Human-Human and Human-Animal Interaction

Some Common Physiological and Psychological Effects

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Human-human and human-animal interaction. Some common physiological and psychological effects

The aim of the present thesis was to investigate hormonal and physiological effects in mothers during a breastfeeding session and in dogs and their owners in response to short-term interaction.

In study one, sixty-six mothers receiving either exogenous oxytocin infusion and/or epidural analgesia (EDA) during labor or intramuscular oxytocin injection post partum were studied. Oxytocin, prolactin, adrenocorticotrophic hormone (ACTH) and cortisol levels, as well as blood pressure were measured during a breastfeeding session two days after birth.

In response to breastfeeding two days after birth, the mothers displayed a pulsatile release of oxytocin and increasing prolactin levels. In addition, the activity in the HPA-axis was reduced and maternal blood pressure decreased. The results also show that EDA administration in combination with oxytocin during labor resulted in significantly lower oxytocin levels and higher cortisol levels, as well as higher blood pressure in response to breastfeeding two days after birth, compared to EDA administration alone. In addition, oxytocin infusions dose-dependently lowered the mothers' endogenous oxytocin levels two days after birth.

In study two, ten female dog owners and their male Labrador dogs participated, together with ten controls. Their levels of oxytocin, cortisol and insulin, as well as their heart rate, were measured. The connection between the quality of the dog-owner relationship and hormone levels was also explored.

Short-term interaction between dogs and their owners resulted in oxytocin release in both species and their cortisol levels and heart rate were also affected. Oxytocin levels and positive attitudes regarding the dog-owner relationship were positively correlated.

In conclusion, both human-human and human-animal interactions induce oxytocin release and promote oxytocin mediated effects, such as decreasing cortisol levels and blood pressure. In addition, social interaction and oxytocin levels are positively related.

Keywords: oxytocin, cortisol, prolactin, ACTH, blood pressure, medical interventions, sensory stimulation, interaction

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Dedication

To my family

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Jonas, W., Johansson, L M., Nissen,E., Ejdebäck, M., Ransjö-Arvidson, AB., Uvnäs-Moberg, K., (2009). Effects of intrapartum oxytocin administration and epidural analgesia on the concentration of plasma oxytocin and prolactin, in response to suckling during the second day post partum. *Breastfeeding Medicine* 4(2), 71-82.

II Handlin, L., Jonas, W., Petersson, M., Ejdebäck, M., Ransjö-Arvidson, AB., Nissen, E., Uvnäs-Moberg, K. (2009). Effects of Sucking and Skin-to-Skin Contact on Maternal ACTH and Cortisol Levels During the Second Day Postpartum—Influence of Epidural Analgesia and Oxytocin in the Perinatal Period. *Breastfeeding medicine* 4(4), 207-220.

III Handlin, L., Jonas, W., Ransjö-Arvidson, AB., Petersson, M., Uvnäs-Moberg, K., Nissen, E. (2010). Influence of common birth interventions on maternal blood pressure patterns during breastfeeding two days after birth. *Submitted*

IV Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdebäck, M., Jansson, A., Uvnäs-Moberg, K. (2010). Short-term interaction between dogs and their owners – effects on oxytocin, cortisol, insulin and heart rate - an exploratory study. *In press. Due to be published in Anthrozoos in 2011*

V Handlin, L., Hydbring-Sandberg, E., Nilsson, A., Ejdebäck, M., Uvnäs-Moberg, K. (2010). Associations between the psychological characteristics of the human-dog relationship and oxytocin and cortisol levels. *Submitted*

Papers I-III are reproduced with the permission of the publishers.

The contribution of Linda Handlin to the papers included in this thesis was as follows:

I. Mainly responsible for chemical analysis of blood samples. Performed statistical analysis of data jointly with Wibke Jonas and wrote the manuscript together with the supervisors.

II. Mainly responsible for chemical analysis of blood samples and performed statistical analysis of data. Wrote the manuscript together with supervisors.

III. Performed statistical analysis of data. Wrote the manuscript jointly with the supervisors.

IV. Mainly responsible for planning and executing the experiment, as well as for chemical analysis of blood samples. Performed part of the statistical analysis (t-test), and wrote the manuscript together with supervisors.

V. Mainly responsible for planning and executing the experiment, as well as for chemical analysis of blood samples. Performed the statistical analysis of data and wrote the manuscript together with the supervisors.

Abbreviations

ACTH	Adrenocorticotrophic hormone
CNS	Central nervous system
CRF	Corticotrophin-releasing-factor
CT	C-tactile afferent
DBP	Diastolic blood pressure
DMX	Dorsal vagal motor nucleus
EDA	Epidural analgesia
EDA^{nonOT}	Epidural analgesia *without* oxytocin infusion
EDA^{OT}	Epidural analgesia *with* oxytocin infusion
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbentassay
HPA-axis	Hypothalamic-pituitary-adrenocortical axis
ICV	Intracerebroventricular
im	Intramuscular
iv	Intravenous
LC	Locus coerulus
MDORS	Monash dog owner relationship scale
NA	Nucleus accumbens
NP	Neurophysin
NTS	Nucleus tractus solitarius
OTim	Oxytocin administered intramuscular
OTiv	Oxytocin administered intravenously
OTR	Oxytocin receptor
PAG	Periaqueductal gray
PVN	Paraventricular nucleus
RIA	Radio-immuno-assay
RN	Raphe nuclei
SBP	Systolic blood pressure

SON	Supraoptical nucleus
SPSS	Statistical Package for the Social Sciences

1 Introduction

1.1 Oxytocin

1.1.1 Synthesis and localization

Oxytocin is a neuropeptide that acts both as a hormone and as a neurotransmitter. It consists of nine amino acids with a very well conserved chemical structure, as is evident by oxytocin being identical in all mammalian species.

Oxytocin is synthesized in the cell bodies of the magnocellular and parvocellular neurons of the paraventricular nuclei (PVN) and of the magnocellular neurons of the supraoptical nuclei (SON) of the hypothalamus.

From the magnocellular neurons in the SON and PVN, oxytocin is transported via axons to the neurohypophysis, from where it is released into the circulation to exert its hormonal effects. Once in the circulation, oxytocin has a half-life of one to two minutes (Ludwig and Leng, 2006, Richard, 1991).

Oxytocinergic nerve fibers originating from the parvocellular neurons in the PVN reach a number of different areas in the central nervous system (CNS) (summarized in table 1), where oxytocin can exert neurogenic effects.

In addition, during extremely strong stimulation, e.g., suckling, oxytocin can also be released directly from the cell bodies and from the dendritic parts of the neurons within the PVN and SON. This direct release allows oxytocin to diffuse into areas that are devoid of oxytocin nerves but richly provided with oxytocin receptors (Ludwig and Leng, 2006).

In the CNS, oxytocin has a half-life of about 20 minutes and it is often degraded into smaller active fragments before binding to receptors to induce

effects (Burbach et al., 2006, Ludwig and Leng, 2006, Richard, 1991, Stancampiano et al., 1991)

Table 1. *Areas of relevance for the present studies in the CNS to which oxytocin neurons reach (Buijs et al., 1985)*

Area in CNS	**Function**
Amygdala	Belongs to the limbic system and is linked to the control of emotions and social interaction
Dorsal vagal motor nucleus (DMX)	The main motor nucleus of the vagal nerve. Innervates the gastrointestinal tract, lungs and cardiovascular systems etc. Cholinergic neurons.
Hippocampus	Belongs to the limbic system and is important for memory, learning, spatial orientation and also for processing information from sensory organs. Participates in the regulation of the HPA-axis.
Locus coerulus (LC)	Located in the brainstem and is related to activity and arousal. Noradrenergic neurons
Nucleus accumbens (NA)	Is located in the forebrain and plays a central role in the reward and reinforcement circuit. Dopaminergic neurons
Nucleus tractus solitarius (NTS)	Is located in the brainstem and is the major sensory nucleus of the vagal nerve. Regulates sympathetic and parasympathetic autonomic functions. Cholinergic neurons.
Periaqueductal gray (PAG)	Located within the midbrain and is important in pain modulation.

In addition to the brain, oxytocin has also been found in, for example, the uterus, ovaries, testis, placenta, thymus, adrenal gland and pancreas, as well as in the heart and blood vessels (Burbach et al., 2006).

Oxytocin is structurally related to the neuropeptide vasopressin. Vasopressin is also synthesized within the SON and PVN of the hypothalamus, but is not located in the same neurons as oxytocin (Vandesande and Dierickx, 1975).

Oxytocin and vasopressin differs by only two amino acids. At positions 3 and 8, oxytocin has isoleucine and leucine, whereas vasopressin has phenylalanine and arginine (lysine in pigs) (Figure 1). These differences are most likely the reason why oxytocin and vasopressin often display opposing effects (Burbach et al., 2006).

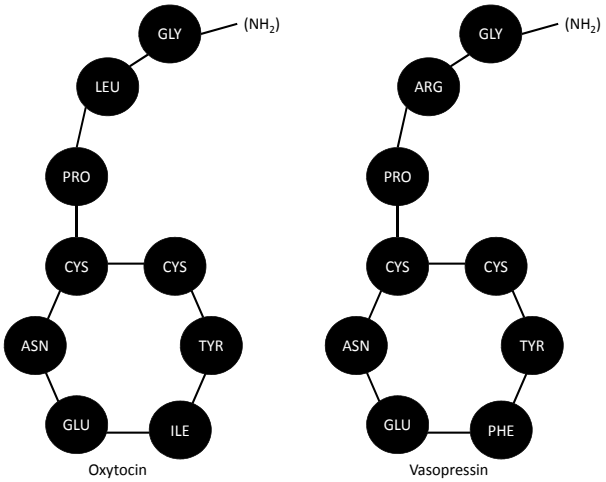

Figure 1. Amino acid sequences of oxytocin and vasopressin

1.1.2 The oxytocin gene

The human oxytocin gene is located on chromosome 20p13 and is located on the same chromosomal locus as the vasopressin gene in a head-to-head orientation. The structural organization of the genes, as well as the post-translational modifications of the two, are very similar (Burbach et al., 2006).

The gene consists of three exons which together is less than 1 kb. The first exon codes for oxytocin itself and a spacer sequence. It also codes for the first nine amino-terminal amino acids of the oxytocin-associated neurophysin (NP). The second and third exons together encode the remaining parts of NP.

In total the gene codes for a 106 amino acid precursor composed of the oxytocin hormone (nine amino acids) and NP (94 amino acids). Oxytocin and NP are connected by a three amino acid long spacer sequence (–Gly-Lys-Arg-). The precursor molecule is split into the final oxytocin molecule and the NP molecule. This process starts in the Golgi apparatus and is finished in the vesicles (Burbach et al., 2006). However, it has been suggested that an oxytocin precursor intermediate circulates in human and monkey plasma in states of estrogen dominance (Amico and Hempel, 1990).

1.1.3 The oxytocin receptor

The gene for the human oxytocin receptor (OTR) is located on chromosomal position 3p25 and codes for a receptor consisting of 389 amino acids. The receptor is a seven transmembrane domain G protein coupled receptor that belongs to the same structural sub-family as the Vasopressin receptors V1a, V1b and V2.

The oxytocin-binding site of the receptor comprises the extracellular amino-terminal fragment E1 together with the extracellular loops E2 and E3 of the receptor. It seems as if the amino acid residue R34 (arginine) within the E1 appears to be essential for high-affinity oxytocin binding.

Once oxytocin has bound to the OTR it can activate signaling pathways such as the classical G-protein pathway. The coupled G-protein activates phospholipase, resulting in accumulation of inositol triphosphate (IP_3) and diacylglycerol. IP_3 then triggers the release of Ca^{2+}, finally resulting in contractions of the smooth muscle tissue and/or increased neurotransmitter release in neurons (Burbach et al., 2006, Gimpl and Fahrenholz, 2001, Gimpl et al., 2008).

The OTR has been detected in various parts of the CNS including the olfactory system, the limbic system, hypothalamus, the thalamus, the basal ganglia, some cortical regions, the brain stem and the spinal cord.

The OTR is also expressed in many tissues throughout the body including blood vessels, kidney, ovary, testis, thymus, heart, pancreas and in adipose tissue (Gimpl and Fahrenholz, 2001).

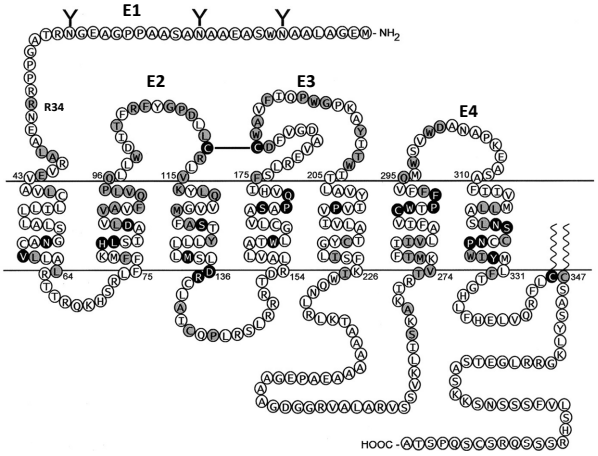

Figure 2. Schematic structure of the human OTR with amino acid residues shown in one-letter code. The Am Physiol Soc, used with permission. Gimpl, G. et al. Physiol. Rev. 81: 629-683 2001.

1.1.4 Release

Oxytocin is released in response to sensory stimulation during, for example, parturition, suckling, feeding, gastric distension, and mating in both males and females (Burbach et al., 2006). In addition, physical warmth, massage-like stroking, and olfactory cues can also induce the release of oxytocin (Agren, 1995, Lund, 2002, Stock, 1988, Uvnas-Moberg, 1998b, Uvnas-Moberg et al., 1993).

Parallel secretion of oxytocin into both the brain and the circulation has been shown during parturition, suckling, feeding, vaginocervical stimulation, and osmotic stimulation (Hattori et al., 1990, Kendrick et al., 1986, Kendrick et al., 1988, Keverne and Kendrick, 1994, Leng et al., 2008).

During intense stimulation of oxytocin release, such as during labor and breastfeeding, the oxytocin system in the PVN and SON undergo morphological and functional changes. The glial covering of the oxytocinergic neurons retracts and the somatic/dendritic parts of the neurons become closer to each other, allowing interaction between these cells. The electrical action potential activity is thereby increased and the firing activity of the magnocellular neurons becomes synchronized. Consequently, the neurons start to burst in synchrony, causing a pulsatile release of oxytocin into the circulation (Hatton and Tweedle, 1982, Theodosis et al., 1986, Theodosis, 2002).

Several substances can influence the release of oxytocin; e.g., estrogens increase oxytocin release and binding to OTR (Schumacher et al., 1993, Yamaguchi et al., 1979). Noradrenalin stimulates oxytocin release through α-adrenreceptors and inhibits the release through β-adrenoreceptors (Tribollet et al., 1978). Dopamine also seems to exert both stimulatory and inhibitory actions on oxytocin (Crowley et al., 1991, Uvnas-Moberg et al., 1995).

From the magnocellular neurons in the PVN and SON oxytocin mediates a positive feedback on its own release (Freund-Mercier and Richard, 1984, Moos et al., 1984).

1.2 Effects of oxytocin

Oxytocin has been shown to exert a multitude of physiological and behavioral effects that depend on the dose and timing schedule as well as the experimental model used. The following is a summary of oxytocin effects of relevance to the present study.

1.2.1 Uterine contractions

The first effect assigned to oxytocin was the induction of uterine contractions (Dale, 1909).

During labor, as the infant's head is pressing against its mother's cervix, nerve fibers in the pelvic and hypogastric nerves become activated (Ferguson reflex), resulting in an increased release of oxytocin into the circulation and brain. Oxytocin is released in a pulsatile fashion at different amplitudes and intervals, with an increasing frequency as the labor progresses (Burbach et al., 2006).

1.2.2 Milk ejection

Oxytocin is released into the circulation each time a mother breastfeeds her child. When the child suckles, the breast sensory fibers in the nipple are activated, leading to release of oxytocin. As a consequence of the circulating oxytocin levels, the blood vessels in the mother's chest become dilated (Burbach et al., 2006).

During suckling, oxytocin is also released into the brain to induce behavioral and physiological adaptations in the mother (Jonas, 2008a, Leng et al., 2008).

1.2.3 Anti-stress responses

In order to facilitate the presentation of the oxytocin-mediated anti-stress effects, a short summary of the stress response is presented, as follows.

When a stressor (either physical or psychological) is presented, two systems dominate in the resulting stress-response.

A first acute response travels through the sympathetic nervous system, where nerves originating in the spinal cord signal for the release of the catecholamines adrenalin and noradrenalin from the adrenal medulla. This response is rapid and the catecholamines are released within seconds.

The second response is activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. When signals of a stressor reach the PVN, corticotrophin-releasing factor (CRF) and vasopressin stimulate adrenocorticotrophic hormone (ACTH) at the pituitary level, which in turn stimulates cortisol at the adrenal level. Cortisol then exerts negative feedback on the axis by acting on the corticotrophs, the PVN, and higher levels in the CNS (Mormede et al., 2007). The increased secretion of CRF occurs within a few seconds, ACTH levels are increased within 15 seconds, and the resulting increase in cortisol levels can be detected within 5-10 minutes after the stressor has been presented (Sapolosky, 2002).

The catecholamines and glucocorticoids released in response to a stressor work together to generate the stress response. This response results in mobilization of energy and its delivery to the parts of the body that need it, and also puts non-essential physiological processes on hold and to blunts inflammation and pain (Sapolosky, 2002).

Oxytocin exerts effects on several sites of the HPA-axis to promote lowering of cortisol levels. In the parvocellular neurons of the PVN, oxytocin decreases CRF secretion; in the adenohypophysis, it decreases the secretion of ACTH from the ACTH producing cells; and in the adrenal glands, oxytocin decreases the secretion of cortisol/corticosterone (Burbach et al., 2006, Neumann et al., 2000, Petersson et al., 2005b, Stachowiak et al., 1995).

Rats receiving oxytocin have lower levels of corticosterone and changes in hippocampal glucocorticoid receptors (Petersson, 1999a, Windle et al., 1997, Petersson et al., 2005b, Petersson et al., 2005a). Oxytocin knock-out mice display an increased release of corticosterone in response to stress (Amico et al., 2008). These results indicate a role for oxytocin in the inhibition of the activity of the HPA axis.

1.2.4 Blood pressure

Oxytocin has a dual effect on blood pressure. Depending on the physiological circumstances and route of administration, oxytocin may either increase or decrease blood pressure (Petersson et al., 1999). After repeated administration of oxytocin, the decrease in blood pressure becomes sustained (Petersson et al., 1999).

Nerves originating in the PVN that contain oxytocin reach the NTS, where oxytocin may exert decreasing effects on the sympathetic nervous tone resulting in lowering of blood pressure. Oxytocin has been shown to

increase the activity of the α2-adrenoreceptors in the NTS, which subsequently attenuates the signaling function of noradrenalin and adrenalin in the brain (Petersson et al., 2005a, Petersson et al., 1998).

1.2.5 Anxiolytic-like and calming effects

Rats and mice receiving low doses of oxytocin become less anxious due to effects exerted in the amygdala (Neumann, 2008). These animals display an increased curiosity towards the environment and higher social interaction with their cage mates (Neumann, 2008, Uvnas-Moberg, 1994b). In contrast, knock-out mice lacking the OTR are more anxious and more sensitive to stress than are normal mice (Amico, 2004).

Higher doses of oxytocin cause the animals to display calming or sedative effects, as indicated by decreased locomotor behavior. These effects probably involve decreased activity in the LC, via stimulation of α2-adrenoceptors, which leads to reductions in stress reactivity, wakefulness, and aggression (Petersson et al., 1996, Petersson et al., 1998, Uvnas-Moberg, 1994b).

1.2.6 Pain threshold, wound healing, and inflammation

Oxytocin increases nociceptive thresholds, as seen by a longer reaction time in the tail-flick test in rats treated with oxytocin. This effect is probably exerted in the PAG and the spinal cord in response to the activation of endogenous opioidergic mechanisms (Petersson et al., 1996).

Higher oxytocin levels also have been linked to faster wound healing (Gouin et al., 2010). In addition, oxytocin is associated with anti-inflammatory effects (Clodi et al., 2008, Szeto et al., 2008, Petersson et al., 2001).

1.2.7 Storing and transfer of nutrients

Oxytocin contributes to an enhanced digestive function and improved use of nutrients for storage, growth, and restoration. Basal levels of insulin, gastrin, and cholecystokinin can be increased or decreased by oxytocin, whereas the feeding-induced release is increased (Holst, 2005, Petersson, 1999b, Uvnas-Moberg, 1994a).

Oxytocin also has insulin-like effects, as displayed by stimulation of glucose uptake in adipocytes and promotion of glucose oxidation and lipogenesis in adipose tissue (Braun et al., 1969).

1.2.8 Oxytocin and other signaling systems

Oxytocin can also influence other signaling systems, such as the dopaminergic, noradrenergic, opiodergic, cholinergic, and serotonergic signaling systems. For example, oxytocin activates the dopamine reward pathways and expression of penile erection (Baskerville and Douglas, 2010). Oxytocin increases the density of $_2$-adrenoceptor agonist binding sites in the hypothalamus, the amygdala, and the NTS (Petersson et al., 2005a). In addition, a functional relationship exists between oxytocin and endogenous opioids, where oxytocin most often is under an inhibitory tone of the opioids (Russell et al., 1989). However, in certain situations, endogenous opioids may stimulate oxytocin release (Keverne and Kendrick, 1991).

1.2.9 Maternal behavior and adaptations

Oxytocin plays a key role in the onset of maternal behavior. When administered ICV, oxytocin induces maternal behavior in virgin rats after priming with oestrogen (Pedersen et al., 1982, Pedersen and Prange, 1979).

Release of endogenous oxytocin in sheep, either in connection with labor or by cervical stimulation, increases maternal care and increases the interaction with the lamb and the bonding to the same. The same effects have also been demonstrated after administration of exogenous oxytocin to sheep.

In contrast, if oxytocin release has been blocked during labor by peridural analgesia or if an oxytocin antagonist is administered, the maternal behaviors will not appear (Kendrick et al., 1987, Kendrick et al., 1986, Keverne and Kendrick, 1994).

Oxytocin is also released into the brain during labor and, as a consequence, the maternal sensation of pain is decreased and the memory of the pain is also made less intense and unpleasant. Oxytocin released into the brain during labor also prepares the mother for motherhood (Burbach et al., 2006).

During breastfeeding, when mother and infant are in close skin-to-skin contact, maternal oxytocin is released in response to the child's massage of the mothers' breast and to the suckling stimulus (Matthiesen, 2001).

During breastfeeding, mothers also experience pleasure and a sense of wellbeing and, in addition, their levels of anxiety are reduced and their social skills are increased (Jonas, 2008a, Nissen, 1998).

Anti-stress patterns are also induced during breastfeeding, as displayed by a fall in cortisol levels and blood pressure (Heinrichs et al., 2001, Jonas, 2008b). The mother's sensitivity to pain is reduced and the levels of some gastrointestinal hormones, such as insulin, CCK, and gastrin, are increased. These changes occur in order to ensure that the mother uses her energy in the most efficient way (Uvnas-Moberg, 1996a).

1.2.10 Social behaviors

Animal experiments have shown that oxytocin is important for a number of social behaviors. For example, it induces grooming and sexual behavior (Arletti et al., 1985, Caldwell et al., 1986), increases social contacts, and decreases aggression (Witt et al., 1992). Oxytocin has also been shown to facilitate bonding between mothers and young as well as pair bonding (Carter, 1998, Insel, 2003, Keverne and Kendrick, 1992).

Human social behaviors are also influenced by oxytocin. People receiving oxytocin in the form of nasal spray become less anxious and they show an attenuated cortisol response in a mental test. They also show a dampened activity in the amygdala (Heinrichs, 2003, Kirsch et al., 2005).

People receiving nasal oxytocin also show increased social skills, such as the ability to read and evaluate the emotional valence of faces or voices (Domes et al., 2007, Hollander et al., 2007). The duration and frequency of gaze directed towards the eye region is increased (Guastella et al., 2008a) and the encoding of positive social information is enhanced (Guastella et al., 2008b, Rimmele et al., 2009). In addition, oxytocin has been shown to increase trust and generosity in men (Kosfeld, 2005) and to reduce abdominal pain and depression in women (Ohlsson et al., 2005).

1.2.11 Long term effects

When oxytocin is administered repeatedly, long- term effects may be induced. Rats receiving repeated injections of oxytocin display reduced blood pressure, increased nociceptive thresholds, and decreased corticosterone levels. These effects can be sustained for up to several weeks after the last injection. In addition, the levels of gastrointestinal hormones are influenced, weight gain is increased, and the time for wound healing is shortened (Uvnas-Moberg, 1998a).

Repeated administration of oxytocin to rats also increases the α2-adrenoreceptor function in several brain regions, such as the amygdala, the NTS, and the LC (Petersson et al., 2005a).

Rat pups receiving repeated oxytocin administration during their first days of life display significantly lower blood pressure in adulthood (Holst, 2002), as well as higher body weight, relatively more adipose tissue in the thigh and interscapular region, and increased nociceptive thresholds (Uvnas-Moberg et al., 1998).

1.3 Sensory stimulation

1.3.1 Sensory fibers

The human skin is innervated with many types of somatosensory afferent fibers; e.g., the myelinated Aβ fibers mediating light touch sensation and the Aδ and C fibers acting as nociceptors, itch, and temperature fibers.

Human hairy skin is also innervated by a class of slowly conducting unmyelinated afferents, the C-tactile (CT) fibers. These fibers respond to innocuous touch and are especially sensitive to gentle, slowly moving touch of the skin and appear to signal the pleasant aspects of touch (Loken et al., 2009, Vallbo et al., 1999). CT fibers are also present in animal hairy skin.

1.3.2 Effects after non-noxious sensory stimulation

Both animals and humans respond to pleasant non-noxious sensory stimulation, which induces a multitude of effects.

Gentle stroking on the backs of anaesthetized rats or afferent electrical stimulation of the sciatic or vagal nerve of anaesthetized rats induce a more than two-fold increase in the rats' plasma oxytocin levels (Stock, 1988). In addition, electroacupuncture, thermal stimulation, or vibration increase the rats' levels of oxytocin in both plasma and cerebral spinal fluid (Uvnas-Moberg et al., 1993).

Brushing or stroking of anaesthetized rats also induces lowering of circulating adrenalin and corticosterone levels and, in addition, blood pressure is reduced (Araki, 1984, Kurosawa, 1995, Kurosawa, 1982, Tsuchiya et al., 1991). Furthermore, efferent vagal nerve activity also increases in response to low-intensity electrical stimulation and stroking (Uvnas-Moberg et al., 1992).

Unanaesthetized rats also respond to the stroking stimuli. Stroking of unanaesthetized rats' abdomens for five minutes induces oxytocin release (Stock, 1988). It also increases the pain threshold and reduces blood pressure (Holst, 2002, Uvnas-Moberg et al., 1993), as well as decreases plasma levels of insulin, gastrin, and somatostatin and lowering energy expenditure (Holst, 2005). In addition, sedative effects are induced, as displayed by reduced spontaneous motor activity (Uvnas-Moberg, 1996b).

Gentle stroking has also been shown to induce long-term antinociceptive and to have blood pressure decreasing effects (Holst, 2002, Lund, 2002)

1.3.3 Sensory stimulation during mother-offspring interaction

Interaction between rat mothers and their pups has been shown to induce calmness in both mother and pups (Holst, 2002).

From a long-term perspective, pups exposed to daily stroking of the ventral side of the abdomen for one week postnatally displayed reduced blood pressure and corticosterone levels in adulthood (Holst, 2002).

Pups exposed to highly interactive mothers during the first week of life become less anxious, more social, and more tolerant to stress compared to pups of mothers who interact less. They also have an increased functioning of the oxytocin receptors in the amygdala, indicating a lifelong effect of stroking (Champagne and Meaney, 2007).

In humans, when a baby lies in skin-to-skin contact on its mother's chest before initiation of suckling, it massages the mother's breast, causing a dose-dependent release of maternal oxytocin. This release does not display the pulsatile pattern that is seen during suckling (Matthiesen, 2001).

1.4 Human-animal interaction (HAI)

1.4.1 History

Over 14 000 years ago, humans started to domesticate wolves, since they were valued for their intelligence, keen senses and loyalty. These early dogs served as guardians, guides, and partners in hunting and fishing. Throughout history, pets, especially dogs and cats, have lived in close contact with humans and have now become central to family life, providing

companionship and pleasure, and are often considered as family members (Walsh, 2009a, Walsh, 2009b). In 2007, 16.8 % of Swedish households had at least one cat and 12.8% had at least one dog, and the numbers are increasing (Manimalis, 2009).

1.4.2 Physiological effects of HAI

During the last decades, research has emerged that shows health benefits associated with interactions with companion animals.

Pet ownership has been shown to lower blood pressure, serum triglycerides, and cholesterol levels (Allen, 2002). Patients suffering from heart attack had significantly higher 1-year survival rates if they had a pet, compared to those without pets, and dog owners were 8.6 times more likely to be alive after 1 year (Friedmann et al., 1980, Friedmann, 1995).

Pets also have a positive impact on the ability to cope with chronic conditions and on the course and treatment of illness such as heart disease, dementia, and cancer (Friedmann and Tsai, 2006, Johnson et al., 2003). Recovery of hospitalized children has been facilitated interaction with companion animals (Walsh, 2009a) and these animals also ease suffering and anxiety at the end of life for those in palliative and hospice care (Geisler, 2004). In addition, children having a dog present in their classroom display increased social competence (Hergovich, 2002, Kotrschal, 2003).

HAI appears to be mutually beneficial, since stroking a dog has been shown to significantly reduce blood pressure in both the person and the animal (Odendaal, 2003).

Interaction between dogs and their owners have been shown to induce oxytocin release in both the dogs and the owners (Miller, 2009, Odendaal, 2003).

2 Aim

The overall aim of the present thesis was to investigate hormonal and physiological effects in mothers during a breastfeeding session and in dogs and their owners in response to short-term interaction.

The specific aims were to investigate

A) In response to breastfeeding:

- Effects on maternal release of oxytocin and prolactin
- Effects on the activity in the maternal HPA-axis
- Possible relationships between maternal hormone levels
- Effect on maternal blood pressure pattern
- If medical interventions, such as EDA and oxytocin infusions, given during labor effect maternal release of oxytocin, prolactin, as well as activity in the HPA-axis and blood pressure.

B) In response to short-term interaction between

- Effects on release patterns of oxytocin, cortisol and insulin in both owners and dogs
- Effects on heart rate in both owners and dogs
- Possible relationships between owners' and their dogs' hormone levels
- Possible relationship between the quality of the dog-owner relationship and hormone levels in owners and/or dogs

3 Material and Methods

The following is a summary of the material and methods used. For a more detailed description, see papers I-V.

3.1 Papers I-III

3.1.1 Participants and setting

The study was conducted at one of the six maternity clinics in Stockholm, Sweden, during January 2002 to December 2003.

Healthy primiparae admitted to the maternity ward on weekdays (Monday to Friday) and fulfilling the inclusion criteria were consecutively informed 10-24 hours after delivery about the study by the two midwives conducting the study.

In total, 86 mothers fulfilled the inclusion criteria. Sixty-three (63) mothers gave their informed consent to both blood sampling and blood pressure measurements and were included in the study. An additional three (3) mothers gave their informed consent to blood pressure measurements only. Twenty (20) mothers declined participation. Two (2) mothers who gave their informed consent had to be excluded for technical reasons during blood sampling/analysis.

Since it is not possible to randomize patients to the different medical interventions studied, we chose a descriptive strategy not to disturb the natural flow of the medical interventions explored.

Labor had started spontaneously in all mothers, but if inertia was diagnosed during labor, oxytocin was administered intravenously (iv) (OTiv group).

If required, epidural analgesia containing the pharmaceuticals bupivacaine and sufentanil (a synthetic opioid that is 5 to 10 times more potent than its

analogue fentanyl) was administered for pain relief. Some mothers received EDA alone (EDA^{nonOT} group), whereas others received EDA combined with exogenous oxytocin infusion (EDA^{OT} group).

According to Swedish practice guidelines, all women receive 8.3 μg (10 IU) oxytocin (Syntocinon®; Novartis AB, Täby, Sweden) intramuscularly (im) after birth to prevent bleeding. During the study period, midwives were instructed not to administer oxytocin im to the women fulfilling the inclusion criteria. However, some midwives did not comply with this instruction and therefore some mothers *did* receive oxytocin im postpartum. These mothers were included as a separate group in the study (OTim group).

Partners stayed together with the mothers and newborns during the subsequent days following birth. The setting was created to be as home like as possible, with no daily hospital routines (unless necessary) taking place and breastfeeding was strongly promoted.

3.1.2 Preparations

All data were collected by the same two research midwives during the morning on the second day post partum, when the newborns were between 24 and 48 hours old.

Each mother was instructed to call for the researchers when the neonate showed rooting and suckling behaviors. A blood pressure monitor (Omron R5-1 Wrist blood pressure monitor, Omron Healthcare, the Netherlands) was then attached to the mother's right wrist for the purpose of blood pressure recordings. An intravenous cannula was inserted in the cubital vein of the mother for the purpose of blood sampling. The midwife then placed the baby in skin-to-skin contact between the mother's breast and the baby was allowed to initiate suckling itself. The infant's legs and trunk was covered with a light blanket in order to keep it warm.

Each mother was asked to stay in skin-to-skin position with her baby for 60 minutes irrespective of duration of suckling and all mothers complied with this instruction.

3.1.3 Blood sampling

In order to catch the pulsatile oxytocin release, blood samples were collected at 30-second intervals during the first 7.5 minutes of suckling, with the first sample taken immediately after the baby started to suck the breast.

The following samples were then taken at 10, 20, 30 and 60 minutes after the baby had started to suck the breast.

A blood sample (5 ml) took approximately 10-15 seconds to collect. All blood samples were collected into ice-chilled tubes containing Trasylol® (Bayer AG Leverkusen, Germany) and Heparin® (LEO Pharma A/S Ballerup, Copenhagen, Denmark). After the experiment, the samples were centrifuged, the plasma was removed, and the samples were stored at –20°C until analysis.

3.1.4 Blood pressure recordings

The basal maternal systolic (SBP) and diastolic (DBP) blood pressure was considered as the measurement taken 5 minutes before the infant was placed skin-to-skin with their mother; subsequent measurements occurred during skin-to-skin contact at 10, 30, and 60 minutes after breastfeeding had been initiated.

3.1.5 Ethical considerations

The ethical committee at the Karolinska Institutet, Stockholm, Sweden, approved the study.

3.1.6 Hormone analysis

Hormone levels in the plasma were determined using commercially available ELISA/EIA-kits following the instructions of the manufacturers. The following kits were used; Correlate-EIA ™ Oxytocin Enzyme Immunoassay kit (Assay designs, Inc. Ann Arbor, USA), Prolactin Enzyme-Linked Immunosorbent Assay kit (Prolactin Enzyme Immunoassay kit, Diagnostic Systems Laboratories, Inc. Texas, USA), ACTH EIA kit from Alpco Diagnostics (Alpco Diagnostics, Salem, USA) and DSL-10-2000 ACTIVE® Cortisol Enzyme Immunoassay kit (Diagnostic Systems Laboratories, Inc. Texas, USA).

3.1.7 Statistical analysis

Data were analyzed using the software Statistical Package for the Social Sciences (SPSS - version 14.0 to 18 (PASW), Chicago, IL, USA, 2010).

Since the study groups were relatively small and a normal distribution could not be taken for granted, all statistical analyses were performed using

non-parametric statistics; i.e., median and interquartile distances ($Q_{25} - Q_{75}$) were used to describe demographic data of mothers and newborns and also for describing hormonal data for the mothers. However, mean values were used for describing maternal blood pressure. The Kruskal-Wallis test for independent samples or the Mann-Whitney U-test for independent samples were used to test differences between the groups and the Wilcoxon signed-rank test was used to test for differences within groups over time. In addition, the Spearman rank coefficient was used for calculating correlations.

3.2 Papers IV–V

3.2.1 Participants and setting

The study was conducted at the Swedish University of Agricultural Sciences in Skara, Sweden.

Included in the study were ten women older than 30 years and their male Labrador dogs who were older than 1 year. Ten female volunteers, within the same age span as the dog-owners, who did not own a dog, served as controls.

The room where the study was conducted was an ordinary room provided with a desk, four chairs, a bookcase, and a water bowl for the dog.

The goal was to perform all experiments, both for the owners and the controls, during the evening, but due to the participants' work schedules, some of the experiments were performed during the morning (4 owners and 5 controls).

3.2.2 Preparations

When the participants arrived at the testing facility, they were equipped with a heart rate monitor (s610i ™, Polar precision performance, Polar Electro) for the purpose of heart rate recordings. In addition, an indwelling catheter was inserted into the cubital vein of the dog owners and the controls and an intravenous catheter was inserted into the cephalic vein of the dogs, for the purpose of blood sampling. Insertion of catheters and sampling of blood in the dogs and humans were performed by the same experienced animal caretaker and nurse, respectively.

Before the experiment started, the owner sat in a chair with the dog loose, sitting or lying beside her. The owner approached her dog at time point zero and started to pet and stroke different parts of the dog's body and talked to the dog for 3 minutes. The owner then remained seated in her chair and did not touch the dog for the rest of the experiment, which lasted for a total of 60 minutes.

The conditions for the control group were the same as for the owners with the exception that there was no dog present during the control experiments.

Due to technical problems, heart rate was only measured in 5 controls.

3.2.3 Blood sampling, Heart rate and video

All blood samples were taken simultaneously from the dog and the owner. The first sample was collected 30 minutes after insertion of the catheters and immediately before the owner started to interact with her dog. Subsequent samples were collected at 1, 3, 5, 15, 30 and 60 minutes after the start of the interaction.

All blood samples (4 ml) were collected into EDTA tubes containing Trasylol® (Bayer AB). The samples were immediately put on ice, centrifuged, and then the plasma was collected and stored at -20°C until analysis.

The heart rate monitors registered the heart rate every 15th second, but only the recordings obtained at each 5th minutes were used in the statistical analysis.

The entire interaction experiment (60 minutes) was videotaped and the videotapes were analyzed in order to control for the dogs' behavior and experience of the situation.

3.2.4 Monash dog owner relationship scale

The dog owners evaluated their relationship with their dog by completing the Monash dog owner relationship scale (MDORS) (translated into Swedish from (Dwyer et al 2006)) during the last 30 minutes of the experiment.

The MDORS contains 28 items concerning both the positive and negative aspects of the relationship with the companion dog. The items are

divided into the three sub-scales Dog-owner interaction, Emotional closeness and Perceived costs.

3.2.5 Ethical considerations

The experimental procedure for the humans was approved by the Local Ethics Committee in Uppsala, and the procedure for the dogs was approved by the Animal Ethics Committee in Uppsala. The use of privately owned dogs was approved by the National Board of Agriculture. For ethical and practical reasons, it was not possible to perform control experiments on the privately owned dogs.

3.2.6 Hormone analysis

Hormone levels in the plasma were determined using commercially available ELISA/EIA-kits by following the instructions of the manufacturers. The following kits were used; Correlate-EIA ™ Oxytocin Enzyme Immunoassay kit (Assay designs, Inc. Ann Arbor, USA), DSL-10-2000 ACTIVE ® Cortisol Enzyme Immunoassay kit (Diagnostic Systems Laboratories, Inc. Texas, USA), Mercodia Canine Insulin ELISA 10-1203-1 and Mercodia Insulin ELISA 10-1113-10 according (Mercodia AB, Uppsala, Sweden).

3.2.7 Statistical analysis

The data were analyzed using the software SAS version 9.1 for Windows (SAS Institute Inc., Cary, NC, USA; 2002) and Statistical Package for the Social Sciences (SPSS/PASW) version 17.0 (SPSS Inc., Chicago, IL, USA; 2009).

The changes in hormone and heart rate levels at specific time points, compared to the start of the dog-owner interaction (0 minutes) were analyzed using linear mixed models in the MIXED procedure of SAS, one model for each trait, with sampling as a categorical predictor. Hormone levels for dogs and humans were normalized by logarithmic transformation ($\log_{10}$) before statistical analysis, in paper IV.

Paired t-tests were calculated to test for differences between the extreme and basal values of oxytocin and cortisol (paper IV).

The Spearman rank coefficient was used for calculating correlations between non-transformed hormone levels and mean values of scores of MDORS in paper V.

4 Results and comments

The first part of this thesis explored if and how exogenous oxytocin infusion and EDA administered during birth, and intramuscular oxytocin injection administered post partum, influence maternal levels of oxytocin and prolactin (paper I), ACTH and cortisol (paper II), as well as maternal blood pressure (paper III) during a breastfeeding session two days after birth.

The second part explored if and how short term sensory interaction between dogs and their owners affect their levels of oxytocin, cortisol, and insulin, as well as their heart rate (paper IV), and if there were any relationships between the hormone levels in the dogs and their owners. In addition, the owners' evaluation of the dog-owner relationship was explored for any connection to oxytocin and cortisol levels in the dogs and owners (paper V).

The most important results are briefly presented and commented upon. For a detailed description, references are made to the original articles (paper I-V).

4.1 Effects of intrapartum oxytocin administration and epidural analgesia on the concentration of plasma oxytocin and prolactin, in response to suckling during the second day post partum (paper I)

4.1.1 Oxytocin

The median (Q25-Q75) oxytocin level at the start of suckling was 119.7 (96.4-217.5) pg/ml for the entire group of women. The median (Q25-Q75) oxytocin levels increased significantly to 166.6 (119.7-215.1) pg/ml within

90 seconds after the start of suckling (p=0.001). During the first 7.5 minutes, a pulsatile oxytocin pattern, with three to four pulses (visually observed), was recorded in all mothers (Figures 3-5).

The median oxytocin levels were lower in the EDA^{OT} group compared to the other groups (OTiv group p=0.005, OTim group p=0.033, EDA^{nonOT} group p=0.051) (Figures 4 & 5).

For the mothers who had received oxytocin infusion during labor (OTiv group and EDA^{OT} group), a significant negative correlation was noted between the amount of exogenous oxytocin they had received and their endogenous oxytocin levels two days later (p=0.019); i.e., the higher the dose of exogenous oxytocin received during labor, the lower their endogenous oxytocin levels two days later (Figure 6).

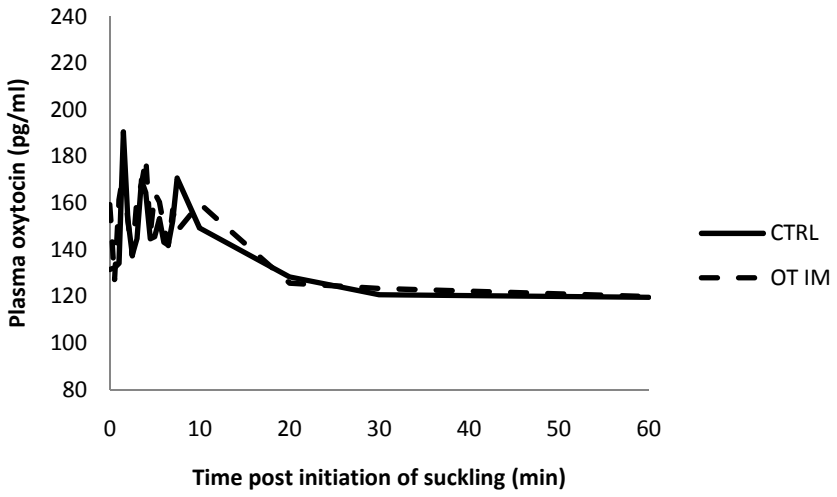

Figure 3. Median oxytocin levels (pg/ml) for the control group (n=20) and the OTim group (n=13) for each time point during the breastfeeding session

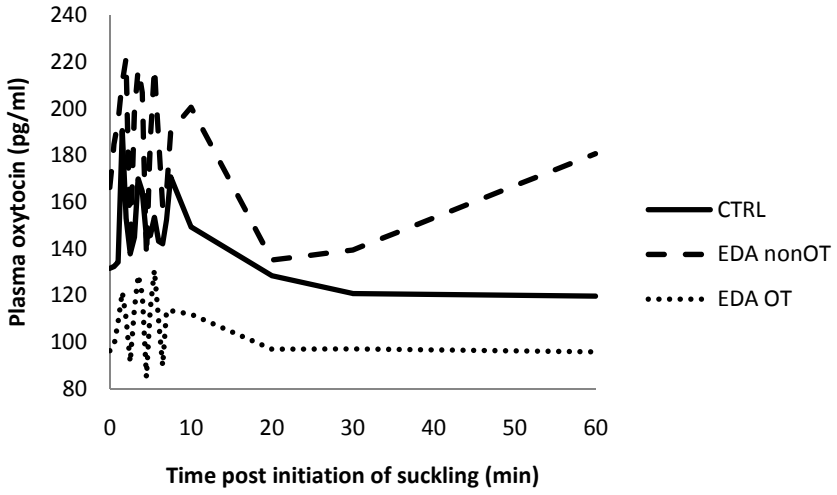

Figure 4. Median oxytocin levels (pg/mL) for the control group (n=20), EDA^{nonOT} group (n=6) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

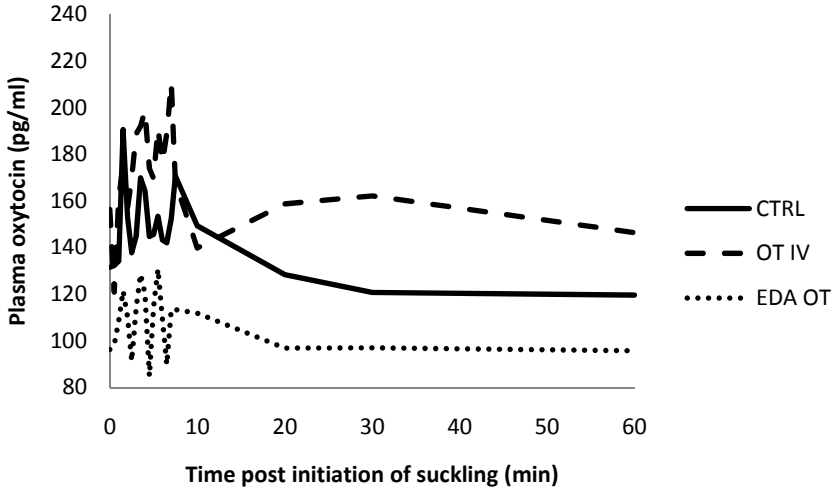

Figure 5. Median oxytocin levels (pg/mL) for the control group (n=20), the OTiv group (n=8) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

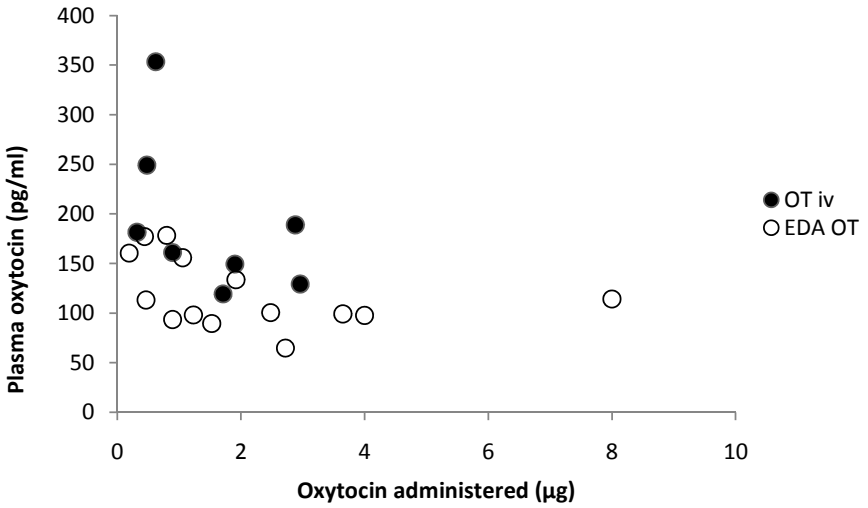

Figure 6. Scatter plot displaying the correlation between the endogenous median oxytocin levels for the women in the OTiv and EDA^{OT} groups and the amount of exogenous oxytocin they received during labor (p=0.019).

Comments on effects on patterns and levels

All mothers in the present study displayed a pulsatile oxytocin pattern during the first 10 minutes, when blood samples were taken with 30-second to 2.5-minute intervals. The pulses, which lasted for about 90 seconds, are likely due to the morphological and functional changes induced in response to the intense suckling stimulus. This reflects the coordinated electrical firing activity in the magnocellular cells of the SON and PVN (Poulain and Wakerley, 1982).

Administration of epidural analgesia or oxytocin infusion during labor did not affect the pulsatile release of oxytocin during breastfeeding. This is in contrast to previously reported effects following cesarean section, which was shown to induce significantly fewer oxytocin pulses during the first ten minutes of breastfeeding (Nissen, 1996b).

A negative relationship was observed between the amount of exogenous oxytocin that the mothers in the OTiv and EDA^{OT} group received during labor and their endogenous oxytocin levels two days later, suggesting that the oxytocin infusion had caused a feedback inhibition of the endogenous oxytocin secretion. However, the EDA^{OT} group had lower oxytocin levels

compared to the OTiv group. This suggests that oxytocin administered iv during labor exerts partly opposing effects on the breastfeeding-related oxytocin response during the second day postpartum: an inhibitory feedback effect, independent of the epidural analgesia, which therefore is likely to be mediated via the circulation, and a second positive feed-forward effect, involving neurotransmission in the spinal cord, which was consequently blocked by the epidural analgesia.

Comments on methodological aspects

Oxytocin levels were higher in this study, where EIA was used to determine oxytocin levels, when compared to results obtained in earlier studies by our group, where oxytocin levels were measured by radio-immuno-assay (RIA). Similar differences between these two techniques have also been reported by other authors (Levine et al., 2007).

It appears that the basal levels of oxytocin are higher when measured with EIA compared to RIA (Uvnäs-Moberg, 2011).

Since standard curves give valid results in EIA, the assumption must be made that something more than oxytocin is measured with this technique. Whether this is because the antibodies used not only recognize oxytocin, but also bind to substances that are similar to oxytocin (e.g., precursors, fragments and metabolites of oxytocin), or something completely different, is not known.

These differences in oxytocin levels when using EIA or RIA make it impossible to compare oxytocin levels reported in different studies where the different techniques have been used.

We believe that the oxytocin values in our study (paper I) are valid for the purpose of that study, since they demonstrate a clear breastfeeding-related pulsatile pattern following initiation of breastfeeding, irrespective of group. In addition, the dose-dependent effects of oxytocin support the validity of the obtained oxytocin values.

4.1.2 Prolactin

The median (Q25-Q75) prolactin level at the start of suckling was 250.4 (180.8-304.1) ng/ml for the entire group of women. The median (Q25-Q75) prolactin level increased significantly to 315.4 (223.2-364.1) ng/ml 20 minutes after start of suckling ($p \leq 0.0001$).

When the different groups were studied separately, all groups displayed a rise in prolactin levels after 20 minutes, except for the EDA^{nonOT} group; however, the rise in the control group was not significant (Figures 7-9).

A significant rise was observed already after 10 minutes in the OTiv and the EDA^{OT} groups ($p=0.012$ and $p=0.008$, respectively). This rise differed significantly from that seen on the Control group ($p=0.006$) (Figure 9).

At the end of the breastfeeding session (after 60 minutes) the prolactin levels in the OTiv and EDA^{OT} groups remained elevated compared to the levels at the start of the session ($p=0.012$ and $p=0.023$, respectively) (Figure 9). This continued elevation in prolactin levels was not seen in the other groups.

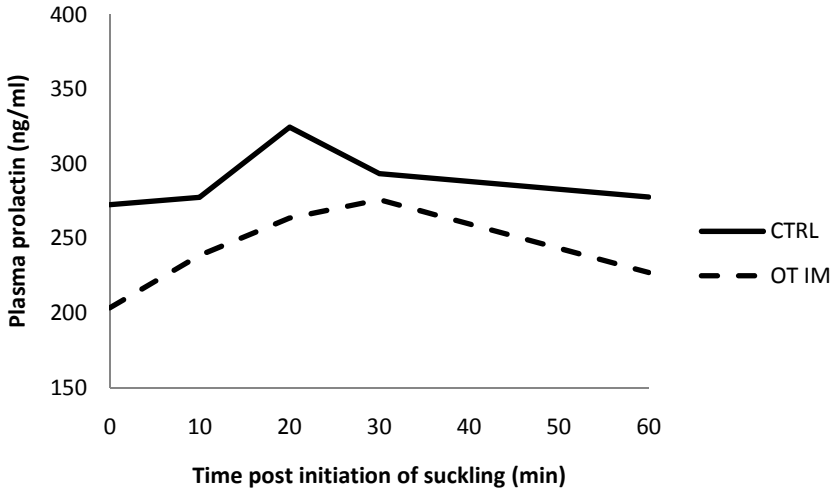

Figure 7. Median prolactin levels (ng/ml) for the control group (n=20) and the OTim group (n=13) for each time point during the breastfeeding session.

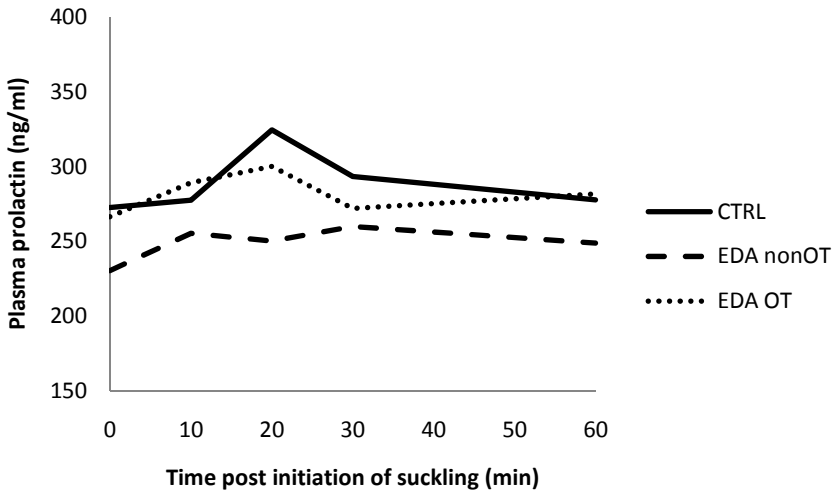

Figure 8. Median prolactin levels (pg/mL) for the control group (n=20), the EDA^{nonOT} group (n=6) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

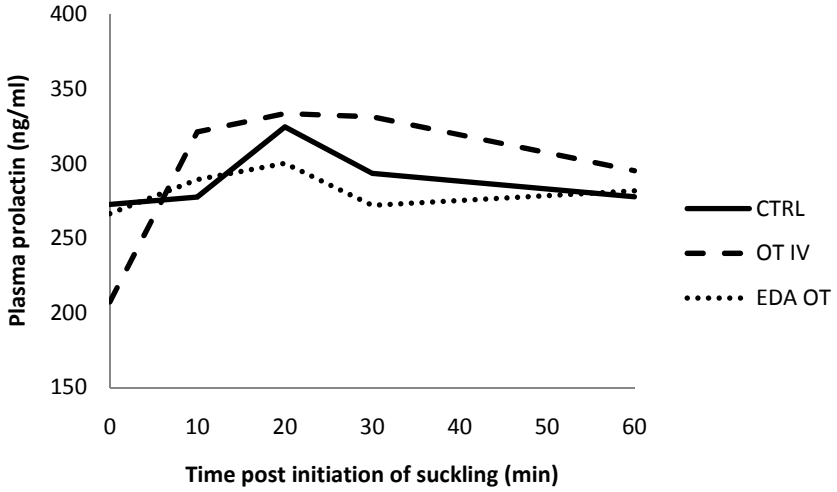

Figure 9. Median prolactin levels (pg/mL) for the control group (n=20), the OTiv group (n=6) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

Comments

Breastfeeding during the second day postpartum was, as expected, associated with a release of prolactin.

The rise in prolactin levels in response to suckling was enhanced in the women who had received oxytocin infusion during labor. These women also displayed a more long-lasting release of prolactin in response to breastfeeding, suggesting that oxytocin released in the brain during labor is of importance for future milk production, since oxytocin stimulates prolactin production via the nervous pathways in the anterior pituitary.

4.2 Effects of Sucking and Skin-to-Skin Contact on Maternal ACTH and Cortisol Levels During the Second Day Postpartum—Influence of Epidural Analgesia and Oxytocin in the Perinatal Period (paper II)

4.2.1 ACTH

For the entire group of women, the median (Q25-Q75) ACTH level at the start of suckling was 12.1 (5.3-19.2) pg/ml. ACTH levels then decreased significantly during the entire breastfeeding session and reached its lowest level at 60 minutes (6.1 pg/ml) ($p=0.001$).

The same significant decrease was also found in the control group ($p=0.044$) and similar, but non-significant, decreases were observed in the other groups (Figures 10-12).

A significant positive correlation was noted between median ACTH levels and median cortisol levels in the entire group of women and in the control group, when the different groups were studied separately; i.e., the higher the ACTH levels, the higher the cortisol levels ($p=0.048$ and $p=0.013$, respectively).

In contrast, significant negative correlations were observed between median oxytocin levels, oxytocin variance, and median ACTH levels in the entire group of women; i.e. the higher the median oxytocin levels and the greater the variance of oxytocin, the lower the median ACTH levels ($p=0.009$ and $p=0.037$, respectively).

The negative relationship between oxytocin variance and median ACTH levels could also be seen in the Control and EDA^{nonOT} groups ($p=0.041$ and $p=0.005$, respectively).

The median ACTH level showed a significantly negative correlation with the duration of the suckling in the entire group of women and also in the OTim group when the different groups were studied separately; i.e., the longer the duration of suckling, the lower the median ACTH level (p=0.041 and p=0.031, respectively).

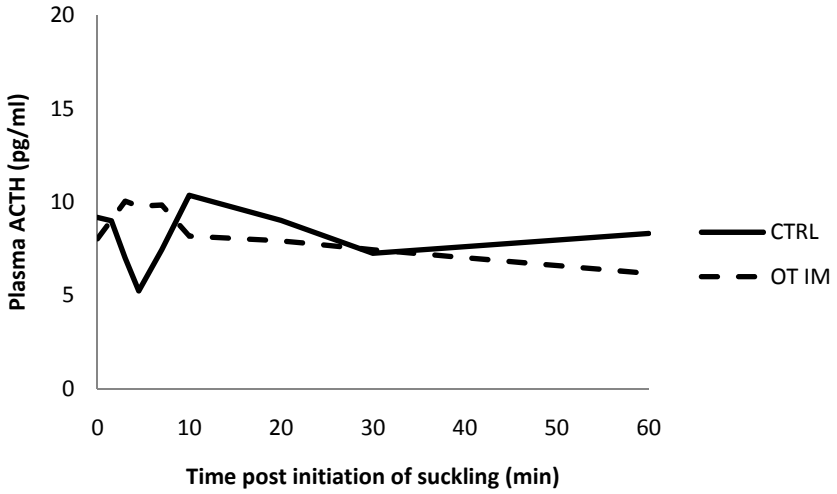

Figure 10. Median ACTH levels (pg/ml) for the control group (n=20) and the OTim group (n=13) for each time point during the breastfeeding session.

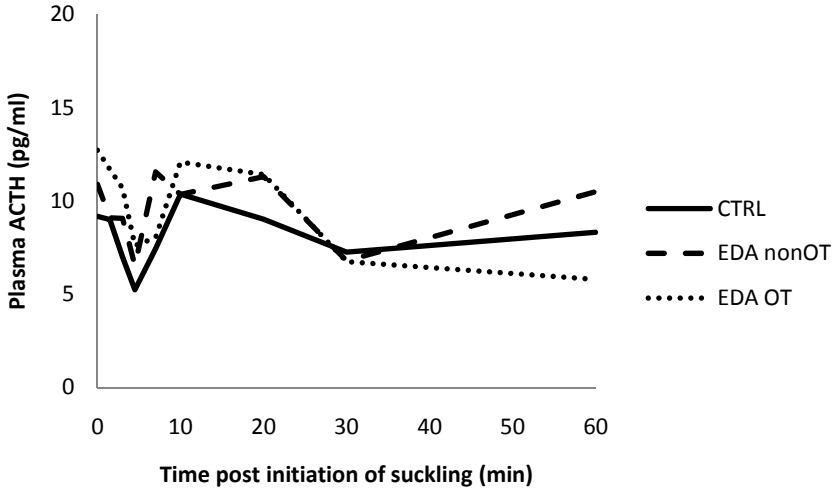

Figure 11. Median ACTH levels (pg/ml) for the control group (n=20), the EDA^{nonOT} group (n=6) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

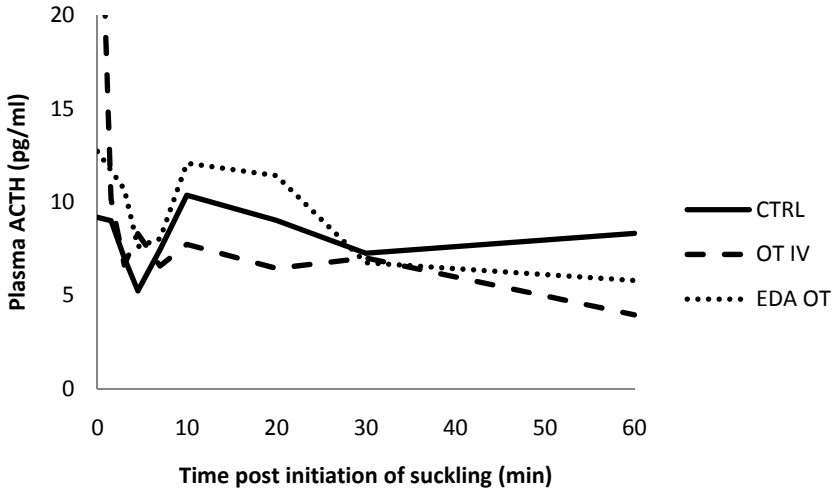

Figure 12. Median ACTH levels (pg/ml) for the control group (n=20), the OTiv group (n=8) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

Comments

Maternal ACTH levels fell significantly during the breastfeeding session studied.

The negative correlations between median oxytocin levels or variance (an expression of oxytocin pulsatility), as well as the duration of the suckling period and median ACTH levels suggest that ACTH is under an inhibitory influence of oxytocin during breastfeeding. This inhibitory effect on ACTH secretion, exerted by oxytocin, may either have been induced by inhibition of CRF secretion in the PVN or via an inhibitory effect on ACTH secretion by oxytocin released into the hypophyseal portal circulation or released from nerves reaching the anterior pituitary.

4.2.2 Cortisol

The median (Q25-Q75) cortisol level at the start of suckling was 911 (766-1119) nmol/l for the entire group of women. Except for a short transient rise at the beginning of the breastfeeding session, cortisol levels fell during the entire session, with the lowest levels reached at 60 minutes ($p<0.0001$).

When the different treatment groups were studied separately, the same significant decrease was also found in the Control and EDA^{OT} groups

($p<0.0001$ and $p=0.028$, respectively) and similar, but non-significant, decreases were observed in the other groups (Figures 13-15).

Cortisol levels at the onset of suckling (0 minutes) as well as the median cortisol level, were significantly lower in the EDA^{nonOT} group compared to the EDA^{OT} group ($p=0.033$ and $p=0.041$, respectively)(Figure 14).

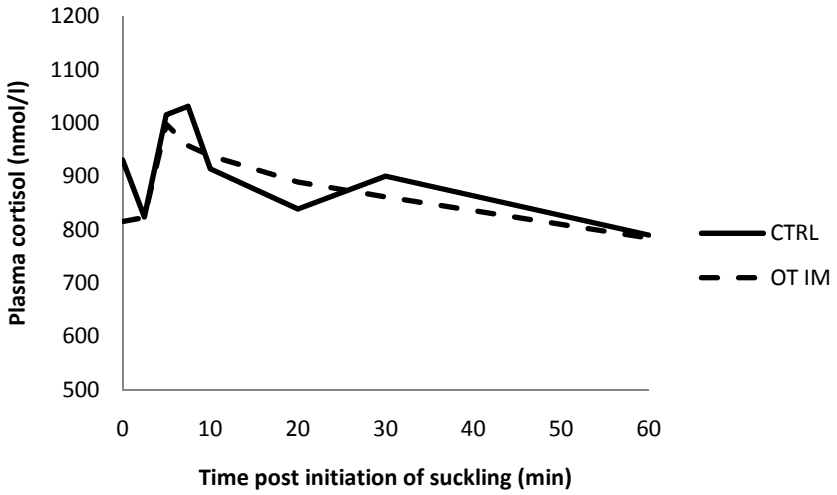

Figure 13. Median cortisol levels (nmol/l) for the control group (n=20) and the OTim group (n=13) for each time point during the breastfeeding session.

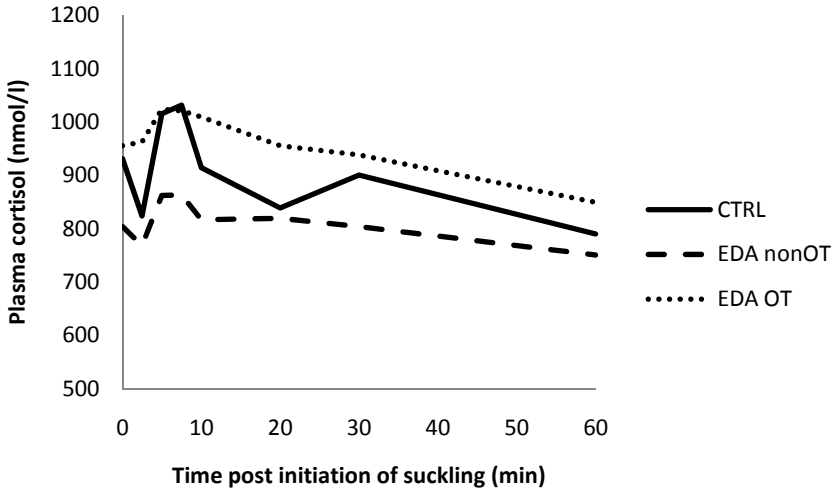

Figure 14. Median cortisol levels (nmol/l) for the control group (n=20), the EDA^{nonOT} group (n=6) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

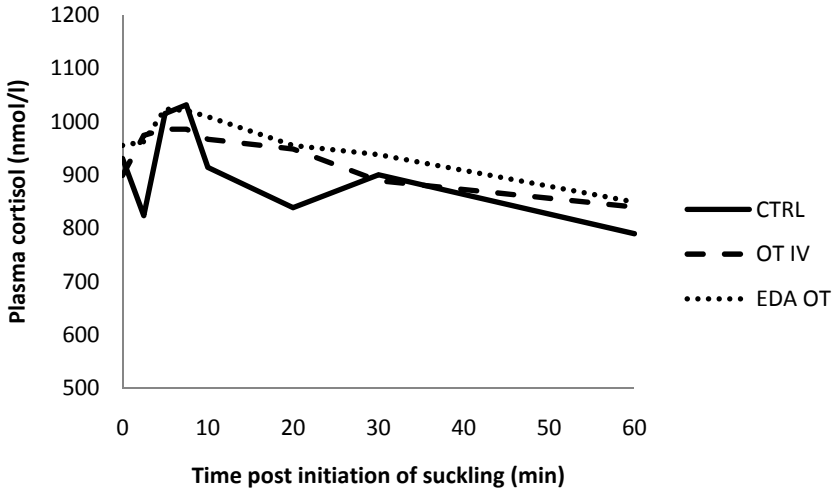

Figure 15. Median cortisol levels (nmol/l) for the control group (n=20), the OTiv group (n=8) and the EDA^{OT} group (n=14) for each time point during the breastfeeding session.

Comments

Cortisol levels fell significantly during the breastfeeding session studied.

The reason for the transient, nonsignificant rise in cortisol levels seen during the first minutes of the breastfeeding session is unclear. It may be due to the fact that the mothers perceived the experimental situation as stressful, resulting in a rise of ACTH and, consequently, of cortisol levels. Alternatively, the rise in cortisol levels is a physiological response to skin-to-skin contact and suckling. Cortisol may promote milk production by increasing the expression of the prolactin-responsive genes (Mizoguchi et al., 1997) and also by stimulating catabolic metabolism, as reported in other mammalian species, thereby allowing recruitment of nutrients for milk production (Svennersten-Sjaunja and Olsson, 2005).

The increased cortisol levels in women having received EDA combined with oxytocin infusions during labor may be an expression of an increased sensitivity to some types of stress as a result of the lowered function in their oxytocinergic system, both in the brain and in the circulation.

4.2.3 Skin-to-skin contact

In the entire group of women, median cortisol levels correlated significantly negatively with the duration of skin-to-skin contact before onset of suckling; i.e., the longer the duration of skin-to-skin contact, the lower the median levels of cortisol ($p=0.044$).

In the OTim group, a longer duration of skin-to-skin contact before onset of suckling was associated with a more pronounced decrease in cortisol levels during the first 2.5 minutes ($p=0.004$).

In contrast, no relationships were noted between ACTH levels and duration of skin-to-skin contact.

Comments

Skin-to-skin contact before onset of suckling contributed to the decrease in maternal cortisol levels and oxytocin administered im postpartum facilitated this decrease.

The results indicate that part of the anti-stress effects induced by breastfeeding is induced by skin-to-skin contact preceding suckling of the breast.

The effects induced by skin-to-skin contact are probably mediated by oxytocinergic neurons emanating from the PVN that reach, for example, the amygdala, the PAG, the DMX, and the NTS.

4.3 Influence of common birth interventions on maternal blood pressure patterns during breastfeeding two days after birth (paper III)

Basal systolic blood pressure (SBP) before breastfeeding did not differ between the different study groups (Figures 16 - 18).

However, basal diastolic blood pressure (DBP) was significantly lower in the $EDA^{non\text{-}OT}$ group compared to the basal DBP in the Control group, the OT IV group and the EDA^{OT} group (p=0.023, p=0.014 & p=0.011, respectively) (Figures 19-21).

Both SBP and DBP fell significantly in the control group, the OT im group, and the EDA^{OT} group, and almost significantly in the OT iv group during breastfeeding. In contrast, no decrease in either SBP or DBP was seen in the $EDA^{non\text{-}OT}$ group (for p-values, see paper III)(Figures 16-21).

In the OTim group, the duration of skin-to-skin contact before suckling was positively correlated with the fall in SBP occurring during the breastfeeding experiment (p=0.046); i.e., the longer the newborns lay in skin-to-skin contact with their mothers, the more the maternal SBP decreased.

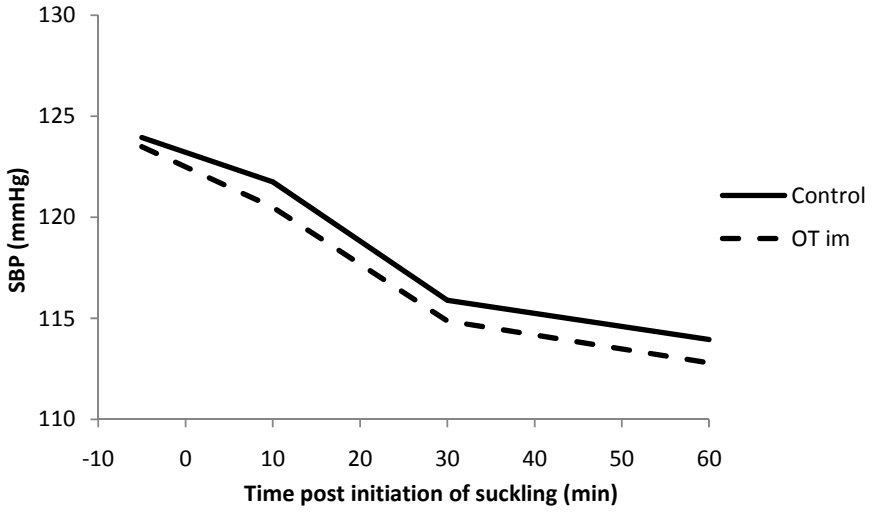

Figure 16. The systolic blood pressure (SBP;mmHg) in the control group (n=21) and the OTim group (n=15) during the 60 minute breastfeeding session.

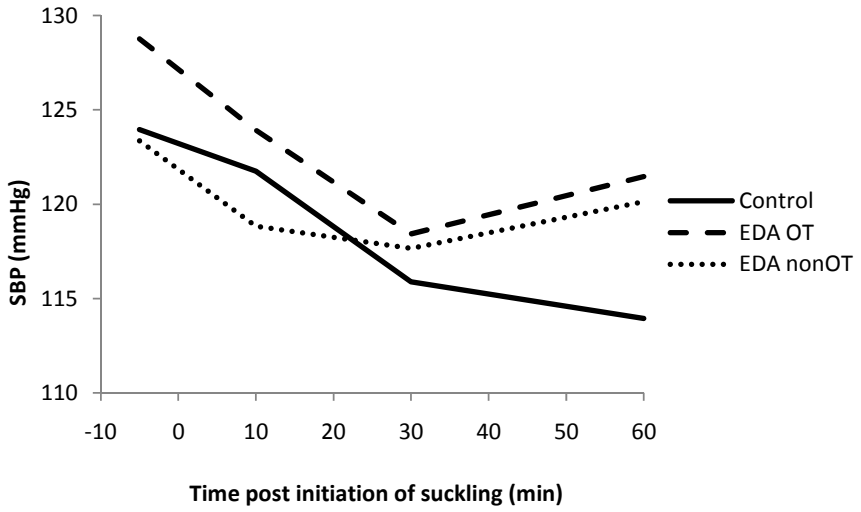

Figure 17. The systolic blood pressure (SBP;mmHg) in the control group (n=21), the EDA^{OT} group (n=14 and the EDA^{nonOT} group (n=7) during the 60 minute breastfeeding session

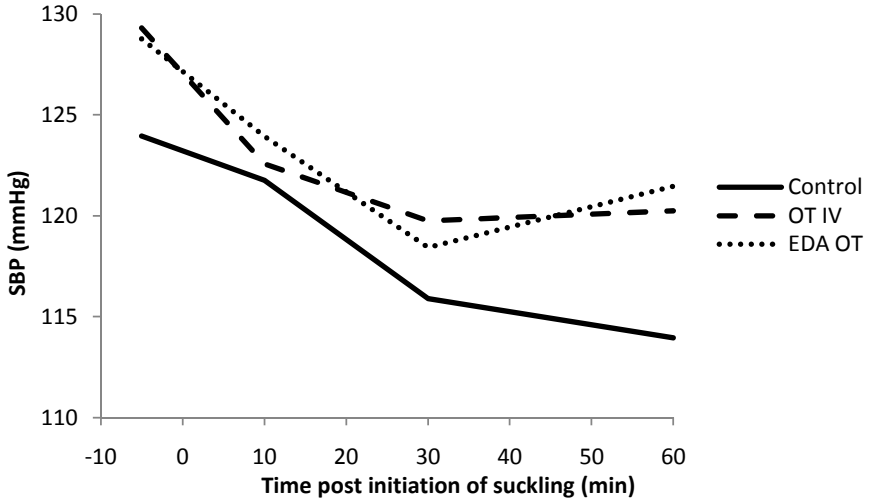

Figure 18. The systolic blood pressure (SBP;mmHg) in the control group (n=21), OTiv group (n=9) and the EDA^{OT} group (n=14) during the 60 minute breastfeeding session

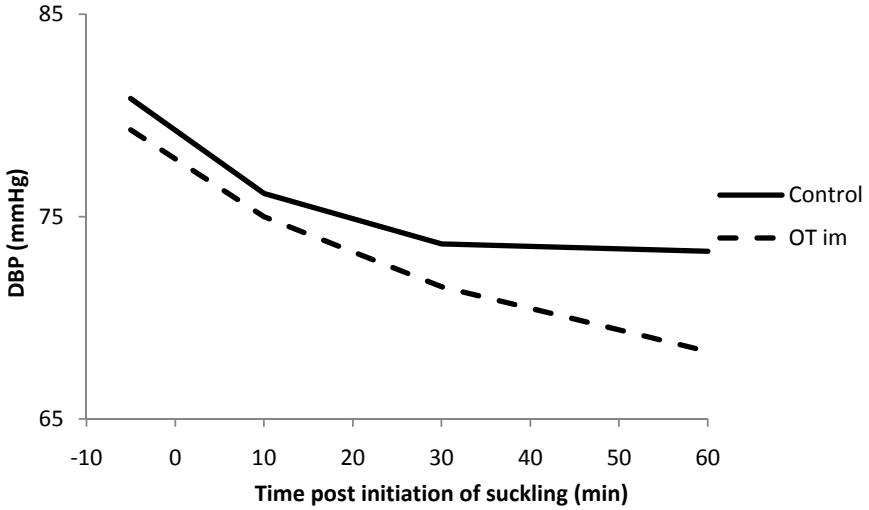

Figure 19. The diastolic blood pressure (DBP;mmHg) in the control group (n=21) and the OTim group (n=15) during the 60 minute breastfeeding session

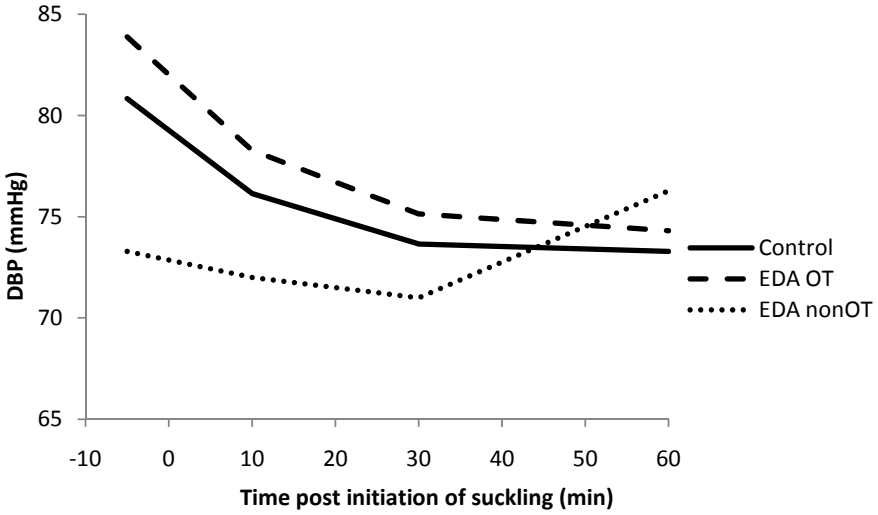

Figure 20. The diastolic blood pressure (DBP;mmHg) in the control group (n=21), the EDA^{OT} group (n=14) and the EDA^{nonOT} (n=7) during the 60 minute breastfeeding session

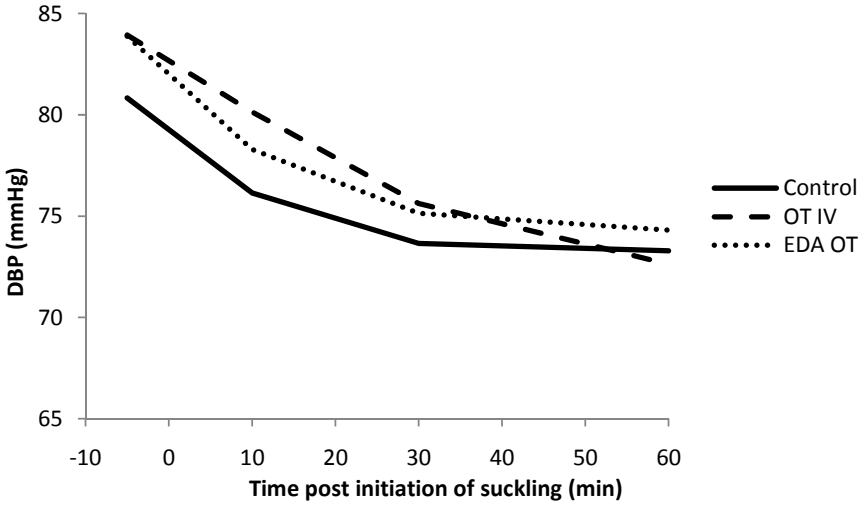

Figure 21. The diastolic blood pressure (DBP;mmHg) in the control group (n=21), the OTiv group (n=9) and the EDA^{OT} group (n=14) during the 60 minute breastfeeding session

Comments

Breastfeeding two days post partum was associated with a decrease in maternal systolic and diastolic blood pressure for all mothers, except those who had received EDA alone during labor. This decrease in blood pressure is most likely due to the oxytocin released into the brain, reaching, for example, NTS and DMX during breastfeeding.

However, the mothers in the EDA^{nonOT} group had significantly lower basal diastolic blood pressure compared to the other groups, which probably made it physiologically impossible for these mothers to decrease their blood pressure further.

The low basal blood pressure in the $EDA^{non\text{-}OT}$ group might be due to a sustained decrease in blood pressure induced by the EDA during labor. Alternatively, the influence on blood pressure could be related to maternal oxytocin levels.

4.4 Short-term interaction between dogs and their owners – effects on oxytocin, cortisol, insulin and heart rate - an exploratory study (paper IV)

4.4.1 The dogs

The dogs' oxytocin levels were significantly increased 3 minutes after start of dog-owner interaction ($p=0.027$), with peak oxytocin levels recorded between 1 and 5 minutes. These levels were significantly higher compared to the levels obtained before the interaction started ($p=0.017$) (Figure 22).

The dogs' cortisol levels were significantly increased 15 and 30 minutes after the start of the dog-owner interaction when compared to levels obtained before the interaction started ($p=0.004$ and $p=0.022$, respectively)(Figure 22).

The dogs' insulin levels did not change significantly during the experiment (Figure 22).

The dogs' heart rate decreased significantly at 55 minutes compared to the rate at the start of the interaction ($p=0.008$).

4.4.2 The owners and controls

The owners' peak oxytocin levels recorded at 1, 3 or 5 minutes were significantly higher compared to the levels obtained before the interaction started ($p=0.026$) (Figure 23).

In contrast, no such effect was seen in the controls ($p=0.417$) (Figure 24).

The minimum cortisol levels recorded at 15 or 30 minutes in both the owners and controls were significantly decreased compared to the levels obtained before the interaction started ($p=0.030$ and $p=0.002$, respectively) (Figure 23 & 24).

In both owners and controls, a significant decrease in insulin levels was noted at 60 minutes ($p=0.0018$ and $p<0.001$, respectively) (Figure 23 & 24).

The owners' heart rate was significantly decreased at 55 and 60 minutes ($p=0.0008$ and $p=0.0008$, respectively).

In contrast, no change in heart rate was seen for the controls.

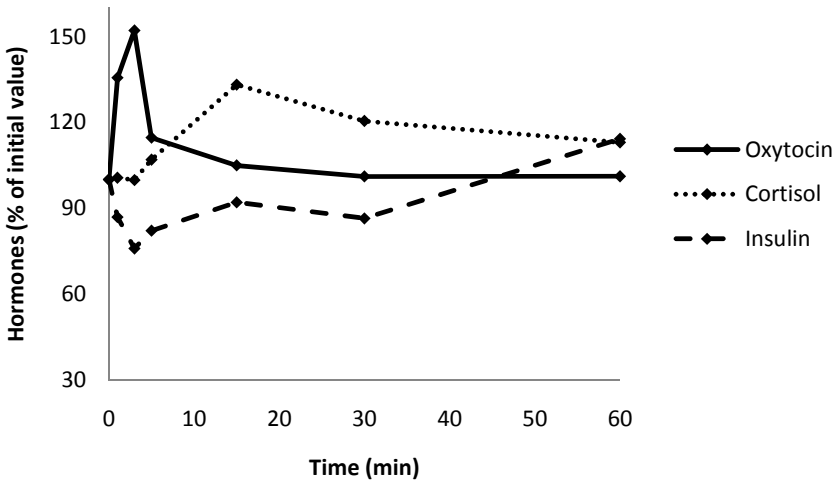

Figure 22. Changes in oxytocin, cortisol, and insulin levels at each time point (% of initial value based on non-transformed data) in ten dogs during the interaction.

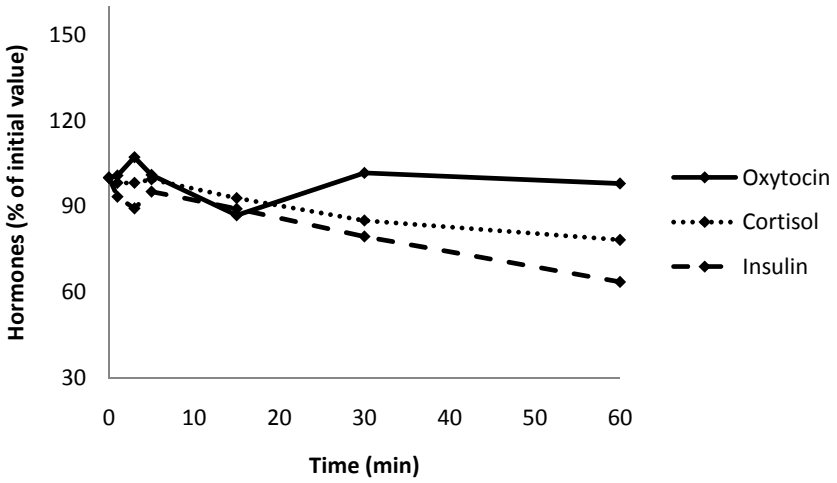

Figure 23. Changes in oxytocin, cortisol, and insulin levels at each time point (% of initial value based on non-transformed data) in ten owners during the interaction.

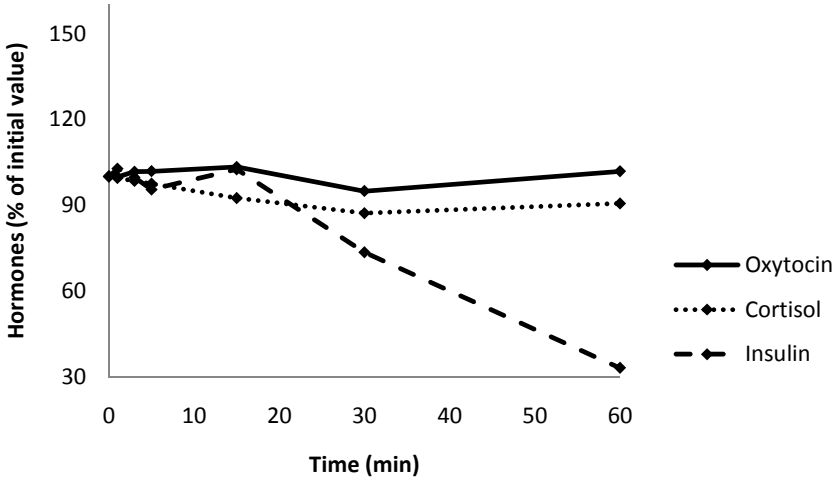

Figure 24. Changes in oxytocin, cortisol, and insulin levels at each time point (% of initial value based on non-transformed data) in ten controls during the interaction.

Comments

Both dogs and owners displayed increasing oxytocin levels during the interaction, a response not seen in the controls, indicating that the increase was most likely induced by the dog-owner interaction.

The dogs' cortisol levels increased during the experiment, which was probably due to the increase in locomotor activity induced by the interaction with the owner.

Both owners and controls displayed decreasing cortisol levels during the experiment, but only owners displayed decreasing heart rate, suggesting that interaction with the dog might have induced an anti-stress effect in the owners.

The dogs' insulin levels did not change during the experiment, but insulin levels in both owners and controls decreased as time progressed. Since no control had been made for feeding, any effects caused by the sensory interaction might have been concealed by parallel feeding-related changes in glucose and insulin levels.

4.5 Associations between the psychological characteristics of the human-dog relationship and oxytocin and cortisol levels (paper V)

4.5.1 Monash dog owner relationship scale

The mean score of the three subscales (Dog-owner interaction, Emotional closeness, and Perceived costs) were 4.1 (SD 0.4), 3.8 (SD 0.4) and 3.8 (SD 0.4), respectively, and the mean total score of MDORS was 4.0 (SE 0.4).

4.5.2 Owners' oxytocin and cortisol levels

The higher the owners' oxytocin levels, the more often they kissed their dog ($p=0.001$) (Figure 25) and the less difficult they thought it was to look after their dog ($p=0.085$).

The lower the owners' cortisol levels, the less bothered they were about the dog stopping them from doing things, the more often they brought their dogs to visit people, and the more traumatic they thought it would be when their dog dies ($p=0.037$, $p=0.044$ and $p=0.025$, respectively).

4.5.3 Dogs' oxytocin and cortisol levels

The higher the dogs' oxytocin levels, the more often the owners kissed their dog (Figure 26), the less often they gave their dog food treats, and the stronger was the bond to the dog ($p=0.019$, $p=0.018$ and $p=0.033$, respectively).

In addition, the higher the dogs' oxytocin levels, the lower the owners' perceived cost and the more positive evaluation of the relationship in total ($p=0.007$ and $p=0.019$, respectively). (Figure 27)

4.5.4 Dogs' and their owners' hormone levels

The dogs' mean oxytocin levels obtained at 60 minutes correlated significantly positively, or tended to correlate significantly positively, with the owners' oxytocin levels obtained at each time point, as well as their mean oxytocin levels; i.e., the higher the dogs' oxytocin levels, the higher the owners' oxytocin levels (for p-values , see paper IV).

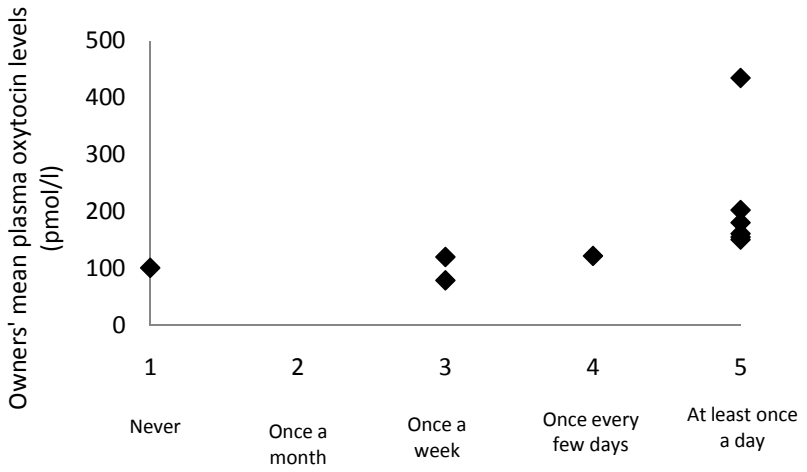

Figure 25. "How often do you kiss your dog?" The more often, the higher were the owners' oxytocin levels (p=0.001)

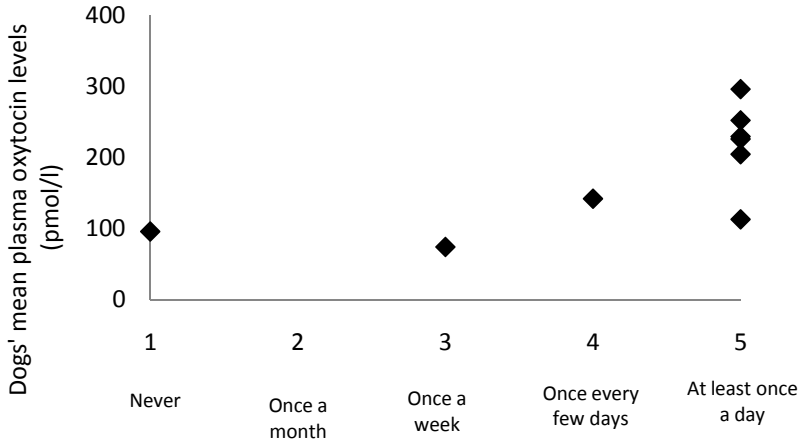

Figure 26. "How often do you kiss your dog?" The more often, the higher the dogs' oxytocin levels (p=0.019).

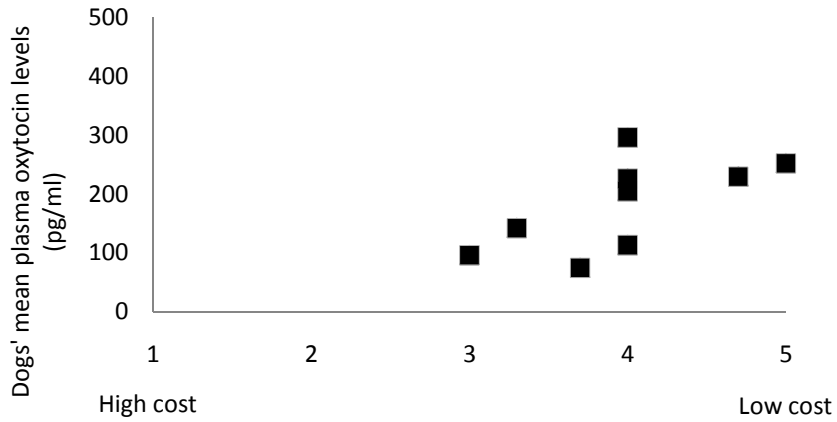

Figure 27. Perceived cost. The lower the cost, the higher the dogs' oxytocin levels (p=0.019).

Comments

The results from the correlation analysis indicate that high levels of oxytocin and low levels of cortisol in dog owners are related to the description of the owner-dog relationship as pleasant and interactive, as well as to a perception of the relationship having few problems.

In addition, the results also indicate that high levels of oxytocin in the dogs are related with increased interaction with the owner and with the owners not seeing the dogs as a burden, but as a positive companion.

The positive relationships between hormone levels in the owners and the dogs indicate a mutual relationship between dog owners and their dogs and that this relationship influences their oxytocin levels.

5 General discussion

5.1 Physiological responses to breastfeeding two days post partum – influence of medical interventions

5.1.1 General response

The results from paper I-III show that during a breastfeeding session two days after birth, the mothers displayed a) an immediate and pulsatile release of oxytocin, b) a rise in prolactin levels, c) a decrease in ACTH and cortisol levels, and d) a decrease in systolic and diastolic blood pressure. These response patterns are in line with those reported in previous studies (Amico et al., 1994, Heinrichs et al., 2001, Jonas, 2008b, Nissen, 1996b).

In addition, some correlations between hormone levels were observed in the present study; median oxytocin and prolactin levels were positively correlated, median oxytocin and ACTH levels were negatively correlated and ACTH and cortisol levels were positively correlated.

Oxytocin has previously been shown to stimulate prolactin release (McKee et al., 2007, Samson et al., 1986). Oxytocin also decrease levels of CRF in the PVN (Neumann et al., 2000), resulting in decreased levels of ACTH and consequently cortisol. In addition, oxytocin exerts inhibitory effects directly on ACTH release in the anterior pituitary (Burbach et al., 2006) and on cortisol release by actions on the adrenal gland (Legros et al., 1988, Stachowiak et al., 1995). Oxytocin also decreases blood pressure, probably via activation of 2-adrenoreceptors in the NTS and adjacent areas in the brain stem involved in the control of the autonomic nervous system (Petersson et al., 2005a).

It is therefore likely that oxytocin released from oxytocinergic neurons during breastfeeding contribute to the increase in prolactin levels, the decrease of ACTH and cortisol levels, as well as the decrease in blood pressure observed during breastfeeding.

Taken together, these results show that oxytocin has a regulatory and integrating role in the response pattern observed during breastfeeding.

5.1.2 Medical interventions during labor

EDA is the most common pharmacological option for efficient pain relief during labor. EDA may attenuate uterine activity (Anim-Somuah et al., 2005) and it is therefore often necessary to also administer oxytocin infusion in order to augment labor.

Oxytocin infusion given to initiate or augment labor increases uterine contractions. This is often related to increased pain, and therefore increasing the need for pain relief. Hence, EDA and oxytocin infusions are strongly connected during labor, irrespective of the order of these medical interventions.

Animal experiments have shown that peridural anesthesia block oxytocin release, both into the circulation and into the brain during labor, and hamper maternal behaviors (Krehbiel et al., 1987, Levy et al., 1992, Williams et al., 2001). In addition, EDA has been shown to counteract the release of oxytocin during labor in humans (Goodfellow et al., 1983, Rahm et al., 2002).

With the present study we wanted to investigate if the medical interventions mothers receive during labor affected the breastfeeding related effects.

5.1.3 Effects of medical interventions

The results from papers I-III show that the maternal release patterns of oxytocin, prolactin, ACTH, and cortisol, as well as maternal blood pressure, in response to breastfeeding two days after birth, are affected by the medical interventions that the mothers received during labor (summarized in Table 2).

Mothers who received EDA alone during labor (EDA^{nonOT} group) had significantly lower basal systolic and diastolic blood pressure and no decrease in blood pressure in response to breastfeeding. These mothers also had significantly lower median cortisol levels compared to the EDA^{OT} group. They also lacked the expected breastfeeding related rise in prolactin levels.

Mothers who received oxytocin infusion during labor (OTiv and EDA^{OT} groups) had dose-dependent decreases in endogenous oxytocin levels two days later. They also had a faster rise in prolatin levels during the

breastfeeding session, compared to mothers who had not received exogenous oxytocin during labor.

In addition, the endogenous oxytocin levels for mothers who received EDA combined with oxytocin (EDA^{OT} group) were significantly lower compared to the other treatment groups (EDA^{nonOT} group and OTiv group).

Table 2. *Summary of comparisons between the effects of medical interventions (EDA^{nonOT}, EDA^{OT} and OTiv groups) in response to breastfeeding two days after birth*

Medical intervention during labor	Effects recorded two days later
EDA alone (EDA^{nonOT} group)	Higher median oxytocin level compared with the EDA^{OT} group (Figure 28)
	Decreases in ACTH and cortisol levels
	Lower median cortisol levels compared with the EDA^{OT} group (Figure 29)
	Lower basal systolic and diastolic blood pressure compared to the EDA^{OT} (Figures 30 and 31) and OTiv group
	No decrease in either systolic or diastolic blood pressure
	No increase in prolactin levels
EDA combined with oxytocin iv (EDA^{OT} group)	Dose-dependent decrease in endogenous oxytocin levels
	Lower median oxytocin levels compared to the EDA^{nonOT} (Figure 28) and OTiv group
	Decreases in ACTH and cortisol levels
	Higher median cortisol levels compared with the EDA^{nonOT} group (Figure 29)
	Decreases in both systolic and diastolic blood pressure (Figures 30 and 31)
	More rapid increase in prolactin levels
Oxytocin iv (OTiv group)	Dose-dependent decrease in endogenous oxytocin levels
	Higher median oxytocin level compared to EDA^{OT} group
	Decreases in ACTH and cortisol levels
	Decreases in both systolic and diastolic blood pressure
	More rapid increase in prolactin levels

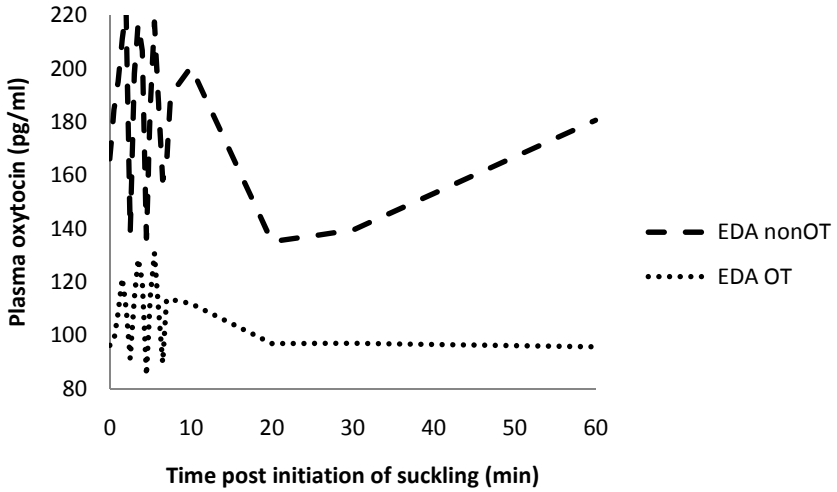

Figure 28. Oxytocin levels in the EDA^{nonOT} and EDA^{OT} groups during the breastfeeding session studied

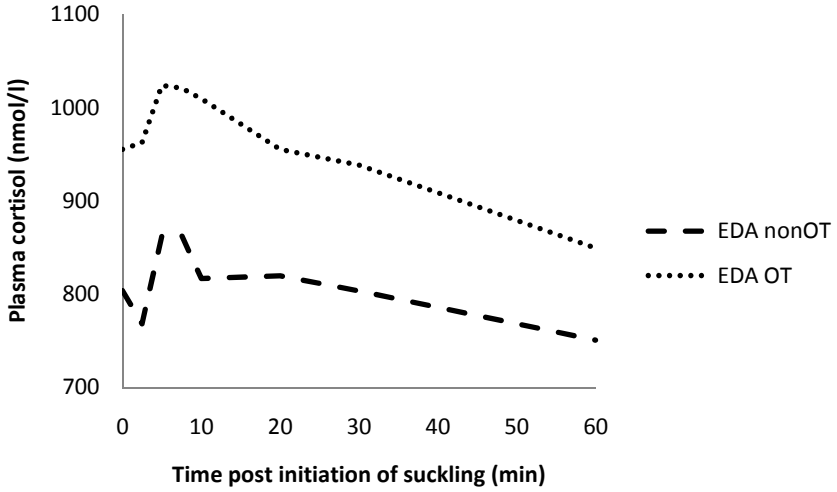

Figure 29. Cortisol levels in the EDA^{nonOT} and EDA^{OT} groups during the breastfeeding session studied

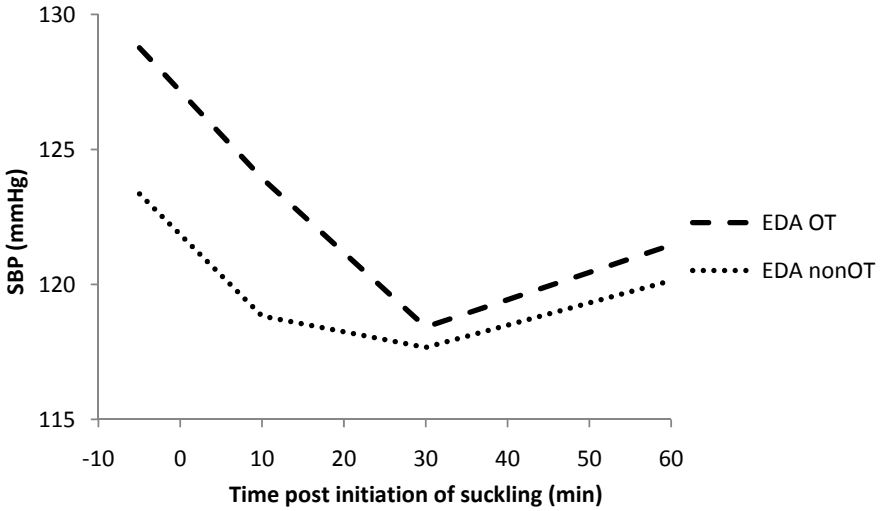

Figure 30. Systolic blood pressure in the EDA^{nonOT} and EDA^{OT} groups during the breastfeeding session studied

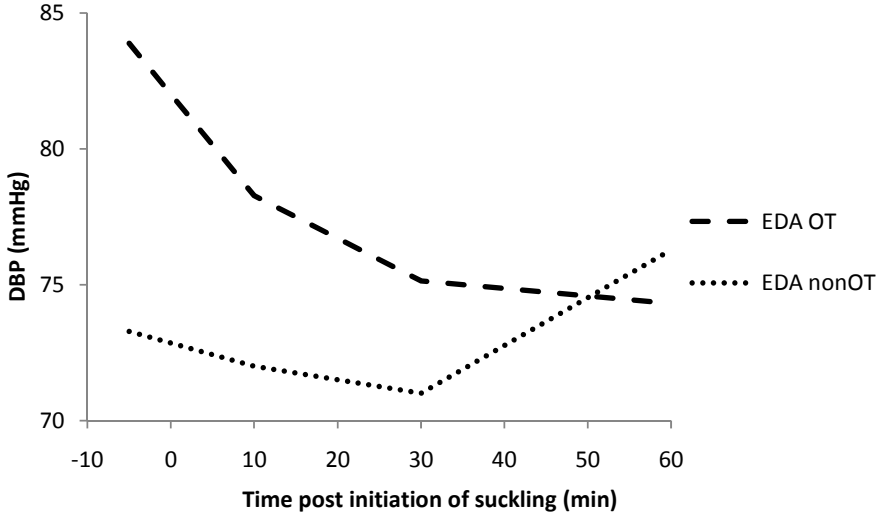

Figure 31. Diastolic blood pressure in the EDA^{nonOT} and EDA^{OT} groups during the breastfeeding session studied

5.1.4 Oxytocin levels at labor and two days later

The first hours after birth are sometimes referred to as the "early sensitive period". It has been shown that sensory stimulation between mother and young during this period result in long-term sustained promotion of social interaction between mother and child (Kennell et al., 1975, Klaus et al., 1972). It also results in increased social interaction in both mother and child and reduced stress behavior in the child at one year (Bystrova et al., 2009).

The high levels of endogenous oxytocin during labor, and the following hours, may facilitate the positive social effects generated during the "early sensitive period".

The mothers who received EDA during labor did not display the normal physiological maternal adaptations, such as decrease in anxiety and increase in social interactive behaviors (Jonas, 2008a).

Animal experiments have shown that there is a parallel release of endogenous oxytocin into the brain and into the circulation in response to parturition and suckling (Kendrick et al., 1986) and that peridural anesthesia during labor decreases both peripheral and central oxytocin release during labor (Levy et al., 1992). Therefore, the absence of maternal psychological adaptations may be due to the low levels of oxytocin during this period.

The results from previous studies and those presented in paper I-III indicate that infusion of oxytocin during labor had two-fold effects. It increased expression of some of the maternal psychological adaptations occurring during the early sensitive period (Jonas, 2008a) . This effect must have been induced in the brain and might have been due to stimulation of afferent nerves (the Ferguson reflex) during labor, leading to increased release of oxytocin in the brain during the early sensitive period.

However, infusion of oxytocin also induced a second type of effects. It induced a dose-dependent decrease of oxytocin levels, and if combined with EDA, decreased levels of endogenous oxytocin two days later. The decrease of endogenous oxytocin levels might have been a result of a feedback inhibition of endogenous oxytocin levels in response to the elevated oxytocin levels during labor.

It is likely that the decreased circulatory levels of oxytocin seen in the mothers in the EDA^{OT} group are paralleled by decreased endogenous oxytocin levels also in the brain, for example the oxytocinergic pathway innervating the NTS. This would explain why mothers in the EDA^{OT} group have higher cortisol levels (Figure 29) and higher blood pressure (Figures 30 and 31) during the breastfeeding session studied.

5.1.5 Effects of skin-to-skin contact

The time the mother and infant spent in skin-to-skin contact before suckling was shown to affect the maternal decrease in cortisol levels and to some extent, the decrease in maternal blood pressure during breastfeeding two days after birth. This means that it is not only the suckling itself, but also the skin-to-skin contact during breastfeeding that contributes to the decrease in cortisol and blood pressure observed in response to breastfeeding.

As described in the introduction, skin-to-skin contact between mother and child induces maternal oxytocin release into the circulation (Matthiesen, 2001) and probably also into the brain. The finding of reduced blood pressure in response to skin-to-skin contact indicates that fibers in the NTS have been activated during skin-to-skin contact. Previous studies have also shown a relationship between higher oxytocin levels and lower blood pressure (Light et al., 2005, Light et al., 2000).

In addition, skin-to-skin contact reduced cortisol levels but not ACTH levels. This is in line with finding in cows where stroking of the abdominal area in front of a cows' udder decrease their cortisol levels without influencing their ACTH levels (Personal communication with Ewa Wredle). These results suggest that the decrease in cortisol levels is induced through a partly different mechanism compared to suckling, which involves a decrease in ACTH.

Perhaps a reduction of sympathetic nervous tone contributes to the decrease in cortisol secretion in response to skin-to-skin contact. The decrease in cortisol levels may also be due to an effect of catecholamines or peptides released from the adrenal medulla or from the sympathetic nerves innervating the adrenal cortex (Lightly et al., 1990, Nussdorfer, 1996). The finding of strong correlations between the fall in blood pressure and cortisol levels in response to skin-to-sin contact supports this (Handlin et al, to be published).

5.2 Physiological and psychological responses to interaction between dogs and their owners

5.2.1 Response patterns

Reports have begun to emerge showing that oxytocin is released in both dogs and humans when they interact (Miller, 2009, Odendaal, 2003). The results from our study (paper IV) further support these findings.

Earlier studies involved practiced pre- and post measurements only of the physiological variables measured in connection interaction. In contrast, our study used repeated measurements throughout the entire experiment. Using this experimental set-up, we were able to show that oxytocin levels peak significantly in both owners and dogs when the owner strokes and caresses her dog. This response is immediate with a significant increase of oxytocin levels being observed within few minutes after start of interaction. When the interaction stops, oxytocin levels in both the owner and the dog return to basal levels within minutes.

These results are in line with previous experiments performed on rats, where gentle stroking on the abdomen induced a significant increase in oxytocin levels (Agren, 1995, Kurosawa, 1995, Stock, 1988, Uvnas-Moberg, 1996b).

Both owners and controls decreased their cortisol levels significantly during the experiment, probably as a consequence of recovery from the experimental stress. We had expected a difference in cortisol levels between owners and controls but it is often difficult to detect stress buffering effects in humans without having the participants go through a stress test prior to the intervention. The cortisol response might have differed between owners and controls if a stress test had been performed before the interaction.

In contrast to the observation in the owners, the dogs' cortisol levels increased during the interaction experiment. A similar result was also described in a study performed by Odendaal and colleagues (Odendaal, 2003). This increase is most likely a result of increased physical activity in response to the interaction and not an expression of stress.

The observation of decreased heart-rate in the dogs at the end of the experiment further strengthens the suggestion that the dogs were not stressed.

As described in the methods section, the owners completed the MDORS during the interaction experiment, in order to document the character of their relationships with their dogs.

The results from the correlation analysis show a mutual relationship between owners and their dogs, where positive (or absence of negative) aspects of the relationship are linked to higher oxytocin levels in both species. In addition, frequent sensory interaction between dog-owners and their dogs was associated with higher oxytocin levels, both in the owners and in the dogs. However, the results from the present study do not allow conclusion on whether it is the increased interaction that generates the higher oxytocin levels or vice versa.

5.2.2 Interaction between and within species

Previous studies have shown that non-noxious sensory stimulation associated with friendly social interaction, such as maternal behavior, pair bonding, and attachment is, linked to activation of the oxytocinergic system (Carter, 1998, Ditzen et al., 2007, Insel, 1998, Insel, 2003, Light et al., 2005, Uvanas-Moberg, 2005, Uvnas-Moberg, 1998b).

However, these studies have focused on interaction between individuals of the same species (e.g., humans, rats, sheep, prairie voles, etc.). The results from our study show that interaction between individuals from different species results in the same effects as previously observed within species. The assumption can be made, therefore, that the mechanisms in the oxytocinergic system and the effects that they cause are part of a "mammalian heritage" and can be activated not only by individuals from the same species but also by individuals from another species.

As mentioned in the introduction, humans and dogs have been living in close contact for a long time and dogs are bred for their keen senses and loyalty (Walsh, 2009a). Perhaps dogs and humans are therefore especially good at activating each others oxytocinergic systems.

5.3 General mechanisms

5.3.1 Acute and long term effects of interaction

The two studies included in this thesis investigated the acute effects of human-human and human-animal interaction. Both types of interaction induced anti-stress effects, displayed by decreasing ACTH, cortisol, and blood pressure in the mothers, and by decreased cortisol and heart rate in the dog owners. These effects are most likely due to a reduction of the activity in the HPA-axis and the sympathetic nervous system.

However, breastfeeding and dog-owner interaction normally occur repeatedly over a long period of time. Since each single interaction session stimulates oxytocin release, the repeated interactions will most likely result in repeated oxytocin release, both into the brain and into the circulation, in breastfeeding mothers, dog owners, dogs, and most likely also in the infants.

As mentioned in the introduction, repeated administration of oxytocin have been shown to induce several long-term effects, such as decreased blood pressure and cortisol levels and increased social interaction (Uvnas-Moberg and Petersson, 2005). In support of this, breastfeeding mothers have a blunted reaction to certain kinds of stress, as displayed by decreases in blood pressure, ACTH and cortisol levels and decreased anxiety (Altemus, 1995, Heinrichs et al., 2001, Light et al., 2000). Breastfeeding is also associated with a long-term decrease in basal blood pressure (Jonas, 2008b). Similarly, pets have been shown to have calming effects and give social support to pet-owners during stressful situations (Allen, 2001, Allen, 2002, Allen et al., 1991).

Both breastfeeding and owning a pet have also been associated with long-term health-promoting effects. For example, women who have given birth and who have breastfed have been shown to be "dose-dependently" protected from hypertension and from development of cardiovascular diseases (Lee et al., 2005, Schwarz et al., 2009, Stuebe et al., 2009). Similar effects are seen in pet owners where owning a pet is associated with lower heart rate and blood pressure (Allen, 2002) and a higher survival rate after a heart attack (Friedmann, 1995, Friedmann et al., 1980).

Taken together, it is possible that the repeated release of oxytocin in response to breastfeeding or interacting with a dog is a key coordinator for mediating the health promoting effects related to human-human and human-animal interactions.

5.3.2 Relationship between oxytocin levels and interactive behaviors

Feldman and colleagues have shown that mothers with high oxytocin levels are more interactive with their children and also more sensitive to their children's cues (Feldman, 2007). In our study, we found a similar pattern, with more interaction between owner and dog being related to higher oxytocin levels. The relationship between a dog-owner and her dog has been found to show several similarities with that seen between a mother and her child (Palmer and Custance, 2008, Topal, 1998), indicating a possibility for the same type of relationship; i.e., dog owners with high oxytocin levels

may be more sensitive to their dogs' cues, just as mothers are to their children's.

5.4 Methodological considerations

5.4.1 Papers I-III

The number of participants in paper I-III is small. However, since the inclusion criteria were strict and procedures were standardized, we believe that important information can be obtained from these studies, as has been the case in other studies performed under similar conditions (Nissen, 1996a, Ransjo-Arvidson et al., 2001, Widstrom et al., 1990).

As described in the methods section, it is not possible to randomize a study of this kind for ethical reasons.

5.4.2 Papers IV-V

The results from the present study should be interpreted with caution, since it is a pilot study with a limited number of participants.

However, since we were strict in only including female owners and male Labrador dogs, the variation due to breed and gender should be minimized. How the results apply to male owners and different breeds of dogs, however, needs to be further investigated.

6 Conclusion

In response to breastfeeding two days after birth, the mothers displayed a pulsatile release of oxytocin and increasing prolactin levels. In addition, the activity in the HPA-axis was reduced and maternal blood pressure decreased.

Oxytocin infusions given during labor were shown to dose-dependently decrease endogenous oxytocin levels two days later. Furthermore, EDA combined with oxytocin infusion during labor resulted in significantly lower oxytocin levels and higher cortisol levels, as well as higher blood pressure in response to breastfeeding two days after birth, compared to EDA administration alone.

Short term interaction between dog-owners and their dogs resulted in increasing oxytocin levels in both the owners and the dogs. The owners also displayed decreasing cortisol levels and heart rate, whereas the dog displayed increasing cortisol levels but decreasing heart rate.

There was a mutual relationship between owners and dogs oxytocin levels and oxytocin levels in both owners and dogs were related to a positive relationship between the two.

Although only acute effects were studied in the present thesis, repeated interactions between mothers and their infants, as well as between owners and their dogs, most likely give rise to long-term oxytocin-mediated effects in both species. This suggests that oxytocin may have an important coordinating and regulatory role for the health promoting effects associated with breastfeeding and pet-ownership.

7 Svensk populärvetenskaplig sammanfattning

Syftet med denna avhandling var att undersöka hormonella och fysiologiska förändringar hos nyblivna mammor i samband med amning och även hos hundägare och hundar under tiden de interagerar med varandra.

Sextio-sex nyblivna mammors nivåer av oxytocin, prolaktin, cortisol och ACTH och även deras blodtryck registrerades under att amningstillfälle två dagar efter förlossningen. I samband med förlossningen hade somliga av mammorna fått antingen oxytocin infusioner för att påskynda värkarbetet och/eller epiduralbedövning (EDA) för smärtlindring.

I samband med amning två dagar senare hade de nyblivna mammorna en pulsatil frisättning av oxytocin och stigande prolaktinnivåer. I samband med amningen sjönk också deras nivåer av ACTH och cortisol och även deras blodtryck, vilket visade att amning har antistresseffekter. Mammor som fått oxytocin infusioner under värkarbetet visades ha dosberoende sänkta kroppsegna nivåer av oxytocin. Hade de dessutom fått EDA hade de också signifikant lägre oxytocin nivåer och signifikant högre cortisol nivåer och blodtryck jämfört med de mammor som fått enbart EDA under värkarbetet.

I den andra studien fick tio kvinnliga labrador- ägare umgås med sina hundar och både hundägarnas och hundarnas nivåer av oxytocin, kortisol, insulin och även deras puls registrerades under försöket. Resultaten visade att oxytocinnivåerna steg signifikant i både hundarna och deras ägare under tiden de interagerade med varandra. Hos både ägare och hundar sjönk pulsen under tiden de umgicks och hos ägarna sjönk även cortisol nivåerna.

Sammanfattningsvis visar resultaten i denna avhandling att effekterna av interaktion människor emellan, men också mellan människor och djur, uppvisar stora likheter. Båda typer av interaktion framkallar oxytocinfrisättning och oxytocin-relaterade effekter, som sänkta cortisolnivåer och sänkt blodtryck men också ökad social interaktion. Dessa

likheter beror antagligen på ett reaktionsmönster som verkar vara gemensamt för de flesta däggdjur.

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