonic opioid-mediated inhibitory influence exerted on the HPA axis. This resembles that of human beings, primates (Volavka and Cho et al., 1979; Blankstein et al., 1980; Morley et al., 1980; Gosselin et al., 1983; Grossman and Moult et al., 1986), horses and dairy cows. However, the mechanisms involved are not clear. In gilts, the ability of naloxone to stimulate cortisol release was abolished following hypophysial stalk-transection (Estienne et al., 1988), suggesting that the opioid antagonist enhances cortisol secretion principally by acting at the level of the central nervous system. Nonetheless, the results do not preclude the additional possibility that naloxone may also antagonize opioid actions at the pituitary or adrenal glands. A direct effect of naloxone on the adrenal cortex has been demonstrated (Lymangrover et al., 1981; Bruni et al., 1985).

To my knowledge, this is the first report which describes the effects of exogenous administration of naloxone on the secretion of cortisol in the fetal and neonatal pigs. In conclusion, the HPA axis of pigs seems to be under suppressive opioidergic control.

7. Summary

Opioids are known to influence organ growth and development as well as the hypothalamo-pituitary-adrenal (HPA) axis. The studies reported in this dissertation were undertaken to evaluate the effects of naloxone on kidney weight, macromolecular constituents of kidney and cortisol secretion in fetal and neonatal pigs and determine if these effects are sex-dependent.

Effects of naloxone on kidney weight and macromolecular constituents of kidney:
Daily intravenous injections of 1 mg/kg naloxone significantly increased both kidney weight and relative kidney weight of male fetuses. Total DNA and RNA content in treated males was significantly higher than in control males. Interestingly, the protein concentration in treated males was significantly lowered by naloxone. However, no significant difference in total protein content between the two groups was observed. In female fetuses, naloxone had no effects on kidney weight or macromolecular constituents of kidney. Although kidney weight in the treated male fetus was not significantly higher in comparison to the treated female fetus, relative kidney weight in males was statistically significant higher than in females. Interestingly, the concentration of RNA was significantly increased by naloxone in males compared with females. Total RNA content of kidney in the male was also significantly higher than in the female. Moreover, in males protein concentration was significantly lower than in females. These findings indicate that during prenatal life naloxone may exert different actions on DNA, RNA and protein synthesis depending on sex.
Following significant differences between 1-day-old and 14-day-old control male newborn piglets were observed in (1) kidney weight; (2) total DNA content of kidney; (3) total RNA content of kidney and (4) the ratio of RNA to DNA. Interestingly, these differences were not any more evident when 1-day-old naloxone treated male newborns were compared with 14-day-old naloxone treated male neonates. These results indicate that naloxone affects the kidney development and interferes with DNA and RNA synthesis in male kidney during prenatal life.

In 14-day-old neonatal pigs, naloxone pump implantation did not significantly influence kidney weight, relative kidney weight and cellular parameters outlined in the study. The results demonstrate that naloxone increased fetal but not neonatal kidney weight in a sex-dependent manner, and point to a role of hyperplasia in renal weight gain of treated fetal males.

Effects of naloxone on cortisol secretion:

No sex difference was evident in the effect of naloxone on cortisol secretion.

In control pig fetuses, no changes in plasma cortisol levels were observed during the four blood sampling periods on days 102, 104, 106, 108 and 110 of gestation. Intravenous naloxone treatment caused a significant increment in cortisol levels on days 102, 104, 106 and 108 of gestation, but not on day 110. Moreover, naloxone had a chronic stimulatory effect on cortisol secretion over the treatment period, when the levels prior to the treatment (levels before naloxone injection on each day) were compared (days 102 to 110 of gestation). In naloxone-treated fetuses, basal cortisol levels (levels before injections) increased significantly from 54.14 ± 7.03 ng/ml on day 102 to 89.75 ± 12.02 ng/ml on day 108. Thus, there was a significant positive correlation between basal cortisol levels and the period of naloxone treatments ($r = 0.296$, $p < 0.05$).

From 1 day after birth to 14 days of age, no changes in plasma levels of cortisol were observed in neonates implanted with naloxone pumps. However, unlike controls, cortisol levels in the treated neonate did not decrease significantly with age. These results indicate that naloxone has a chronic stimulatory effect on cortisol release in neonatal pigs.

The data illustrate that the HPA axis in fetal and neonatal pigs is most probably under inhibitory influence of opioids.

8. Zusammenfassung

Opioide beeinflussen neben der Hypothalamus-Hypophysen-Nebennieren-Achse auch direkt Organwachstum und -entwicklung. Die vorliegende Arbeit beschäftigt sich mit der Wirkung des Opioid-Antagonist Naloxon auf die Nieren, sowie die Cortisolfreisetzung in fötalen und neugeborenen Ferkeln.

Wirkung von Naloxon auf das Nierengewicht und den makromolekularen Aufbau der Niere:

Tägliche intravenöse Injektionen von 1mg Naloxon pro Kilogramm Körpergewicht führen bei männlichen Föten zu einem signifikanten Anstieg sowohl des absoluten Nierengewichts, als auch des Organgewichts relativ zum Körpergewicht. Zusätzlich zeigen die mit Naloxon behandelten Tiere eine Erhöhung des Gesamt-DNA sowie -RNA Gehalts im Vergleich zu den Kontrolltieren. Die Proteinkonzentration im Gewebe wird durch die Gabe von Naloxon signifikant reduziert, obwohl der Vergleich des Gesamtproteingehalts von behandelten und unbehandelten Tieren keinen bedeutsamen Unterschied aufweist. Weibliche Föten zeigen im Gegensatz zu den männlichen Tieren keinerlei Beeinflussung des Nierengewichts oder des makromolekularen Aufbaus der Niere nach Naloxongabe. Statistisch weisen behandelte weibliche Föten ein signifikant geringeres Organgewicht relativ zum Gesamtkörpergewicht als behandelte männliche Tiere auf, obwohl das absolute Nierengewicht keinen Unterschied zeigt. Im Vergleich zu den weiblichen zeigen die Nieren männlicher Naloxon behandelter Föten eine erhöhte Gesamt-RNA Konzentration sowie einen erhöhten Gehalt an Gesamt-RNA. Die Proteinkonzentration hingegen ist gegenüber den weiblichen Tieren verringert.

Neugeborene Ferkel im Alter von einem Tag zeigen im direkten Vergleich mit 14 Tage alten Ferkeln signifikante Unterschiede bezüglich

1. des Nierengewichts
2. des Gesamt-DNA Gehalts in der Niere
3. des Gesamt-RNA Gehalts in der Niere und
4. der RNA/ DNA Ratio

Nach der Behandlung mit Naloxon hingegen sind diese Differenzen in den unterschiedlichen Altersstufen nicht mehr nachzuweisen, was die Einflussnahme von Naloxon auf die Nierenentwicklung zeigt.
Bei 14 Tage alten Ferkeln zeigen Naloxonimplantate (Alzet Pumpe) keine Wirkung auf absolutes- und Nierengewicht relativ zum Gesamtkörpergewicht oder auf andere untersuchte zelluläre Parameter. Diese Ergebnisse geben Hinweise auf eine Beteiligung der Opioide in der DNA und RNA Synthese pränatal und in Abhängigkeit vom Geschlecht.

Wirkung von Naloxon auf die Cortisolsekretion:

Die Wirkung von Naloxon auf die Cortisolsekretion weist keinen geschlechtsgebundenen Unterschied auf.
Kontrollföten der pränatalen Tage 102, 104, 106, 108 und 110 zeigen keine Veränderung des Cortisolgehalt im Plasma, wohingegen in Naloxon behandelten Tieren ein signifikanter Anstieg der Cortisolkonzentration an Tag 102pc, 104pc, 106pc, 108pc, aber nicht an Tag 110 nach der Konzeption nachzuweisen ist. Außerdem lässt sich im Vergleich der untersuchten Altersstufen von Beginn (102dpc) bis zum Ende (110dpc) der Behandlung ein chronischer stimulierender Effekt für Naloxon auf die Cortisolsekretion ableiten. Der Cortisolgehalt steigt von einem basalen Wert vor Beginn der täglichen Naloxoninjektionen von 54.14 ± 7.03 ng/ml an Tag 102pc auf 89.75 ± 12.02 ng/ml an Tag 108pc. Diese Werte ergeben eine positive Korrelation zwischen Cortisolkonzentration und der Dauer der Naloxonbehandlung ($r=0.296$, $p< 0.05$).
Postnatal zeigen die Tiere mit Naloxonimplantat weder an Tag eins noch an Tag 14 eine Veränderung des Plasmacortisollevels, im Gegensatz zu den Kontrolltieren, in welchen sich der Cortisolgehalt des Plasmas im Laufe des untersuchten Zeitraumes signifikant verringert. Anhand dieser Resultate kann man auch für die postnatale Entwicklung im neugeborenen Schwein von einem chronischen stimulierenden Effekt des Naloxon ausgehen.

Die vorgelegten Daten zeigen, dass die Hypothalamus-Hypophysen-Nebennieren-Achse sowohl von neugeborenen, als auch von fötalen Schweinen einem inhibierendem Einfluss von Opioiden unterliegt.

9. List of Abbreviations

ACTH	adrenocorticotrophin
ATP	adenosine triphosphate
AVP	arginine vasopressin
BSA	bovine serum albumin
°C	Celsius degree
CCK	cholecystokinin
cm	centimeter
CNS	central nervous system
Con	control
CRF	corticotropin-releasing factor
D	day
DAGO	[D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin
DEPC	diethylpyrocarbonate
DNA	deoxyribonucleic acid
DSLET	[D-Ser^2, Leu^5]enkephalin, Thr^6
EIA	enzyme immunoassay
ENK	[D-Met^2,Pro^5]enkephalinamide
EtBr	ethidium bromide
Fig	figure
g	unit of centrifugal force
g	gram
GTP	guanine triphosphate
h	hour(s)
^{3}H	tritrium
HPA	hypothalamo-pituitary-adrenal
Ht	hematocrit
[^{3}H]TdR	[6-^{3}H]-thymidine
i.c.	intracisternal
i.c.v.	intracerebroventricular
i.e.	that is
i.m.	intramuscular
ir	immunoreactive
i.t.	intrathecal
IU	international unit
i.v.	intravenous
kg	kilogram
Leu	leucine
LI	labeling index
M	molar
MC	mesangial cell
Met	methionine
METH	methadone
mg	milligram
min	minutes

ml	milliliter
MOPS	3-morpholinopropanesulfonic acid
mRNA	messenger ribonucleic acid
µg	microgram
µl	microliter
µM	micromolar
n	number of animals
NAL	naloxone
ng	nanogram
nm	nanometer
NTX	naltrexone
OE	oestradiol
OGF	opioid growth factor
P	period
p.c.	post coitus
PEA	proenkephalin A
PGE_2	prostaglandin E_2
p.p.	postpartum
RMIC	renal medullary interstitial cell
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
s.c.	subcutaneous
SEL	subependymal layer
SEM	standard error mean
TAE	Tris-aminomethane-acetate-EDTA buffer
V	voltage
vs	versus
UV	ultraviolet
ß	beta
δ	delta
κ	kappa
µ	mu
ζ	zeta

10. Chemical Reagents and Composition of Solutions and Buffers

10.1. Chemical Reagents

Material	Company, Order No.	Locus of Company
Agarose ultra pure electrophoresis grad	Life Technologies, 15510-027	Gaithersburg, MD, USA
Chloroform	Applichem, A3691	Darmstadt, Germany
Diethylpyrocarbonate (DEPC)	Applichem, A0881	Darmstadt, Germany
EDTA (Dinatrium salt) (Titriplex III)	Merck, 1.08421	Darmstadt, Germany
Ethanol	Merck, 1.00986	Darmstadt, Germany
Ethidium bromide	Sigma Aldrich, E1510	Deisenhofen, Germany
Ficoll Typ 400	Sigma Aldrich, F4375	Deisenhofen, Germany
Formaldehyde	Merck, 4002	Darmstadt, Germany
Formamide	Fluka 47670	Fluka, Neu Ulm, Vertrieb Sigma Aldrich, Deisen-hofen
Guanidine thiocyanate	Carl Roth, 0017.2	Karlsruhe, Germany
Isoamyl alcohol	Applichem, A0875	Darmstadt, Germany
Isopropanol	Merck, 1.09634	Darmstadt, Germany
ß-Mercaptoethanol	Sigma Aldrich, M3148	Deisenhofen, Germany
MOPS	Applichem, A2947	Darmstadt, Germany
N-lauroylsarcosine Na-salt	Sigma Aldrich, L-9150	Deisenhofen, Germany
Orange G (Certistein)	Merck, 15925	Darmstadt, Germany
Phenol, water-saturated, not stabilized	Applichem, A1578	Darmstadt, Germany

10.2. Composition of Solutions and Buffers

Guanidine thiocyanate solution (GTC):

Guanidine thiocyanate	4 M
Na-Citrat, pH 7.0	25 mM
Na-lauroylsarcosine (Sarcosyl)	0.5%
ß-Mercaptoethanol	0.1 M

Stored without addition of ß-Mercaptoethanol: 3 months;
with addition of ß-Mercaptoethanol: 1 month

10X MOPS:

MOPS	0.2 M
Na-acetate	50 mM
EDTA	1 mM

NaOH ad pH 7.0

Stock solution 10X MOPS/1 l

MOPS	41.8 g

NaOH ad pH 7.0
16.6 ml 3 M Na-acetate solution
20 ml 0.5 M EDTA, pH 8
Sterilized by filtration
MOPS solution kept dark at RT

5X denaturing RNA loading buffer:
(Quiagen, RNeasy Mini Handbook 1997, modified)

Saturated bromphenol blue solution	226 µl
500 mM EDTA, pH 8	20 µl
EtBr (10 mg/ml)	15 µl
37% Formaldehyde (pH > 4)	180 µl
100% Glycerin	500 ml
Deionized formamide	771 µl
10X MOPS	1000 µl

Store: 3 months at 4°C

1.2% agarose gel for denaturing RNA gel electrophoresis:

Agarose	1.4 g
H_2O	101.5 ml
10X MOPS	11.7 ml
37% Formaldehyde (pH > 4)	3.5 ml

1X TAE buffer:

Tris-acetate	0.04 M
EDTA, pH 8	0.001 M

Stock solution 50X TAE/1 l:

Tris base	242 g
Glacial acetic acid	57.1 ml
0.5 M EDTA, pH 8	100 ml

10X DNA loading buffer: Orange Mix (Reeben, 1992)

20%	Ficoll
0.25%	Orange G
20 mM	EDTA, pH 8.0

EDTA 0.5 M pH 8.0/1 l:

EDTA	186.1 g
H_2O	800 ml

NaOH ad pH 8, add about 20 g of NaOH pellets
(pH < 8.0 - no solution)

TE buffer pH 7.5:

Tris-HCl	10 mM
EDTA, pH 8.0	1 mM

11. References

Adamson WT, Windh RT, Blackford S and Kuhn CM. 1991. Ontogeny of mu- and kappa-opiate receptor control of the hypothalamo-pituitary-adrenal axis in rats. Endocrinology 129: 959-964.

Alcaraz C, Vargas ML, Fuente T and Milanés MV. 1993. Chronic naloxone treatment induced supersensitivity to a mu but not to a kappa agonist at the hypothalamus-pituitary-adrenocortical axis level. J Pharmacol Exp Ther. 266: 1602-1606.

Al-Damluji S, Bouloux P, White A and Besser M. 1990. The role of alpha-2-adrenoceptors in the control of ACTH secretion; interaction with the opioid system. Neuroendocrinology. 51: 76-81.

Alexander SL and Irvine CH. 1995. The effect of naloxone administration on the secretion of corticotropin-releasing hormone, arginine vasopressin, and adrenocorticotropin in unperturbed horses. Endocrinology. 136: 5139-5147.

Allolio B, Schulte HM, Deuss U, Kallabis D, Hamel E and Winkelman W. 1987. Effect of oral morphine and naloxone on pituitary-adrenal response in man induced by human corticotropin-releasing hormone. Acta Endocrinol (Copenh). 114: 509-514.

Allolio B, Winkelmann W, Hipp FX, Kaulen D and Mies R. 1982. Effects of a met-enkephalin analog on adrenocorticotropin (ACTH), growth hormone, and prolactin in patients with ACTH hypersecretion. J Clin Endocrinol Metab. 55: 1-7.

Ardaillou N, Nivez MP, Bellon G, Combe C and Ardaillou R. 1990. Effect of prostaglandin E_2 on proline uptake and protein synthesis by cultured human mesangial cells. Kidney Int. 38: 1151-1158.

Auernhammer CJ, Riepl RL, Schopohl J, Lehnert P, Muller OA and Stalla GK. 1994. In man the mu-opiate agonist loperamide specifically inhibits ACTH secretion induced by the cholecystokinin-like peptide ceruletide. Neuroendocrinology. 60: 16-22.

Auernhammer CJ, Stalla GK, Lange M, Pfeiffer A and Müller OA. 1992. Effects of loperamide on the human hypothalamo-pituitary-adrenal axis in vivo and in vitro. J Clin Endocrinol Metab. 75: 552-557.

Avery ME. 1975. Pharmacological approaches to the acceleration of fetal lung maturation. Br Med Bull. 31: 13-17.

Bajusz S, Ronai AZ, Szekely JI, Graf L, Dunai-Kovacs Z and Berzetei I. 1977. A superactive antinociceptive pentapeptide, (D-Met^2, Pro^5)-enkephalinamide. FEBS Lett. 76: 91-92.

Barb CR, Kraeling RR, Rampacek GB and Whisnant CS. 1986. Influence of stage of the estrous cycle on endogenous opioid modulation of luteinizing hormone, prolactin, and cortisol secretion in the gilt. Biol Reprod. 35: 1162-1167.

Bareis DL and Slotkin TA. 1978. Responses of heart ornithine decarboxylase and adrenal catecholamines to methadone and sympathetic stimulants in developing and adults rats. J Pharmacol Exp Ther. 205: 164-174.

Barg J, Belcheva MM and Coscia CJ. 1992. Evidence for the implication of phosphoinositol signal transduction in μ-opioid inhibition of DNA synthesis. J Neurochem. 59: 1145-1152.

Barg J, Belcheva MM, Rowinski J and Coscia CJ. 1993. κ-Opioid agonist modulation of [^{3}H]thymidine incorporation into DNA: evidence for the involvement of pertussis toxin-sensitive G protein-coupled phosphoinositide turnover. J Neurochem. 60: 1505-1511.

Barg J, Nah SY, Levy R, Saya D and Vogel Z. 1993. Modulation of thymidine incorporation by κ-opioid ligands in rat spinal cord-dorsal root ganglion co-cultures. Brain Res. 629: 109-114.

Bartolome JV and Bartolome MB. 1994. Role of the spinal cord in intracisternal ß-endorphin-evoked suppression of liver DNA synthesis in 10-day-old rats. Brain Res. 642: 311-315.

Bartolome JV, Alicke B and Bartolome MB. 1997. Central administration of morphine inhibits brain and liver ornithine decarboxylase activity in neonatal rats: involvement of transcription- and non-transcription-dependent mechanisms. Eur J Pharmacol. 331: 145-153.

Bartolome JV, Bartolome MB, Daltner LA, Evans CJ, Barchas JD, Kuhn CM and Schanberg SM. 1986. Effects of ß-endorphin on ornithine decarboxylase in tissues of developing rats: a potential role for this endogenous neuropeptide in the modulation of tissue growth. Life Sci. 38: 2355-2362.

Bartolome JV, Bartolome MB, Lorber BA, Dileo SJ and Schanberg SM. 1991. Effects of central administration of beta-endorphin on brain and liver DNA synthesis in preweaning rats. Neuroscience. 40: 289-294.

Bartolome JV, Lorber BA and Bartolome MB. 1994. Brain cholecystokinin and ß-endorphin systems may antagonistically interact to regulate tissue DNA synthesis in rat pups. Brain Res. 661: 19-24.

Bauer M and Parvizi N. 1996. Pulsatile and diurnal secretion of GH and IGF-I in the chronically catheterized pig fetus. J Endocrinol. 149: 125-133.

Becker FF, Rossman T, Reiss B and Simon EJ. 1972. The effect of levorphanol tartrate on ribonucleic acid synthesis in normal and regenerating rat liver. Res Commun Chem Pathol Pharmacol. 3: 105-116.

Bero LA, Lurie SN and Kuhn CM. 1987. Early ontogeny of kappa-opioid receptor regulation of prolactin secretion in the rat. Brain Res. 465: 189-196.

Bhargava HN, Matwyshyn GA, Hanissian S and Tejwani GA. 1988. Opioid peptides in pituitary gland, brain regions and peripheral tissues of spontaneously hypertensive and Wistar-Kyoto normotensive rats. Brain Res. 440: 333-340.

Bisignani GJ, McLaughlin PJ, Ordille SD, Beltz MS, Jarowenko MV and Zagon IS. 1999. Human renal cell cancer proliferation in tissue culture is tonically inhibited by opioid growth factor. J Urol. 162: 2186-2191.

Blankstein J, Reges FI and Winter JSD. 1978. Opioid control of anterior pituitary function in hyperprolactinemic women. Endocrinology (Suppl). 102: 349A.

Blankstein J, Reyes FI, Winter JS and Faiman C. 1980. Effects of naloxone upon prolactin and costisol in normal women. Proc Soc Exp Biol Med. 164: 363-365.

Bolt RJ, van Weissenbruch MM, Lafeber HN and Delemarre-van de Waal HA. 2002. Development of the hypothalamic-pituitary-adrenal axis in the fetus and preterm infant. J Pediatr Endocrinol Metab. 15: 759-769.

Bouloux PM, Grossman A, Allolio B, Delitala G and Besser GM. 1986. Effect of adrenergic antagonism on the growth hormone, prolactin and cortisol response to a synthetic opioid agonist in man. Horm Res. 23: 83-90.

Bousquet J, Lye SJ and Challis JR. 1984. Comparison of leucine enkephalin and adrenocorticotrophin effects on adrenal function in fetal and adult sheep. J Reprod Fertil. 70: 499-506.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254.

Briggs FN and Munson PL. 1955. Studies on the mechanism of stimulation of ACTH secretion with the aid of morphine as a blocking agent. Endocrinology. 57: 205-219.

Brooks AN and Challis JR. 1988. Exogenous opioid regulation of the hypothalamic-pituitary-adrenal axis in fetal sheep. J Endocrinol. 119: 389-395.

Brooks AN and Challis JRG. 1989. Effects of CRF, AVP and opioid peptides on pituitary-adrenal responses in sheep. Peptides. 10: 1291-1293.

Brooks AN and Challis JRG. 1991. Effects of naloxone on the preparturient increase in adrenocorticotrophin and cortisol in fetal sheep. J Neuroendocrinol. 3: 419-424.

Broughton-Pipkin F, Hull D and Stephenson T. 1994. Fetal Physiology. In Marshall's Physiology of Reproduction. Lamming GE Ed. vol. 3, 4th Ed. London: Chapman & Hall. 767-861.

Brown DR, Blank MS and Holtzman SG. 1980. Suppression by naloxone of water intake induced by deprivation and hypertonic saline in intact and hypophysectomized rats. Life Sci. 26: 1535-1542.

Bruni G, Dal Pra P, Fiaschi AI and Segre G. 1985. Effect of ACTH, ß-endorphin, morphine and naloxone on the release of cortisol by isolated adrenal glands. Pharmacol Res Commun. 17: 847-853.

Buckingham JC and Cooper TA. 1984a. Differences in hypothalamo-pituitary-adrenocortical activity in the rat after acute and prolonged treatment with morphine. Neuroendocrinology. 38: 411-417.

Buckingham JC and Cooper TA. 1984b. Influence of opioid substances on hypothalamo-pituitary-adrenocortical activity in the rat. In Opioid Modulation of Endocrine Function. Delitala G, Motta M and Serio M Ed. Raven Press, New York. 81-87.

Buckingham JC and Cooper TA. 1986. Pharmacological characterization of opioid receptors influencing the secretion of corticotrophin releasing factor in the rat. Neuroendocrinology. 44: 36-40.

Buckingham JC. 1982. Secretion of corticotrophin and its hypothalamic releasing factor in response to morphine and opioid peptides. Neuroendocrinology. 35: 111-116.

Buckingham JC. 1986. Stimulation and inhibition of corticotrophin releasing factor secretion by beta-endorphin. Neuroendocrinology. 42: 148-152.

Carson GD and Challis JR. 1982. Changes in plasma cortisol and progesterone after administration of enkephalins to newborn lambs. Can J Physiol Pharmacol. 60: 1166-1170.

Carter D and Lightman SL. 1986. Opiates. In Neuroendocrinology. Ch. 5. Lightman SL and Everitt BJ Ed. Blackwell Scientific Publications, Oxford.

Castro MI, Valego NK, Zehnder TJ and Rose JC. 1992. The ratio of plasma bioactive to immunoreactive ACTH-like activity increases with gestational age in the fetal lamb. J Dev Physiol. 18: 193-201.

Catlin DH, Poland RE, Gorelick DA, Gerner RH, Hui KK, Rubin RT and Li CH. 1980. Intravenous infusion of ß-endorphin increases serum prolactin, but not growth hormone or cortisol, in depressed subjects and withdrawing methadone addicts. J Clin Endocrinol Metab. 50: 1021-1025.

Challis J, Sloboda D, Matthews S, Holloway A, Alfaidy N, Howe D, Fraser M and Newnham J. 2000. Fetal hypothalamic-pituitary adrenal (HPA) development and activation as a determinant of the timing of birth, and of postnatal disease. Endocr Res. 26: 489-504.

Challis JR and Brooks AN. 1989. Maturation and activation of hypothalamic-pituitary adrenal function in fetal sheep. Endocr Rev. 10: 182-204.

Challis JRG, Matthews SG, Gibb W and Lye SJ. 2000. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev. 21: 514-550.

Chirgwin JM, Przybyla AE, MacDonald RJ and Rutter WJ. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry. 18: 5294-5299.

Chomczynski P and Sacchi N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 162: 156-159.

Cicero TJ, O'Connor L, Nock B, Adams ML, Miller BT, Bell RD and Meyer ER. 1989. Age-related differences in the sensitivity to opiate-induced perturbations in reproductive endocrinology in the developing and adult male rat. J Pharmacol Exp Ther. 248: 256-261.

Clouet DH and Ratner M. 1968. The effect of morphine administration on the incorporation of [14-C]leucine into protein in cell-free systems from rat brain and liver. J Neurochem. 15: 17-23.

Clouet DH. 1970. The effects of drugs on protein synthesis in the nervous system. In Protein Metabolism of the Nervous System. Lajtha A Ed. Plenum Press, New York. 699-713.

Clouet DH. 1971. Protein and nucleic acid metabolism. In Narcotic Drugs: Biochemical Pharmacology. Clouet DH Ed. Plenum Press, New York. 216-228.

Cohen MR, Cohen RM, Pickar D, Kreger D, McLellan C and Murphy DL. 1985. Hormonal effects of high dose naloxone in humans. Neuropeptides. 6: 373-380.

Cohen MR, Cohen RM, Pickar D, Weingartner H and Murphy DL. 1983. High-dose naloxone infusions in normals. Dose-dependent behavioral, hormonal, and physiological responses. Arch Gen Psychiatry. 40: 613-619.

Coiro V, Chiodera P, Volpi R, Salati G, Ferrari P, Caiazza A and Rossi G. 1984. Histaminergic H_1 and H_2 receptors do not mediate cortisol release in response to naloxone in normal men. Biomed Pharmacother. 38: 358-360.

Conaglen JV, Donald RA, Espiner EA, Livesey JH and Nicholls MG. 1985. Effect of naloxone on the hormone response to CRF in normal man. Endocr Res. 11: 39-44.

Crofford M and Smith AA. 1973. Growth retardation in young mice treated with d1-methadone. Science. 181: 947-949.

Cushman P Jr. 1972. Growth hormone in narcotic addiction. J Clin Endocrinol Metab. 35: 352-358.

De Souza EB, El-Tayeb KMA and Van Loon GR. 1981. Endogenous opioid peptides alter pituitary-adrenocortical secretion (Abstract). 63rd Annual Meeting of the Endocrine Society. Cincinnati OH. 182.

del Pozo E, Martin-Perez J, Stadelmann A, Girard J and Brownell J. 1980. Inhibitory action of a met-enkephalin on ACTH release in man. J Clin Invest. 65: 1531-1534.

del Pozo E, von Graffenried B, Brownell J, Derrer F and Marbach P. 1980. Endocrine effect of a methionine-enkephalin derivative (FK 33-824) in man. Horm Res. 13: 90-97.

Delitala G, Devilla L and Arata L. 1981. Opiate receptors and anterior pituitary hormone secretion in man. Effect of naloxone infusion. Acta Endocrinol (Copenh). 97: 150-156.

Delitala G, Giusti M, Rodriguez G, Mazzocchi G, Ferrini S, Baccelliere L, Montano V, Rosadini G and Giordano G. 1982. Growth hormone, prolactin and cortisol nyctohemeral variations during naloxone-induced opiate receptor blockade in man. Acta Endocrinol (Copenh). 100: 321-326.

Delitala G, Grossman A and Besser GM. 1981a. The modulation of opiate-induced changes in anterior pituitary function by a dopamine agonist and antagonist. Neuroendocrinol Lett. 3: 147-152.

Delitala G, Grossman A and Besser GM. 1981b. Changes in pituitary hormone levels induced by met-enkephalin in man-the role of dopamine. Life Sci. 29: 1537-1544.

Delitala G, Grossman A and Besser M. 1983. Differential effects of opiate peptides and alkaloids on anterior pituitary hormone secretion. Neuroendocrinology. 37: 275-279.

Delitala G, Palermo M, Tomasi P, Besser M and Grossman A. 1991. Adrenergic stimulation of the human pituitary-adrenal axis is attenuated by an analog of met-enkephalin. Neuroendocrinology. 53: 41-46.

Delitala G, Trainer PJ, Oliva O, Fanciulli G and Grossman AB. 1994. Opioid peptide and α-adrenoceptor pathways in the regulation of the pituitary-adrenal axis in man. J Endocrinol. 141: 163-168.

Demura R, Suda T, Wakabayashi I, Yoshimura M, Jibiki K, Odagiri E, Demura H and Shizume K. 1981. Plasma pituitary hormone responses to the synthetic enkephalin analog (FK 33-824) in normal subjects and patients with pituitary diseases. J Clin Endocrinol Metab. 52: 263-266.

Di Francesco P, Gaziano R, Casalinuovo IA, Palamara AT and Favalli C. 1994. Inhibitory effect of morphine on the control of rat fibroblast cell proliferation. Int J Tissue React. 16: 205-210.

Domanski E, Romanowicz K and Kerdelhue B. 1993. Enhancing effect of intracerebrally infused ß-endorphin antiserum on the secretion of cortisol in foot-shocked sheep. Neuroendocrinology. 57: 127-131.

Dutriez-Casteloot I, Bernet F, Dedieu JF, Croix D, Laborie C, Montel V, Lesage J, Beauvillain JC and Dupouy JP. 1999. Hypothalamic-pituitary-adrenocortical and gonadal axes and sympathoadrenal activity of adult male rats prenatally exposed to morphine. Neurosci Lett. 263: 1-4.

Ehrenreich H, Kolmar C, Muller OA and Goebel FD. 1987. Potentiation of the hCRF-induced release of ACTH in man by an opioid antagonist. Klin Wochenschr. 65: 453-457.

Eisenberg RM. 1980. Effects of naloxone on plasma corticosterone in the opiate-naive rat. Life Sci. 26: 935-943.

Eisenberg RM. 1985. Plasma corticosterone changes in response to central or peripheral administration of kappa and sigma opiate agonists. J Pharmacol Exp Ther. 233: 863-869.

Ela C, Barg J, Vogel Z, Hasin Y and Eilam Y. 1997. Distinct components of morphine effects on cardiac myocytes are mediated by the kappa and delta opioid receptors. J Mol Cell Cardiol. 29: 711-720.

Engquist A, Hicquet J, Saurbrey N and Blichert-Toft M. 1981. Cortisol, glucose, and hemodynamic responses to surgery after naloxone administration. Acta Anaesthesiol Scand. 25: 17-20.

Estienne MJ, Kesner JS, Barb CR, Kraeling RR and Rampacek GB. 1988. On the site of action of naloxone-stimulated cortisol secretion in gilts. Life Sci. 43: 161-166.

Farren CK, O'Malley S, Grebski G, Maniar S, Porter M and Kreek MJ. 1999. Variable dose naltrexone-induced hypothalamic-pituitary-adrenal stimulation in abstinent alcoholics: a preliminary study. Alcohol Clin Exp Res. 23: 502-508.

Fevre J. 1975. Maternal and fetal corticosteroids in the sow at the end of gestation. C R Acad Sci Hebd Seances Acad Sci D. 281: 2009-2012.

Fowden AL, Li J and Forhead AJ. 1998. Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? Proc Nutr Soc. 57: 113-122.

Friedman L, Gaines DW, Newell RF, Sager AO, Matthews RN and Braunberg RC. 1994. Body and organ growth of the developing Hormel-Hanford strain of male miniature swine. Lab Anim. 28: 376-379.

Friedman L, Gaines DW, Newell RF, Smith MC, Braunberg RC, Flynn TJ and O'Donnell Jr MW. 1995. Growth patterns in selected organs of the miniature swine as determined by gross macromolecular composition. J Anim Sci. 73: 1340-1350.

Gaillard RC, Grossman A, Smith R, Rees LH and Besser GM. 1981. The effects of a met-enkephalin analogue on ACTH, ß-LPH, ß-endorphin and Met-enkephalin in patients with adrenocortical disease. Clin Endocrinol (Oxf). 14: 471-478.

Geber WF, Lefkowitz SS and Hung CY. 1976. Action of naloxone on the interferon-lowering activity of morphine in the mouse. Pharmacology. 14: 322-329.

George JM, Reier CE, Lanese RR and Rower M. 1974. Morphine anesthesia blocks cortisol and growth hormone response to surgical stress in humans. J Clin Endocrinol Metab. 38: 736-741.

George R and Way EL. 1955. Studies on the mechanism of pituitary-adrenal activation by morphine. Br J Pharmacol. 10: 260-264.

George R and Way EL. 1959. The role of the hypothalamus in pituitary-adrenal activation and antidiuresis by morphine. J Pharmacol Exp Ther. 125: 111-115.

Gluckman PD, Sizonenko SV and Bassett NS. 1999. The transition from fetus to neonate-an endocrine perspective. Acta Paediatr Suppl. 88: 7-11.

Godfredson JA, Holland MD, Odde KG and Hossner KL. 1991. Hypertrophy and hyperplasia of bovine fetal tissues during development: Fetal liver insulin-like growth factor I mRNA expression. J Anim Sci. 69: 1074-1081.

Gonzálvez ML, Milanés MV and Vargas ML. 1991. Effects of acute and chronic administration of μ- and δ-opioid agonists on the hypothalamic-pituitary-adrenocortical (HPA) axis in the rat. Eur J Pharmacol. 200: 155-158.

Gonzálvez ML, Milanés MV, Martínez-Piñero MG, Marín MT and Vargas ML. 1994. Effects of intracerebroventricular clonidine on the hypothalamic noradrenaline and plasma corticosterone levels of opiate naive rats and after naloxone-induced withdrawal. Brain Res. 647: 199-203.

Gore MT, Young RB, Claeys MC, Chromiak JA, Rahe CH, Marple DN, Hough JD, Griffin JL and Mulvaney DR. 1994. Growth and development of bovine fetuses and neonates representing three genotypes. J Anim Sci. 72: 2307-2318.

Gorodinsky A, Barg J, Belcheva MM, Levy R, McHale RJ, Vogel Z and Coscia CJ. 1995. Dynorphins modulate DNA synthesis in fetal brain cell aggregates. J Neurochem. 65: 1481-1486.

Gosselin RE, Blankstein J, Dent DW, Hobson WC, Fuller GB, Reyes FI, Winter JS and Faiman C. 1983. Effects of naloxone and an enkephalin analog on serum prolactin, cortisol, and gonadotropins in the chimpanzee. Endocrinology. 112: 2168-2173.

Gosztonyi G, Schmidt V, Nickel R, Rothschild MA, Camacho S, Siegel G, Zill E, Pauli G and Schneider V. 1993. Neuropathologic analysis of postmortal brain samples of HIV-seropositive and -seronegative i.v. drug addicts. Forensic Sci Int. 62: 101-105.

Grignolo A, Seidler FJ, Bartolome M, Kuhn CM, Slotkin TA and Schanberg SM. 1982. Norepinephrine content of the rat kidney during development: alterations induced by perinatal methadone. Life Sci. 31: 3009-3016.

Grossman A and Besser GM. 1982. Opiates control ACTH through a noradrenergic mechanism. Clin Endocrinol (Oxf). 17: 287-290.

Grossman A and Clement-Jones V. 1983a. Endorphins and enkephalins. In Clinics in Endocrinology and Metabolism – Receptors in Health and Disease. Clayton RN Ed. Saunders, London.

Grossman A and Clement-Jones V. 1983b. Opiate receptors: enkephalins and endorphins. Clin Endocrinol Metab. 12: 31-56.

Grossman A, Delitala G, Mannelli M, Al-Damluji S, Coy DH and Besser GM. 1986. An analogue of met-enkephalin attenuates the pituitary-adrenal response to ovine corticotrophin releasing factor. Clin Endocrinol (Oxf). 25: 421-426.

Grossman A, Gaillard RC, McCartney P, Rees LH and Besser GM. 1982. Opiate modulation of the pituitary-adrenal axis: effects of stress and circadian rhythm. Clin Endocrinol (Oxf). 17: 279-286.

Grossman A, Moult PJ, Cunnah D and Besser M. 1986. Different opioid mechanisms are involved in the modulation of ACTH and gonadotrophin release in man. Neuroendocrinology. 42: 357-360.

Grossman A, Moult PJ, Gaillard RC, Delitala G, Toff WD, Rees LH and Besser GM. 1981. The opioid control of LH and FSH release: effects of a met-enkephalin analogue and naloxone. Clin Endocrinol (Oxf). 14: 41-47.

Grossman A, Stubbs WA, Gaillard RC, Delitala G, Rees LH and Besser GM. 1981. Studies of the opiate control of prolactin, GH and TSH. Clin Endocrinol (Oxf). 14: 381-386.

Grossman A. 1983. Brain opiates and neuroendocrine function. Clin Endocrinol Metab. 12: 725-746.

Hansson T, Naslund B, Blanck A and Rane A. 1989. Perinatal opiate exposure. Effects on metabolism of xenobiotics and steroids in adult rat liver. Dev Pharmacol Ther. 12: 146-152.

Haracz JL, Bloom AS, Wang RI and Tseng LF. 1981. Effect of intraventricular ß-endorphin and morphine on hypothalamic-pituitary-adrenal activity and the release of pituitary ß-endorphin. Neuroendocrinology. 33: 170-175.

Harris RA, Dunn A and Harris LS. 1974. Effects of acute and chronic morphine administration on the incorporation of (^{3}H)-lysine into mouse brain and liver proteins. Res Commun Chem Pathol Pharmacol. 9: 299-306.

Harris RA, Harris LS and Dunn A. 1975. Effect of narcotic drugs on ribonucleic acid and nucleotide metabolism in mouse brain. J Pharmacol Exp Ther. 192: 280-287.

Hashiguchi Y, Molina PE, Dorton S, McNurlan MA, Garlick PJ, Reddy D and Abumrad NN. 1997. Central opiate μ-receptor-mediated suppression of tissue protein synthesis. Am J Physiol. 273: R920-927.

Hashiguchi Y, Molina PE, Preedy VR, Sugden PH, McNurlan MA, Garlick PJ and Abumrad NN. 1996. Central effects of morphine and morphine-6-glucuronide on tissue protein synthesis. Am J Physiol. 271: R619-625.

Hatzoglou A, Bakogeorgou E, Papakonstanti E, Stournaras C, Emmanouel DS and Castanas E. 1996. Identification and characterization of opioid and somatostatin binding sites in the opossum kidney (OK) cell line and their effect on growth. J Cell Biochem. 63: 410-421.

Hauser KF, McLaughlin PJ and Zagon IS. 1987. Endogenous opioids regulate dendritic growth and spine formation in developing rat brain. Brain Res. 416: 157-161.

Hauser KF. 1992. Morphine regulates DNA synthesis in rat cerebellar neuroblasts in vitro. Brain Res Dev Brain Res. 70: 291-297.

Hayford SM, Epps RP and Dahl-Regis M. 1988. Behavior and development patterns in children born to heroin-addicted and methadone-addicted mothers. J Natl Med Assoc. 80: 1197-1200.

Haynes LW. 1984. Opioid systems and the developing brain. TIPS. 453-454.

Hellman L, Fukushima DK, Roffwarg H and Fishman J. 1975. Changes in estradiol and cortisol production rates in men under the influence of narcotics. J Clin Endocrinol Metab. 41: 1014-1019.

Ho WK, Lam S, Leung KC, Au KK, Wong HK, Tsang YF and Wen HL. 1978. Effect of naloxone on morphine-induced changes in ACTH, corticosterone and cyclic nucleotides. Neuropharmacology. 17: 397-400.

Hockings GI, Grice JE, Walters MM, Crosbie GV, Torpy DJ and Jackson RV. 1995. A synergistic adrenocorticotropin response to naloxone and vasopressin in normal human: evidence that naloxone stimulates endogenous corticotropin-releasing hormone. Neuroendocrinology. 61: 198-206.

Höllt V, Przewlocki R and Herz A. 1978. ß-Endorphin-like immunoreactivity in plasma, pituitaries and hypothalamus of rats following treatment with opiates. Life Sci. 23: 1057-1065.

Homma T, Ichikawa I and Hoover RI. 1988. Prostaglandins of mesangial origin inhibit mesangial cell proliferation and matrix synthesis (Abstract). Kidney Int. 33: 268A.

Howlett TA and Rees LH. 1986. Endogenous opioid peptides and hypothalamo-pituitary function. Annu Rev Physiol. 48: 527-536.

Hutchings DE. 1982. Methadone and heroin during pregnancy: a review of behavioral effects in human and animal offspring. Neurobehav Toxicol Teratol. 4: 429-434.

Ignar DM and Kuhn CM. 1990. Effects of specific mu and kappa opiate tolerance and abstinence on hypothalamo-pituitary-adrenal axis secretion in the rat. J Pharmacol Exp Ther. 255: 1287-1295.

Isayama T, McLaughlin PJ and Zagon IS. 1991. Endogenous opioids regulate cell proliferation in the retina of developing rat. Brain Res. 544: 79-85.

Iyengar S, Kim HS and Wood PL. 1986. Kappa opiate agonists modulate the hypothalamic-pituitary-adrenal axis in the rat. J Pharmacol Exp Ther. 238: 429-436.

Jackson RV, Grice JE, Hockings GI and Torpy DJ. 1995. Naloxone-induced ACTH release: mechanism of action in humans. Clin Endocrinol (Oxf). 43: 423-424.

Jackson RV, Grice JE, Jackson AJ and Hockings GI. 1990. Naloxone-induced ACTH release in man is inhibited by clonidine. Clin Exp Pharmacol Physiol. 17: 179-184.

Jackson RV, Grice JE, Jackson AJ and Vella RD. 1989. Potentiation of fenfluramine-induced ACTH release in man by naloxone. Clin Exp Pharmacol Physiol. 16: 263-267.

Jakubovic A, McGeer EG and McGeer PL. 1978. Comparative inhibition of biosynthetic processes in rat liver testicular cells by D-, L- and DL-methadone. Biochem Pharmacol. 27: 123-125.

Jarvis S, Lawrence AB, McLean KA, Chirnside J, Deans LA and Calvert SK. 1998. The effect of environment on plasma cortisol and ß-endorphin in the parturient pig and the involvement of endogenous opioids. Anim Reprod Sci. 52: 139-151.

Jefferson LS, Wolpert EB, Giger KE and Morgan HE. 1971. Regulation of protein synthesis in heart muscle. 3. Effect of anoxia on protein synthesis. J Biol Chem. 246: 2171-2178.

Jezova D, Vigas M and Jurcovicova J. 1982. ACTH and corticosterone response to naloxone and morphine in normal, hypophysectomized and dexamethasone-treated rats. Life Sci. 31: 307-314.

Jin DF, Muffly KE, Okulicz WC and Kilpatrick DL. 1988. Estrous cycle- and pregnancy-related differences in expression of the proenkephalin and proopiomelanocortin genes in the ovary and uterus. Endocrinology. 122: 1466-1471.

Kaltenbach K and Finnegan LP. 1987. Perinatal and developmental outcome of infants exposed to methadone in-utero. Neurotoxicol Teratol. 9: 311-313.

Kampa M, Papakonstanti EA, Alexaki VI, Hatzoglou A, Stournaras C and Castanas E. 2004. The opioid agonist ethylketocyclazocine reverts the rapid, non-genomic effects of membrane testosterone receptors in the human prostate LNCaP cell line. Exp Cell Res. 294: 434-445.

Keshet E, Polakiewicz RD, Itin A, Ornoy A and Rosen H. 1989. Proenkephalin A is expressed in mesodermal lineages during organogenesis. EMBO J. 8: 2917-2923.

Kew D and Kilpatrick DL. 1990. Widespread organ expression of the rat proenkephalin gene during early postnatal development. Mol Endocrinol. 4: 337-340.

Kilpatrick DL and Rosenthal JL. 1986. The proenkephalin gene is widely expressed within the male and female reproductive systems of the rat and hamster. Endocrinology. 119: 370-374.

King AC, Schluger J, Gunduz M, Borg L, Perret G, Ho A and Kreek MJ. 2002. Hypothalamic-pituitary-adrenocortical (HPA) axis response and biotransformation of oral naltrexone: preliminary examination of relationship to family history of alcoholism. Neuropsychopharmacology. 26: 778-788.

Kokka N, Garcia JF and Elliott HW. 1994. Effects of acute and chronic administration of narcotic analgesics on growth hormone and corticotrophin (ACTH) secretion in rats. Proc Natl Acad Sci U S A. 347: 359.

Kornblum HI, Loughlin SE and Leslie FM. 1987. Effects of morphine on DNA synthesis in neonatal rat brain. Developmental Brain Res. 31: 45-52.

Kosten TR, Kreek MJ, Raghunath J and Kleber HD. 1986a. Cortisol levels during chronic naltrexone maintenance treatment in ex-opiate addicts. Biol Psych. 21: 217-220.

Kosten TR, Kreek MJ, Raghunath J and Kleber HD. 1986b. A preliminary study of beta-endorphin during chronic naltrexone maintenance treatment in ex-opiate addicts. Life Sci. 39: 55-59.

Kosterlitz HW and Paterson SJ. 1985. Types of opioid receptors: relation to antinociception. Philos Trans R Soc Lond B Biol Sci. 308: 291-297.

Kosterlitz HW, Paterson SJ and Robson LE. 1981. Characterization of the kappa-subtype of the opiate receptor in the guinea-pig brain. Br J Pharmacol. 73: 939-949.

Kream RM and Zukin RS. 1979. Binding characteristics of a potent enkephalin analog. Biochem Biophys Res Commun. 90: 99-109.

Lamberts SW, Bons EG and Del Pozo E. 1983. The met-enkephalin analog FK 33-824 and naloxone do not directly influence cortisol secretion by cultured human adrenocortical cells. Life Sci. 32: 755-758.

Lang DW, Darrah HK, Hedley-Whyte J and Laasberg LH. 1975. Uptake into brain proteins of ^{35}S-methionine during morphine tolerance. J Pharmacol Exp Ther. 192: 521-530.

Lesage J, Grino M, Bernet F, Dutriez-Casteloot I, Montel V and Dupouy JP. 1998. Consequences of prenatal morphine exposure on the hypothalamo-pituitary-adrenal axis in the newborn rat: effect of maternal adrenalectomy. J Neuroendocrinol. 10: 331-342.

Leslie FM, Chen Y and Winzer-Serhan UH. 1998. Opioid receptor and peptide mRNA expression in proliferative zones of fetal rat central nervous system. Can J Physiol Pharmacol. 76: 284-293.

Leslie FM. 1987. Methods used for the study of opioid receptors. Pharmacol Rev. 39: 197-249.

Leslie RD, Prescott RW, Kendall-Taylor P, Cook D, Weightman D, Ratcliffe J and Ingram MC. 1985. Opiate receptor blockade and diurnal pituitary and adrenal hormone levels. Horm Metab Res. 17: 86-89.

Levin ER, Sharp B, Meyer NV and Carlson HE. 1981. Morphine and naloxone: effects on ß-endorphin immunoreactivity in canine plasma and secretions from rat pituitaries. Endocrinology. 109: 146-151.

Lifschitz MH, Wilson GS, Smith EO and Desmond MM. 1983. Fetal and postnatal growth of children born to narcotic-dependent women. J Pediatr. 102: 686-691.

Liggins GC. 1976. Adrenocortical-related maturational events in the fetus. Am J Obstet Gynecol. 126: 931-941.

Lightman SL and Young WS. 1988. Corticotrophin releasing factor, vasopressin and pro-opiomelanocortin mRNA responses to stress and opiates in the rat. J Physiol. 403: 511-523.

Lorber BA, Freitag SK and Bartolome JV. 1990. Effects of beta-endorphin on DNA synthesis in brain regions of preweanling rats. Brain Res. 531: 329-332.

Lotti VJ, Kokka N and George R. 1969. Pituitary-adrenal activation following intrahypothalamic microinjection of morphine. Neuroendocrinology. 4: 326-332.

Lymangrover JR, Dokas LA, Kong A, Martin R and Saffran M. 1981. Naloxone has a direct effect on the adrenal cortex. Endocrinology. 109: 1132-1137.

Magyar DM, Fridshal D, Elsner CW, Glatz T, Eliot J, Klein AH, Lowe KC, Buster JE and Nathanielsz PW. 1980. Time-trend analysis of plasma cortisol concentrations in the fetal sheep in relation to parturition. Endocrinology. 107: 155-159.

Maldonado R, Stinus L, Gold LH and Koob GF. 1992. Role of different brain structures in the expression of the physical morphine withdrawal syndrome. J Pharmacol Exp Ther. 261: 669-677.

Maneckjee R and Minna JD. 1990. Opioid and nicotine receptors affect growth regulation of human lung cancer cell lines. Proc Natl Acad Sci U S A. 87: 3294-3298.

Maneckjee R, Biswas R and Vonderhaar BK. 1990. Binding of opioids to human MCF-7 breast cancer cells and their effects on growth. Cancer Res. 50: 2234-2238.

Marc M, Parvizi N, Ellendorff F, Kallweit E and Elsaesser F. 2000. Plasma cortisol and ACTH concentrations in the warmblood horse in response to a standardized

treadmill exercise test as physiological markers for evaluation of training status. J Anim Sci. 78: 1936-1946.

Martin del Campo AF, Dowson JH, Herbert J and Paykel ES. 1994. Effects of naloxone on diurnal rhythms in mood and endocrine function: a dose-response study in man. Psychopharmacology (Berl). 114: 583-590.

Martínez JA, Vargas ML, Fuente T, del Rio-García J and Milanés MV. 1990. Plasma beta-endorphin and cortisol levels in morphine-tolerant rats and in naloxone-induced withdrawal. Eur J Pharmacol. 182: 117-123.

Marx D, Kolb E and Salomon FV. 1992. The fresh weight and the concentration of DNA, RNA and protein in different tissues of swine of different ages. Zentralbl Veterinarmed A. 39: 11-25.

Matthews SG and Parrott RF. 1990. Naloxone infusions increase cortisol secretion in grouped and isolation–stressed sheep. J Endocr. 127 (Suppl Abstr): 78.

McDonald RK, Evans FT, Weise VK and Patrick RW. 1959. Effect of morphine and nalorphine on plasma hydrocortisone levels in man. J Pharmacol Exp Ther. 125: 241-247.

McLaughlin PJ, Tobias SW, Lang CM and Zagon IS. 1997. Chronic exposure to the opioid antagonist naltrexone during pregnancy: maternal and offspring effects. Physiol Behav. 62: 501-508.

McLaughlin PJ. 1994. Opioid antagonist modulation of rat heart development. Life Sci. 54: 1423-1431.

McLaughlin PJ. 1996. Regulation of DNA synthesis of myocardial and epicardial cells in developing rat heart by [Met^5]enkephalin. Am J Physiol. 271: R122-129.

McLaughlin PJ. 2002. Exposure to the opioid antagonist naltrexone throughout gestation alters postnatal heart development. Biol Neonate. 82: 207-216.

Mendelson JH, Mello NK, Cristofaro P, Skupny A and Ellingboe J. 1986. Use of naltrexone as a provocative test for hypothalamic-pituitary hormone function. Pharmacol Biochem Behav. 24: 309-313.

Mesiano S and Jaffe RB. 1997. Developmental and functional biology of the primate fetal adrenal cortex. Endocr Rev. 18: 378-403.

Michelson D, Altemus M, Galliven E, Hill L, Greenberg BD and Gold P. 1996. Naloxone-induced pituitary-adrenal activation does not differ in patients with depression, obsessive compulsive disorder, and healthy controls. Neuropsychopharmacology. 15: 207-212.

Mielenz M. 2002. Studies on the ontogeny of the growth hormone-receptor gene expression in pig (sus scrofa domestica). Ph.D. thesis. The faculty of biological sciences at the University of Hannover-Germany.

Milanes MV, Gonzalvez ML, Fuente T and Vargas ML. 1991. Pituitary-adrenocortical response to acute and chronic administration of U-50,488H in the rat. Neuropeptides. 20: 95-102.

Morley JE, Baranetsky NG, Wingert TD, Carlson HE, Hershman JM, Melmed S, Levin SR, Jamison KR, Weitzman R, Chang RJ and Varner AA. 1980. Endocrine effects of naloxone-induced opiate receptor blockade. J Clin Endocrinol Metab. 50: 251-257.

Morley JE. 1981. The endocrinology of the opiates and opioid peptides. Metabolism. 30: 195-209.

Muffly KE, Jin DF, Okulicz WC and Kilpatrick DL. 1988. Gonadal steroids regulate proenkephalin gene expression in a tissue-specific manner within the female reproductive system. Mol Endocrinol. 2: 979-985.

Munson PL. 1973. Effects of morphine and related drugs on the corticotrophin (ACTH)-stress reaction. Prog Brain Res. 39: 361-372.

Naber D, Pickar D, Davis GC, Cohen RM, Jimerson DC, Elchisak MA, Defraites EG, Kalin NH, Risch SC and Buchsbaum MS. 1981. Naloxone effects on ß-endorphin, cortisol, prolactin, growth hormone, HVA and MHPG in plasma of normal volunteers. Psychopharmacology (Berl). 74: 125-128.

Naeye RL and Blanc WA. 1971. Organ and body growth in anencephaly. A quantitative, morphological study. Arch Pathol. 91: 140-147.

Naeye RL, Blanc W, Leblanc W and Khatamee MA. 1973. Fetal complications of maternal heroin addiction: abnormal growth, infections, and episodes of stress. J Pediatr. 83: 1055-1061.

Nakamoto T, Usui A, Oshima K, Ikemoto H, Mitani S and Usui T. 1988. Analysis of growth factors in renal cell carcinoma. Biochem Biophys Res Commun. 153: 818-824.

Nanda AS, Dobson H and Ward WR. 1992. Opioid modulation of the hypothalamo-pituitary-adrenal axis in dairy cows. Domest Anim Endocrinol. 9: 181-186.

Nathanielsz PW. 1998. Comparative studies on the initiation of labor. Eur J Obstet Gynecol Reprod Biol. 78: 127-132.

Neidle A, Manigault I and Wajda IJ. 1979. Distribution of opiate-like substances in rat tissues. Neurochem Res. 4: 399-410.

Nikolarakis K, Pfeiffer A, Stalla GK and Herz A. 1987. The role of CRF in the release of ACTH by opiate agonists and antagonists in rats. Brain Res. 421: 373-376.

Nikolarakis KE, Pfeiffer A, Stalla GK and Herz A. 1989. Facilitation of ACTH secretion by morphine is mediated by activation of CRF releasing neurons and sympathetic neuronal pathways. Brain Res. 498: 385-388.

O WS. 1990. The effect of beta-endorphin on rat oocyte maturation in vitro. Mol Cell Endocrinol. 68: 181-185.

Orth JM. 1986. FSH-induced Sertoli cell proliferation in the developing rat is modified by beta-endorphin produced in the testis. Endocrinology. 119: 1876-1878.

Oyama T, Yamaya R, Jin T and Kudo T. 1982. Effect of exogenous ß-endorphin on anterior pituitary hormone secretion in man. Acta Endocrinol (Copenh). 99: 9-13.

Parrott RF and Goode JA. 1992. Effects of intracerebroventricular corticotropin-releasing hormone and intravenous morphine on cortisol, prolactin and growth hormone secretion in sheep. Domest Anim Endocrinol. 9: 141-149.

Parrott RF and Goode JA. 1993. Central effects of naloxone and selected opioid agonists on cortisol and prolactin secretion in non-stressed sheep. Gen Pharmacol. 24: 101-103.

Parrott RF and Thornton SN. 1988. Effects of psychological stress and dehydration on plasma cortisol, oxytocin and vasopressin in Clun Forest sheep. J Physiol. 399: 91P.

Parrott RF and Thornton SN. 1989. Opioid influences on pituitary function in sheep under basal conditions and during psychological stress. Psychoneuroendocrinology. 14: 451-459.

Parrott RF, Ebenezer IS, Baldwin BA and Forsling ML. 1991. Hormonal effects of apomorphine and cholecystokinin in pigs: modification of the response to cholecystokinin by a dopamine antagonist (metoclopramide) and a kappa opioid agonist (PD117302). Acta Endocrinol (Copenh). 125: 420-426.

Parvizi N Ed. 1993. Opioids in farm animals. Landwirtschaftsverlag Münster.

Paterson SJ, Robson LE and Kosterlitz HW. 1983. Classification of opioid receptors. Br Med Bull. 39: 31-36.

Pechnick RN. 1993. Effects of opioids on the hypothalamo-pituitary-adrenal axis. Annu Rev Pharmacol Toxicol. 32: 353-382.

Petraglia F, Facchinetti F, M'Futa K, Ruspa M, Bonavera JJ, Gandolfi F and Genazzani AR. 1986. Endogenous opioid peptides in uterine fluid. Fertil Steril. 46: 247-251.

Pfeiffer A and Herz A. 1984. Endocrine actions of opioids. Horm Metab Res. 16: 386-397.

Pfeiffer A, Herz A, Loriaux DL and Pfeiffer DG. 1985. Central kappa- and mu-opiate receptors mediate ACTH-release in rats. Endocrinology. 116: 2688-2690.

Pfeiffer A, Knepel W, Braun S, Meyer HD, Lohmann H and Brantl V. 1986. Effects of a kappa-opioid agonist on adrenocorticotropic and diuretic function in man. Horm Metab Res. 18: 842-848.

Pontiroli AE, Baio G, Stella L, Crescenti A and Girardi AM. 1982. Effects of naloxone on prolactin, luteinizing hormone, and cortisol responses to surgical stress in humans. J Clin Endocrinol Metab. 55: 378-380.

Powell R and Gannon F. 2002. Purification of DNA by phenol extraction and ethanol precipitation. Oxford Practical Approach Series. Oxford University Press.

Przekop F, Mateusiak K, Stupnicka E, Romanowicz K and Domanski E. 1990. Suppressive effect of ß-endorphin and naloxone on the secretion of cortisol under stress conditions in sheep. Exp Clin Endocrinol. 95: 210-216.

Quirion R, Finkel MS, Mendelsohn FA and Zamir N. 1983. Localization of opiate binding sites in kidney and adrenal gland of the rat. Life Sci. 33 Suppl 1: 299-302.

Rahe CH, Owens RE, Fleeger JL, Newton HJ and Harms PG. 1980. Pattern of plasma luteinizing hormone in the cyclic cow: dependence upon the period of the cycle. Endocrinology. 107: 498-503.

Randall GCB, Kendall JZ, Tsang BK and Taverne MAM. 1990. Endocrine changes following infusion of fetal pigs with corticotrophin in litters of reduced numbers. Anim Rep Sci. 23: 109-122.

Redekopp CA, Livesey JH and Donald RA. 1985. Inhibition of spontaneous ACTH release and improved response to CRF in sheep premedicated with the enkephalin analogue DAMME. Horm Metab Res. 17: 646-649.

Reeben M. 1992. bionet-molbio.methods-reagents archives 27.07.

Rehfeld JF. 1985. Neuronal cholecystokinin: one or multiple transmitters? J Neurochem. 44: 1-10.

Rolandi E, Magnani G, Sannia A and Barreca T. 1981. Hormonal changes induced by a partial opiate antagonist, nalorphine. Evaluation of PRL, GH, TSH, LH, FSH and cortisol secretion. Eur J Clin Pharmacol. 21: 23-25.

Rolandi E, Marabini A, Franceschini R, Messina V, Bongera P and Barreca T. 1983. Changes in pituitary secretion induced by an agonist-antagonist opioid drug, buprenorphine. Acta Endocrinol (Copenh). 104: 257-260.

Rossman T, Becker FF and Vilcek J. 1971. An investigation into the mechanism of cytotoxicity of levorphanol. Mol Pharmacol. 7: 480-483.

Rushen J and Ladewig J. 1991. Stress-induced hypoalgesia and opioid inhibition of pigs responses to restraint. Physiol Behav. 50: 1093-1096.

Rushen J, Nay TS, Wright LR, Payne DC and Foxcroft GR. 1995. Stress and nursing in the pig: role of HPA axis and endogenous opioid peptides. Physiol Behav. 58: 43-48.

Rushen J, Schwarze N, Ladewig J and Foxcroft G. 1993. Opioid modulation of the effect of repeated stress on ACTH, cortisol, prolactin and GH in pigs. Physiol Behav. 53: 923-928.

Sakiyama S, Usui S and Miura Y. 1969. Regulation of ribosomal RNA synthesis. Adv Enzyme Regul. 7: 207-218.

Sambrook J, Fritsch EF and Maniatis T. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2.Aufl.

Sands J, Dobbing J and Gratrix CA. 1979. Cell number and cell size: organ growth and development and the control of catch-up growth in rats. Lancet. 2: 503-505.

Sarkar NK, Lodge GA and Friend DW. 1977. Hyperplasic and hypertrophic growth in organs and tissues of the neonatal pig. J Anim Sci. 45: 722-728.

Sassani J, McLaughlin PJ and Zagon IS. 1993. Regulation of corneal epithelial wound healing in vitro by opioid growth factor (Abstract)**.** Invest Ophthalmol Visual Sci. 34: 1376.

Sawynok J, Pinsky C and LaBella FS. 1979. Minireview on the specificity of naloxone as an opiate antagonist. Life Sci. 25: 1621-1632.

Schluger J, Ho A, Porter M, Maniar S, Gunduz M, Perret G, King A and Kreek MJ. 1998. Nalmefene causes greater hypothalamic-pituitary-adrenal axis activation than naloxone in normal volunteers: implications for the treatment of alcoholism. Alcohol Clin Exp Res. 22: 1430-1436.

Schmahl W, Funk R, Miaskowski U and Plendl J. 1989. Long-lasting effects of naltrexone, an opioid receptor antagonist, on cell proliferation in developing rat forebrain. Brain Res. 486: 297-300.

Schmidt M, Sangild PT, Blum JW, Andersen JB and Greve T. 2004. Combined ACTH and glucocorticoid treatment improves survival and organ maturation in premature newborn calves. Theriogenology. 61: 1729-1744.

Serri O, Rasio E and Somma M. 1981. Effects of naloxone on insulin-induced release of pituitary hormones. J Clin Endocrinol Metab. 53: 206-208.

Siegel RA, Chowers I, Conforti N, Feldman S and Weidenfeld J. 1982. Effects of naloxone on basal and stress-induced ACTH and corticosterone secretion in the male rat-site and mechanism of action. Brain Res. 249: 103-109.

Silver M, Barnes RJ, Comline RS, Fowden AL, Clover L and Mitchell MD. 1979. Prostaglandins in the fetal pig and prepartum endocrine changes in mother and fetus. Anim Reprod Sci. 2: 305-322.

Silver M, Comline RS and Fowden AL. 1983. Fetal and maternal endocrine changes during the induction of parturition with the PGF analogue, cloprostenol, in chronically catheterized sows and fetuses. J Dev Physiol. 5: 307-321.

Simantov R, Childers SR and Snyder SH. 1978. (^{3}H) Opiate binding: anomalous properties in kidney and liver membranes. Mol Pharmacol. 14: 69-76.

Singhal PC, Gibbons N and Abramovici M. 1992. Long term effects of morphine on mesangial cell proliferation and matrix synthesis. Kidney Int. 41: 1560-1570.

Singhal PC, Sharma P, Gibbons N, Franki N, Kapasi A and Wagner JD. 1997. Effect of morphine on renomedullary interstitial cell proliferation and matrix accumulation. Nephron. 77: 225-234.

Singhal PC, Sharma P, Sanwal V, Prasad A, Kapasi A, Ranjan R, Franki N, Reddy K and Gibbons N. 1998. Morphine modulates proliferation of kidney fibroblasts. Kidney Int. 53: 350-357.

Skjaerlund DM, Mulvaney DR, Bergen WG and Merkel RA. 1994. Skeletal muscle growth and protein turnover in neonatal boars and barrows. J Anim Sci. 72: 315-321.

Slamberova R. 2003. Drugs during pregnancy-effects on the mother and next generation. Cesk Fysiol. 52: 15-21.

Slizgi GR and Ludens JH. 1985. Displacement of ^{3}H-EKC binding by opioids in rat kidney: a correlate to diuretic activity. Life Sci. 36: 2189-2193.

Smith AA, Hui FW and Crofford MJ. 1977. Inhibition of growth in young mice treated with d,1-methadone. Eur J Pharmacol. 43: 307-314.

Smythe GA, McGinley R, Chisholm DJ and Lazarus L. 1980. Effect of Leu-enkephalin on serum cortisol levels in man. Clin Exp Pharmacol Physiol. 7: 47.

Solomon S and Bengele HH. 1973. Growth rates and organ weights of rats. Biol. Neonate. 22: 222-229.

Spiler IJ and Molitch ME. 1980. Lack of modulation of pituitary hormone stress response by neural pathways involving opiate receptors. J Clin Endocrinol Metab. 50: 516-520.

Steven WJ, Lamberts SW, Janssens EN, Bons EG, Uitterlinden P, Zuiderwijk JM and Del Pozo E. 1983. The met-enkephalin analog FK 33-824 directly inhibits ACTH release by the rat pituitary gland in vitro. Life Sci. 32: 1167-1173.

Stolman S and Loh HH. 1971. Proceedings of the NAS-NRC Committee on Problems of Drug Dependence. 1: 803-814.

Stubbs WA, Delitala G, Jones A, Jeffcoate WJ, Edwards CR, Ratter SJ, Besser GM, Bloom SR and Alberti KG. 1978. Hormonal and metabolic responses to an enkephalin analogue in normal man. Lancet. 2: 1225-1227.

Sugden PH and Fuller SJ. 1991. Correlations between cardiac protein synthesis rates, intracellular pH and the concentrations of creatine metabolites. Biochem J. 273: 339-346.

Sushkova VV, Stognii NA, Kas'ianova NN, Shevtsova NF and Gulyi MF. 1992. Change in the intensity of biosynthesis of proteins, lipids and glycogen in animal tissues during long-term administration of morphine. Ukr Biokhim Zh. 64: 116-119.

Szekely JI, Ronai AZ, Dunai-Kovacs Z, Miglecz E, Berzetri I, Bajusz S and Graf L. 1977. (D-met^2, pro^5)-enkephalinamide: a potent morphine-like analgesic. Eur J Pharmacol. 43: 293-294.

Taub DD, Eisenstein TK, Geller EB, Adler MW and Rogers TJ. 1991. Immunomodulatory activity of mu- and kappa-selective opioid agonists. Proc Natl Acad Sci U S A. 88: 360-364.

Taylor CC, Soong Y, Wu D and Szeto HH. 1994. Differential effects of mu, delta, and kappa opioid agonists on fetal plasma cortisol levels. Reg Pept. 54: 297-298.

Taylor CC, Soong YI, Wu D, Yee JS and Szeto HH. 1997. Morphine stimulates adrenocorticotropin and cortisol release in the late-term ovine fetus. Pediatr Res. 41: 411-415.

Taylor CC, Wu D, Soong Y, Yee JS and Szeto HH. 1996. κ-Opioid agonist, U50,488H, stimulates ovine fetal pituitary-adrenal function via hypothalamic arginine-vasopressin and corticotrophin-releasing factor. J Pharmacol Exp Ther. 277: 877-884.

Taylor CC, Wu D, Soong Y, Yee JS and Szeto HH. 1997a. Dynorphin A_{1-13} stimulates ovine fetal pituitary-adrenal function through a novel nonopioid mechanism. J Pharmacol Exp Ther. 280: 416-421.

Taylor CC, Wu D, Soong Y, Yee JS and Szeto HH. 1997b. Opioid modulation of the fetal hypothalamic-pituitary-adrenal axis: the role of receptor subtypes and route of administration. J Pharmacol Exp Ther. 281: 129-135.

Taylor T, Dluhy RG and Williams GH. 1983. ß-endorphin suppresses adrenocorticotropin and cortisol levels in normal human subjects. J Clin Endocrinol Metab. 57: 592-596.

Tencheva Z, Velichkova A and Angelova A. 1995. Morphine-induced alterations on DNA-binding proteins in developing rat brain. Methods Find Exp Clin Pharmacol. 17: 449-454.

Thomas JA and Dombrosky JT. 1975. Effect of methadone on the male reproductive system. Arch Int Pharmacodyn Ther. 215: 215-221.

Thomas JM and Beamer JL. 1971. Age-weight relationships of selected organs and body weight for miniature swine. Growth. 35: 259-272.

Thornton SN and Parrott RF. 1989. Naloxone affects the release of cortisol, but not of vasopressin or oxytocin, in dehydrated sheep. Acta Endocrinol (Copenh). 120: 50-54.

Torpy DJ, Grice JE, Hockings GI, Crosbie GV, Walters MM and Jackson RV. 1995. Effect of sodium valproate on naloxone-stimulated ACTH and cortisol release in humans. Clin Exp Pharmacol Physiol. 22: 441-443.

Torpy DJ, Grice JE, Hockings GI, Walters MM, Crosbie GV and Jackson RV. 1997. Diurnal effects of fluoxetine and naloxone on the human hypothalamic-pituitary-adrenal axis. Clin Exp Pharmacol Physiol. 24: 421-423.

Torpy DJ, Jackson RV, Grice JE, Hockings GI, Crosbie GV and Walters MM. 1994. Effect of flumazenil on basal and naloxone-stimulated ACTH and cortisol release in humans. Clin Exp Pharmacol Physiol. 21: 157-161.

Tsagarakis S, Navarra P, Rees LH, Besser M, Grossman A and Navara P. 1989. Morphine directly modulates the release of stimulated corticotrophin-releasing factor-41 from rat hypothalamus in vitro. Endocrinology. 124: 2330-2335.

Tsagarakis S, Rees LH, Besser M and Grossman A. 1990. Opiate receptor subtype regulation of CRF-41 release from rat hypothalamus in vitro. Neuroendocrinology. 51: 599-605.

Tulunay FC and Takemori AE. 1975. The effect of morphine on the incorporation of ^{3}H-leucine into retinal proteins and subsequent axonal, transport in the optic system of rats. Life Sci. 16: 551-559.

Ur E, Wright DM, Bouloux PM and Grossman A. 1997. The effects of spiradoline (U-62066E), a kappa-opioid receptor agonist, on neuroendocrine function in man. Br J Pharmacol. 120: 781-784.

Van Loon GR and De Souza EB. 1978. Development of tolerance to the ACTH-releasing effects of ß-endorphin. Res Commun Chem Pathol Pharmacol. 22: 203-204.

Vargas ML, Martínez-Piñero MG and Milanés MV. 1997. Neurochemical activity of noradrenergic neurons and pituitary-adrenal response after naloxone-induced withdrawal: the role of calcium channels. Naunyn-Schmiedeberg's Arch Pharmacol. 355: 501-506.

Vertes Z, Melegh G, Vertes M and Kovacs S. 1982. Effect of naloxone and D-Met^{2}-Pro^{5}-enkephalinamide treatment on the DNA synthesis in the developing rat brain. Life Sci. 31: 119-126.

Volavka J, Bauman J, Pevnick J, Reker D, James B and Cho D. 1980. Short-term hormonal effects of naloxone in man. Psychoneuroendocrinology. 5: 225-234.

Volavka J, Cho D, Mallya A and Bauman J. 1979. Naloxone increases ACTH and cortisol levels in man. N Engl J Med. 300: 1056-1057.

Volavka J, Mallya A, Bauman J, Pevnick J, Cho D, Reker D, James B and Dornbush R. 1979. Hormonal and other effects of naltrexone in normal men. Adv Exp Med Biol. 116: 291-305.

Volavka J, Resnick RB, Kestenbaum RS and Freedman AM. 1976. Short-term effects of naltrexone in 155 heroin ex-addicts. Biol Psychiatry. 11: 679-685.

Vythilingam M, Anderson GM, Owens MJ, Halaszynski TM, Bremner JD, Carpenter LL, Heninger GR, Nemeroff CB and Charney DS. 2000. Cerebrospinal fluid corticotropin-releasing hormone in healthy humans: effects of yohimbine and naloxone. J Clin Endocrinol Metab. 85: 4138-4145.

Wakabayashi I, Demura R, Miki N, Ohmura E, Miyoshi H and Shizume K. 1980. Failure of naloxone to influence plasma growth hormone, prolactin, and cortisol secretions induced by insulin hypoglycemia. J Clin Endocrinol Metab. 50: 597-599.

Wang D, Teichtahl H, Drummer O, Goodman C, Cherry G, Cunnington D and Kronborg I. 2005. Central sleep apnea in stable methadone maintenance treatment patients. Chest. 128: 1348-1356.

Wang X, Tresham JJ, Scoggins BA and Coghlan JP. 1988. Met-enkephalin and the enkephalin analogue FK-33824 centrally inhibit adrenocorticotrophic hormone secretion in sheep. Clin Exp Pharmacol Physiol. 15: 865-873.

Wang XM, Coghlan JP, Congiu M, Scoggins BA and Wintour EM. 1986. Met-enkephalin stimulates secretion of ACTH in sheep. J Endocrinol. 111: 463-467.

Wang XQ, Imaki T, Shibasaki T, Yamauchi N and Demura H. 1996. Intracerebroventricular administration of ß-endorphin increases the expression of c-fos and of corticotropin-releasing factor messenger ribonucleic acid in the paraventricular nucleus of the rat. Brain Res. 707: 189-195.

Weisinger G, Zinder O and Simantov R. 1995. Antisense knockout of a neuropeptide gene reduces fibroblast proliferation. Biochem Biophys Res Commun. 214: 530-537.

Williams KL, Ko MC, Rice KC and Woods JH. 2003. Effect of opioid receptor antagonists on hypothalamic-pituitary-adrenal activity in rhesus monkeys. Psychoneuroendocrinology. 28: 513-528.

Wilson GS, McCreary R, Kean J and Baxter JC. 1979. The development of preschool children of heroin-addicted mothers: a controlled study. Pediatrics. 63: 135-141.

Wilson GS. 1975. Somatic growth effects of perinatal addiction. Addict Dis. 2: 333-345.

Wise T, Stone RT and Vernon MW. 1991. Relationships of serum estriol, cortisol and albumin concentrations with pig weight at 110 days of gestation and at birth. Biol Neonate. 59: 114-119.

Wittert G, Hope P and Pyle D. 1996. Tissue distribution of opioid receptor gene expression in the rat. Biochem Biophys Res Commun. 218: 877-881.

Yang KP and Challis JR. 1991. Opioid receptors are present in the hypothalamus but not detectable in the anterior pituitary of the developing ovine fetus. Neuroendocrinology. 53: 623-627.

Yeung MY and Smyth JP. 2002. Hormonal factors in the morbidities associated with extreme prematurity and the potential benefits of hormonal supplement. Biol Neonate. 81: 1-15.

Zagon IS and McLaughlin PJ. 1981. Heroin prolongs survival time and retards tumor growth in mice with neuroblastoma. Brain Res Bull. 7: 25-32.

Zagon IS and McLaughlin PJ. 1982. Comparative effects of postnatal undernutrition and methadone exposure on protein and nucleic acid contents of the brain and cerebellum in rats. Dev Neurosci. 5: 385-393.

Zagon IS and McLaughlin PJ. 1983a. Naltrexone modulates growth in infant rats. Life Sci. 33: 2449-2454.

Zagon IS and McLaughlin PJ. 1983b. Increased brain size and cellular content in infant rats treated with an opiate antagonist. Science. 221: 1179-1180.

Zagon IS and McLaughlin PJ. 1984. Naltrexone modulates body and brain development in rats: a role for endogenous opioid systems in growth. Life Sci. 35: 2057-2064.

Zagon IS and McLaughlin PJ. 1985. Opioid antagonist-induced regulation of organ development. Physiol Behav. 34: 507-511.

Zagon IS and McLaughlin PJ. 1987. Endogenous opioid systems regulate cell proliferation in the developing rat brain. Brain Res. 412: 68-72.

Zagon IS and McLaughlin PJ. 1989a. Opioid antagonist modulation of murine neuroblastoma: a profile of cell proliferation and opioid peptides and receptors. Brain Res. 480: 16-28.

Zagon IS and McLaughlin PJ. 1989b. Naloxone modulates body and organ growth of rats: dependency on the duration of opioid receptor blockade and stereospecificity. Pharmacol Biochem Behav. 33: 325-328.

Zagon IS and McLaughlin PJ. 1989c. Endogenous opioid systems regulate growth of neural tumor cells in culture. Brain Res. 490: 14-25.

Zagon IS and McLaughlin PJ. 1991a. The role of endogenous opioids and opioid receptors in human and animal cancers. In Stress and Immunity. Plotnikoff NP, Murgo AJ, Faith RE and Wybran J Ed. Caldwell, NJ: CRC. 343-356.

Zagon IS and McLaughlin PJ. 1991b. Identification of opioid peptides regulating proliferation of neurons and glia in the developing nervous system. Brain Res. 542: 318-323.

Zagon IS and McLaughlin PJ. 1992. An opioid growth factor regulates the replication of microorganisms. Life Sci. 50: 1179-1187.

Zagon IS and McLaughlin PJ. 1993. Opioid growth factor in the developing nervous system. In Receptors in the Developing Nervous System. Growth Factors and Hormones. Zagon IS and McLaughlin PJ Ed. London: Chapman & Hall. vol. 1, 39-62.

Zagon IS, Gibo DM and McLaughlin PJ. 1990. Adult and developing human cerebella exhibit different profiles of opioid binding sites. Brain Res. 523: 62-68.

Zagon IS, Goodman SR and McLaughlin PJ. 1990. Demonstration and characterization of zeta (ζ), a growth-related opioid receptor, in a neuroblastoma cell line. Brain Res. 511: 181-186.

Zagon IS, Sassani JW and McLaughlin PJ. 1995. Opioid growth factor modulates corneal epithelial outgrowth in tissue culture. Am J Physiol. 268: R942-950.

Zagon IS, Wu Y and McLaughlin PJ. 1994a. Opioid antagonist modulation of DNA synthesis in mouse tongue epithelium is circadian dependent. Pharmacol Biochem Behav. 48: 709-714.

Zagon IS, Wu Y and McLaughlin PJ. 1994b. Opioid growth factor inhibits DNA synthesis in mouse tongue epithelium in a circadian rhythm-dependent manner. Am J Physiol. 267: R645-652.

Zagon IS, Wu Y and McLaughlin PJ. 1996. Opioid growth factor-dependent DNA synthesis in the neonatal rat aorta. Am J Physiol. 270: R 22-32.

Zagon IS, Wu Y and McLaughlin PJ. 1997. Opioid growth factor is present in human and mouse gastrointestinal tract and inhibits DNA synthesis. Am J Physiol. 272: R1094-1104.

Zagon IS, Wu Y and McLaughlin PJ. 1999. Opioid growth factor and organ development in rat and human embryos. Brain Res. 839: 313-322.

Zanoboni A and Zanoboni Muciaccia W. 1987. Effects of naloxone and coffee on anterior pituitary hormones. Drugs Exp Clin Res. 13: 443-446.

Zanoboni A, Zecca L, Zanussi C and Zanoboni-Muciaccia W. 1981. Naloxone and anterior pituitary hormones: effect on TRH stimulation test. Neuroendocrinology. 33: 140-143.

Zeman FJ, Heng H, Hoogenboom ER, Kavlock RJ and Mahboob S. 1986. Cell number and size in selected organs of fetuses of rats malnourished and exposed to nitrofen. Teratog Carcinog Mutagen. 6: 339-347.

Zeman FJ. 1983. The effect of prenatal protein-calorie malnutrition on kidney development in the rat. Prog Clin Biol Res. 140: 309-338.

Zhou Y, Spangler R, Maggos CE, Wang XM, Han JS, Ho A and Kreek MJ. 1999. Hypothalamic-pituitary-adrenal activity and pro-opiomelanocortin mRNA levels in the

hypothalamus and pituitary of the rat are differentially modulated by acute intermittent morphine with or without water restriction stress. J Endocrinol. 163: 261-267.

Zhu Y and Pintar JE. 1998. Expression of opioid receptors and ligands in pregnant mouse uterus and placenta. Biol Reprod. 59 : 925-932.

Zis AP, Haskett RF, Albala AA and Carroll BJ. 1984. Morphine inhibits cortisol and stimulates prolactin secretion in man. Psychoneuroendocrinology. 9: 423-427.

12. Acknowledgement

This thesis has benefited greatly from the contribution of many people. I would like to acknowledge all of them.

I am very grateful to my supervisor, Prof. Dr. Dr. Nahid Parvizi for her useful suggestions, helpful advice and nice support. The way she and her family, Roland and Ralph, treated me during my stay was pleasant and I appreciate it.

I would like to thank Prof. Dr. Dr. h.c. Franz Ellendorff, former head of the institute, for giving me the opportunity to study and work in the Federal Research Center for Agriculture, Institute for Animal Science, Mariensee-Neustadt, Germany. My immense thanks also go to his wife.

I express sincere thanks to Prof. Dr. Hans-Jörg Abel for being my supervisor, for his friendship, encouragement, great support and help.

I extend my gratitude to Prof. Dr. Dr. Matthias Gauly for showing interest in my work, for his friendship and great help.

I also extend my gratitude to Prof. Dr. Wolfgang Holtz for showing interest in my work. His understanding is gratefully acknowledged.

Ute Beermann, I would like to thank her for being a friend. She also shared her experience in fetal surgery with me and was always ready to help whenever I needed it. She, her mother, Achim, Anika and Arne gave me a warm home feeling and I am grateful for it.

Iris Stelter, Meike Stünkel, Ronald Wittig and Melanie Müller, their help during the surgeries is gratefully acknowledged.

Patrick Aldag, thanks a lot for helping me translate several articles into English.

Karin Klingemann and Erich Hildewerth, thanks for taking good care of the sows and their piglets.

I also would like to express my deep appreciations to the other laboratory technicians and colleagues of the Department of Functional Genomics and Bioregulation, Sabine Klein, Gudrun Neuhaus, Rita Lechler, Bärbel Hildewerth, Melanie Niegisch, Beate Bünger, Liselotte Schütte, Birgit Mielenz, Karin von Kittlitz, Uta Buntrock and Ingrid Bergman, for their assistance that led me to the success of the Ph.D. program.

I learn a lot, especially about the technique, from Manfred Mielenz and for this I am very grateful to him.

I thank Sandra Kriegelstein for her excellent translation. It is a great help for me.

Thanks also go to the other doctorands (Nezka Petric, Enowmpey Enowtambong, Pornchai Sanyathitiseree, Marco Marc and Shuly Cohen) at the Department of Functional Genomics and Bioregulation, Institute of Animal Science, for interesting, unforgettable discussions and being fun to be with.

Irina Lebedeva and Vladimir Lebedeva cared about my health and lent me their medical instrument. I am grateful for it.

I wish to extend special thanks to the two other people in the Institute for Animal Science, Elisabeth Falge and Gisela Schleibaum. I received a lot of help from them. Their understanding and support are gratefully acknowledged.

I thank my parents for giving me life in the first place, for unconditional love, support and encouragement to pursue my interests. This dissertation is dedicated to them.

Finally, I wish to express my profound gratitude to other members of my family and friends for their support and help during this period.

CURRICULUM VITAE

Name:	Dingjian LI
Date of Birth:	21st November, 1967
Nationality:	Chinese
Marital Status:	Single

EDUCATION

1974-1979	Huaxi Primary School, Guiyang
1979-1985	Huaxi Secondary School, Guiyang
Sept. 1985-July 1989	Bachelor of Science in Agriculture, Guizhou Agricultural College
Sept. 1989-June 1992	Master of Science in Agriculture, Nanjing Agricultural University
Sept. 1996-Jan. 1997	Graduate Student, Chongqing Foreign Languages University
April 2002-July 2007	Ph.D. Student, Institute of Animal Physiology and Animal Nutrition, Georg-August-University Göttingen, Germany

PROFESSIONAL CAREER

July 1992-Aug. 1997	Lecturer, Department of Animal Husbandry and Veterinary Medicine, Guizhou Agricultural College
Aug. 1997-Aug. 1998	Lecturer , Department of Animal Husbandry and Veterinary Medicine, Guizhou University
Since August 1998	Visiting Scientist, Institute of Animal Science Mariensee, Federal Agricultural Research Center (FAL), Germany