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Oxytocin is a principal hormone that exerts part of its effects by active fragments

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ABSTRACT

Oxytocin is a nonapeptide consisting of a cyclic six amino-acid structure and a tail of three amino acids. It was originally known for its ability to induce milk ejection and to stimulate uterine contractions. More recently, oxytocin has been shown to stimulate social behaviors, and exert pain-relieving, anti-stress/anti-inflammatory and restorative effects.

We hypothesize that oxytocin is a principal hormone that, in part, exerts its effects after degradation to active fragments with more specific effect profiles.

Experimental findings on rats show that administered oxytocin exerts biphasic effects. For example, after an initial increase in pain threshold, a second more long-lasting increase follows. Blood pressure and cortisol levels initially increase and then reverse into a long-lasting decrease in blood pressure and cortisol. Whereas the initial effects are, the second-phase effects are not blocked by an oxytocin antagonist, but by an opioid mu-antagonist and by an alpha 2-adrenoreceptor antagonist, respectively, suggesting that other receptors are involved. Repeated administration of oxytocin induces multiple anti-stress effects, which are mediated by alpha 2-adrenoreceptors. Repeated administration of linear oxytocin and linear oxytocin fragments with a retained C-terminal reduce spontaneous motor activity, a sedative or anti-stress effect, suggesting that alpha 2-adrenoreceptors have been activated. In contrast, linear mid-fragments stimulate motor activity.

Low-intensity stimulation of cutaneous nerves in rats, as well as breastfeeding and skin-to-skin contact between mothers and babies, trigger immediate anti-stress effects. Some of these effects are likely caused by open ring/linear C-terminal fragments activating alpha 2-adrenoreceptors.

Oxytocin fragments may be pre-formed and released in the brain or created by metabolic conversion of the principal hormone oxytocin in the central nervous system. Oxytocin and its fragments may also be released from peripheral sites, such as peripheral nerves, the gastrointestinal tract, and blood vessels in response to decreased sympathetic or increased parasympathetic nervous tone. Smaller fragments of oxytocin produced in the periphery may easily pass the blood-brain barrier to induce effects in the brain.

In conclusion, oxytocin is linked to many different, sometimes opposite effects. The intact cyclic molecule may act to initiate social interaction and associated psychophysiological effects, whereas linear oxytocin and C-terminal fragments may induce relaxation and anti-stress effects following social interaction. In this way, the principal hormone oxytocin and its fragments may take part in a behavioral sequence, ranging from approach and interaction to calm and relaxation. Linear fragments, with an exposed cysteine-residue, may exert anti-inflammatory and antioxidant effects and thereby contribute to the health-promoting effects of oxytocin.

Introduction

Oxytocin

Chemical and morphological structure

Sir Henry Dale discovered oxytocin at the beginning of the 20th century, noting that it stimulated contractions of both the uterine muscles and the myoepithelial cells of the mammary gland, thus establishing its important role during labour and lactation.

In the 1950s, Du Vignaud discovered that oxytocin is a nonapeptide consisting of a cyclic part with a disulfide bond and a three amino-acid-long extension. Before being released, oxytocin is clipped off from a longer molecule that includes neurophysin (a carrier protein) in the

endoplasmic reticulum [1]. Sometimes more immature and longer oxytocin molecules extending from the carboxy-terminal end of the oxytocin molecule are found in the circulation [2].

Oxytocin is produced in neurons originating in the supraoptic (SON) and paraventricular nucleus (PVN) of the hypothalamus. Magnocellular neurons from the SON and the PVN project to the posterior pituitary, releasing oxytocin into the circulation to act as a hormone. Oxytocin is also released into the brain from dendrites and cell-bodies within the SON and PVN [3], as well as from axon-collaterals of the magnocellular neurons projecting to the posterior pituitary [4]. Also, oxytocin influences behavioural, physiological, and neuroendocrine functions via oxytocin neurons projecting from the PVN to different regulatory areas in the brain [5]. Recently, a local production of oxytocin has been

demonstrated in many peripheral organs, such as the cardiovascular system, gastrointestinal tract, uterus, ovaries, and adrenal gland. Oxytocin is also produced in certain cell types, such as endothelial cells and the keratinocytes of the epidermal layer of the skin [6–8].

Release of oxytocin

Oxytocin release is under multifactorial control. Other neurotransmitters, hormones, and sensory stimuli influence its release [9].

The oxytocin receptor

Today, researchers have only described one known oxytocin receptor that mediates oxytocin-linked effects. The oxytocin receptor is a G-protein coupled receptor, which was originally demonstrated in uterine muscular tissue. The same type of oxytocin receptor has been demonstrated in the brain and other peripheral tissues. However, human oxytocin receptors are connected to different types of G-proteins, which are associated with different second messengers within the cell and therefore, to different effects. The oxytocin receptor has been labelled the "promiscuous receptor" [10].

Effects of oxytocin

Behavioural and anti-stress effects

Oxytocin causes uterine contractility and milk ejection. It also stimulates various types of social-interactive behaviours, including maternal caring behaviour, and bonding between mothers and-infants and within couples [11–13].

Oxytocin reduces the levels of anxiety and pain [12,14,15]. Also, it may decrease stress levels, by reducing the activity in the hypothalamic-pituitaryadrenal (HPA) axis and sympathetic nervous system (for example, decreasing blood pressure) [16–18]. By contrast, it stimulates the activity in the parasympathetic nervous system including that of the vagal nerve leading to an increased digestive, anabolic, and restorative capacity [19,20].

Moreover, oxytocin increases wound healing and plasma levels of some growth factors and decreases inflammation [21,22]. Oxytocin delivered to rats via osmotic mini-pumps decreases both inflammation and atherosclerosis [23].

As oxytocin integrates effects linked to increased social interaction, decreases stress levels, and stimulates of restoration and growth, it has been labelled the hormone of growth and relaxation, or calm and connection [9].

Long-term effects in response to repeated treatment with oxytocin

Repeated administration of oxytocin has been shown to give rise to several sustained or long-term effects. For example, five daily injections of oxytocin (1 mg/kg subcutaneously (s.c.) or 1 microg/kg intracerebroventricularly (i.c.v)) to rats increased nociceptive thresholds, decreased blood pressure, pulse rate, and corticosterone (the equivalent to cortisol in the rat) levels for 1–3 weeks after administration of the last dose of oxytocin. In the same way, oxytocin changed the sensitivity of glucocorticoid and mineralocorticoid receptors in the brain and decreased the secretion of thyroid stimulating hormone (TSH) and thyroid hormones [15,17,18,24,25].

Also, oxytocin lowered basal levels of the gastrointestinal hormones, gastrin, and insulin, but enhanced feeding-induced responses of these hormones. Rats showed a sustained increase in the amount of spontaneous motor activity in response to repeated treatments with oxytocin [19,26]. All these long-term effects of oxytocin must have been induced within the brain since only 1–3 per ml of a dose of oxytocin given peripherally penetrates the blood-brain barrier, and a thousand-fold higher dose of oxytocin had to be given s.c. compared to i.c.v. to achieve the same effects.

Reversed effects of oxytocin in threatening or unfamiliar situations

Oxytocin may induce not only friendly, interactive behaviours but also defensive behaviours. When a mother experiences that her offspring is threatened, she may react with a defence reaction or maternal aggression towards the intruder. In these situations, blood pressure and cortisol levels would rise rather than decrease in response to oxytocin. This reversed effect of oxytocin can be observed in both males and females in certain stressful situations. Most often oxytocin stimulates the calm-and-connection response, or the growth-and- relaxation response [9], but if the environment is perceived as unfamiliar and hostile, opposite reaction patterns may be induced in defence of the individual or the offspring [27].

Administration of oxytocin to humans

Intravenous oxytocin has been used clinically since the 1960s to initiate and augment labour and to reduce bleeding after birth. In addition, intranasal administration of oxytocin has been used to promote milk let-down in breastfeeding women.

More recently, intranasal oxytocin spray has been administered to humans to stimulate various aspects of social-interactive behaviour and decrease anxiety and stress [28]. As a consequence of these results, intranasal oxytocin is being tested in clinical trials for the treatment of autism, schizophrenia, and stress-related disorders [29].

Local intravaginal application of oxytocin in a gel has been demonstrated to rejuvenate the vaginal mucosa and to relieve subjective symptoms of vaginal atrophy in menopausal disorders [30].

Hypothesis

We hypothesize that oxytocin is a principal hormone that, in part, exerts its effects after degradation to active fragments, which mediate different aspects of the effect pattern that can be induced by the intact, cyclic oxytocin molecule. The effects induced by the fragments may or may not be mediated via the classical oxytocin receptor. Opening of the cyclic structure of oxytocin produces linear oxytocin fragments, which may bind to other receptors than the classical oxytocin receptor. When linear fragments are formed, a cysteine residue will be exposed, which may play an important role in the effects caused by linear oxytocin and linear oxytocin fragments.

We also hypothesize that oxytocin fragments can be produced and released within the brain either directly or following immediate degradation of the principal hormone and that oxytocin fragments are formed not only in the brain but also at peripheral sites, such as peripheral nerves, the gastrointestinal tract and blood vessels. This latter pool of oxytocin/oxytocin fragments is readily activated following an increase in parasympathetic nervous tone or a decrease in sympathetic nervous tone. Smaller oxytocin fragments produced at peripheral sites may pass the blood-brain barrier and exert effects in the brain.

Evaluation of the hypothesis

Conclusions regarding the existence of active oxytocin fragments based on data obtained from animal experiments

Administration of oxytocin may induce a biphasic effect pattern. The effects observed during the second, delayed phase may or may not be identical to the one observed during the initial phase

As summarized in the introduction, oxytocin can induce long-lasting effects on the nociceptive threshold, blood pressure, and levels of cortisol (corticosterone in the rat) [15,17,18].

To establish the time-course for the appearance of these long-term effects generated by oxytocin, nociceptive thresholds, blood pressure, and cortisol levels were recorded repeatedly during 24 h after administration of oxytocin (1 mg/kg, s.c. or 1 microg/kg i.c.v.) to rats. These studies showed that the effects of oxytocin are, in fact, biphasic.

Administration of oxytocin induced an immediate, approximately 90 min long elevation of the nociceptive threshold. Six hours later, a second-phase increase of nociceptive threshold occurred, which was

sustained for 24 h after administration of oxytocin. If repeated injections of oxytocin (1 mg/kg, s.c. or 1 microg/kg i.c.v.) were given (once daily for five consecutive days), the effect on nociceptive thresholds was sustained for at least 10 days. In some experiments, an effect was still seen three weeks after the last injection.

The effects of oxytocin on blood pressure and corticosterone levels were also biphasic when oxytocin was administered s.c. The second-phase effects, however, differed from the initial responses caused by administration of oxytocin. Both blood pressure and corticosterone levels increased immediately following the administration of oxytocin s.c. This first-phase response was transient and was followed by a decrease in blood pressure and corticosterone levels after 6–10 h. These second-phase effects remained significant 24 h later and were also seen in response to oxytocin administered i.c.v. No increase in blood pressure or corticosterone levels was seen in response to oxytocin i.c.v.

Also, the effects in response to i.c.v. administration of oxytocin lasted for several weeks after the last administration of oxytocin [15,17,18,31].

If the same oxytocin treatment was administered postnatally to rat pups, the effects of oxytocin became even more long-lasting. When tested in adulthood, blood pressure and cortisol levels were lower, and nociceptive thresholds were increased in comparison to controls. Interestingly, also spontaneously hypertensive rats (SHR) had decreased blood pressure in adulthood if treated with oxytocin postnatally [32–35].

The delayed and long-term effects of oxytocin involve other signalling mechanisms than the oxytocin/oxytocin receptor interaction

Nociceptive thresholds. The immediate effect of oxytocin (1 mg/kg, s.c.) on nociceptive thresholds was antagonized by an oxytocin antagonist (Atosiban), indicating that this effect was mediated by activation of the oxytocin receptor. When the oxytocin antagonist was given concomitantly with five daily injections of oxytocin the development of the delayed/sustained increase in nociceptive thresholds was partly antagonized. By contrast, administration of the oxytocin antagonist did not counteract the established, long-term elevation of the nociceptive thresholds induced by five daily injections of oxytocin. Instead, administration of naloxone, a mu–receptor opioid antagonist, did [15].

In summary, the immediate increase in nociceptive thresholds seems to be mediated by the classical oxytocin receptor, whereas the delayed and sustained effect caused by repeated exposure to oxytocin is due to an increased function of mu–receptor signalling. In fact, both Atosiban and naloxone blocked the immediate effect of oxytocin on nociceptive thresholds, suggesting that oxytocin increases nociceptive thresholds by secondary activation of mu–receptors [15]. The sustained increase in nociceptive thresholds is mediated by a manifest increase in mu–receptor signalling and no longer linked to an activation of the oxytocin receptor. Indeed, several studies show that oxytocin increases the activity of mu-receptors [36].

Blood pressure. The initial rise of blood pressure caused by administration of oxytocin (1 mg/kg, s.c) was also at least partially antagonized by the administration of the oxytocin antagonist, Atosiban, suggesting that this effect was, in part, mediated by the oxytocin receptor. Oxytocin administered i.c.v. did not induce any immediate change in blood pressure, but blood pressure was decreased in response to five daily injections of oxytocin (1 mg/kg s.c or 1 microg/kg i.c.v.) [17]. Simultaneous administration of Atosiban did not block the long-term decrease in blood pressure. Nor was the established reduction in blood pressure following this treatment reversed by administration of the oxytocin antagonist. These results suggest that while the first-phase increase in blood pressure in response to oxytocin s.c. is mediated by, or at least involves, the classical oxytocin receptor, the second-phase decrease of blood pressure, as well as the prolonged decrease in blood pressure, are not [17].

To explore the mechanism behind the delayed, second-phase lowering of blood pressure, and the long-term lowering of blood pressure in response to repeated administration of oxytocin, the potential effect of different agonists and antagonists of (nor)adrenergic transmission, known to be involved in the regulation of blood pressure, were tested. The extensive pharmacological testing showed that oxytocin's effect on the lowering of blood pressure was inhibited by administration of the alpha 2-adrenoreceptor antagonist idazoxan [37]. These findings suggest that oxytocin increases the function of alpha 2- adrenoreceptors when decreasing blood pressure. In the brain, alpha 2-adrenoreceptors most often serve as inhibitors of noradrenergic transmission, which plays an important role in the regulation of stress levels, including blood pressure. Indeed, when an alpha 2-adrenoreceptor agonist (Clonidine) was administered to rats that had been treated with oxytocin for five days, the decrease in blood pressure induced by the clonidine treatment was enhanced, demonstrating that oxytocin had increased the function of alpha 2-adrenoreceptors [37].

Oxytocin-induced activation of alpha 2-adrenoreceptors gives rise to its anti-stress effects

Oxytocin's ability to activate alpha 2-adrenoreceptors was further studied using single-cell recordings of the noradrenergic cells in the locus coeruleus (LC), a nucleus in the brainstem that is of central importance for the control of stress reactions and blood pressure. Five daily injections of oxytocin (1 mg/kg s.c), previously shown to lower blood pressure and cortisol levels, decreased the firing of the noradrenergic cells in the LC by 50%, in comparison to saline-treated controls [38].

Immuno-histological techniques have also established a changed function of brain alpha 2-adrenoreceptors in response to oxytocin treatment. Treatment with oxytocin according to the schedule above increased the density of alpha 2-adrenoreceptors (e.g., within the hypothalamus, amygdala, and the nucleus of the solitary tract (NTS)). These areas of the brain control stress reactions, including the HPA axis and the sympathetic nervous system, and thereby the regulation of cortisol levels and blood pressure [39,40].

Rat pups treated with five daily injections of oxytocin (1 mg/kg s.c) in the postnatal period were also found to have an increased density of alpha 2-adrenoreceptors when they were studied later in adulthood [41]. This effect may be linked to the life-long reduction in stress levels observed after postnatal administration of oxytocin [32–35].

Conclusion A 1–3

We interpret these findings to mean that oxytocin produced immediate as well as delayed effects on nociceptive thresholds, blood pressure, and corticosterone levels.

These results further indicate that some of the delayed and long-term effects of oxytocin are mediated by other receptors than the classical oxytocin receptor, e.g., mu-opioid receptors (pain) and alpha 2-adrenoreceptors (anti-stress effects, such as lower blood pressure and cortisol levels). We suggest that these effects may be induced by fragments of the principal hormone oxytocin that interact more specifically with these receptors than the principal hormone itself.

Conclusions regarding the existence of active oxytocin fragments based on the metabolic pathways involved in the degradation of oxytocin and the chemical structure of oxytocin and its fragments

Formation of active oxytocin fragments via enzymatic degradation of the principal hormone oxytocin

Peptide hormones are often metabolized to active fragments by enzymatic degradation. The peptide fragments formed can be more or less active, or even have opposite effects to the original principal peptide. Enzymes cause the degradation that can be located in the circulation, or specific organs, such as the brain and liver [42].

Oxytocin degrades along several different metabolic pathways. Single amino acids may be clipped off from the carboxy-terminal three amino acid long extension of the molecule, and then oxytocin 1–8 and

1–7 may be formed. Another pathway by which oxytocin may be degraded is by opening of the cyclic structure of the oxytocin molecule between the tyrosine and cysteine residues. Thereby, oxytocin loses its cyclic structure and is transformed into a linear type of oxytocin (cyt6-oxytocin2-9). Thus, in spite of containing the same amino acids, the cyclic oxytocin and the linear molecule (cyt6-oxytocin2-9) have different molecular configurations. From this linear oxytocin molecule amino acids may be clipped off from both the amino and carboxy-terminal ends to create a multitude of fragments including (cyt6-oxytocin3-9), (cyt6-oxytocin4-9), and (cyt6-oxytocin3-7) [43,44]. Some of the oxytocin fragments are biologically active and have an amnesic effect, in particular (cyt6-oxytocin4-9) [45–48].

The enzymes (aminopeptidases) responsible for the conversion of oxytocin into fragments have been demonstrated, both in the periphery and the brain, which support the assumption that oxytocin may be degraded into one or several active fragments in all types of tissues [43,44,49]. Which fragment(s) is/are produced may vary depending on the type and amounts of degrading enzymes that are present in the different locations. Interestingly, the function of the enzymes that degrade oxytocin can be affected by other peptides, as well as by the sympathetic nervous system, indicating that different fragments may be produced under different circumstances [50].

Conclusion B1

Oxytocin is degraded into smaller fragments by well-identified enzymatic mechanisms.

Two types of fragments are formed, those that retain the cyclic structure of the molecule, but in which the tail of three amino acids is shortened (cyclic fragments), and those in which the cyclic structure of oxytocin is opened up to form linear oxytocin or linear fragments. The size of the linear fragment can be reduced in length by amino acids being cleaved off from both the amino and carboxy-terminal sides of the molecule.

Administration of an inhibitor of enzymatic degradation may block the development of active fragments and their effects

To support the assumption that some of the secondary and long-lasting effects of oxytocin are caused by active fragments of oxytocin, pilot experiments were performed to study the role of enzymatic degradation of oxytocin. An inhibitor of enzymatic degradation, pepstatin A, was administered to rats together with oxytocin (1 mg/kg s.c) for five days and, after that, blood pressure was measured. Interestingly, the decrease in blood pressure normally seen in response to repeated administration of oxytocin did not occur in the animals that were treated with the inhibitor of enzymatic degradation. The results of these pilot experiments support the assumption that oxytocin must be degraded into an active fragment to decrease blood pressure. These results are also in agreement with experiments showing that the antidepressant effects of oxytocin are blocked by the previous administration by an enzymatic inhibitor [46].

Administration of oxytocin and oxytocin fragments influence spontaneous locomotor behaviour differently

Administration of oxytocin influences spontaneous motor behaviour in rats in a complex way. A single dose of oxytocin increases (low dose) or decreases (high dose) spontaneous motor activity in rats [51]. Repeated administration of oxytocin (1 mg/kg s.c. once daily for five days) increased spontaneous motor activity [26]. To investigate if different fragments of oxytocin affect spontaneous motor activity differently, we performed pilot experiments in which we recorded spontaneous locomotor behaviour in a photocell-equipped open-field arena. Six different fragments—the cyclical fragments, oxytocin (1–8) and (1–7); and the linear fragments (cyt6-oxytocin2-9), (cyt6-oxytocin3-9), (cyt6-oxytocin4-9), and (cyt6-oxytocin3-7) were studied after daily administration i.c.v. of 0.3 nmol of each fragment over five days to male Sprague-Dawley rats ($n = 5$–6). Controls (receiving saline) were run in parallel to all groups. The animals visited the photocell-equipped open-field arena for the first time and were observed for 15 min.
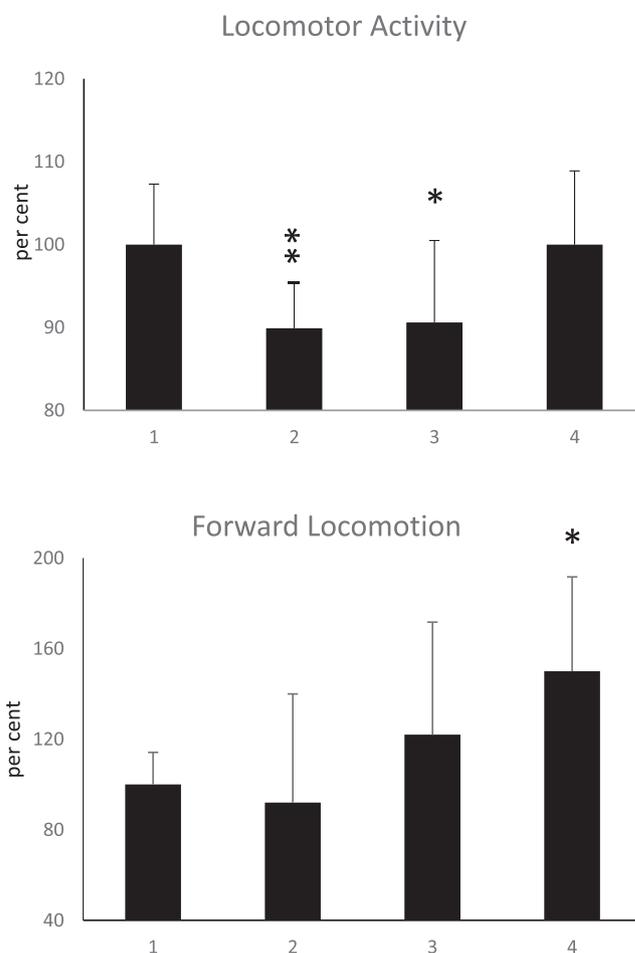

Fig. 1. Locomotor activity and forward locomotion during 15 min in male rats 2–3 days after a 5-day treatment period i.c.v. with 1. NaCl (n = 16), 2. Cyt6-oxytocin(2-9) (0.3 nM) (n = 6), 3. Cyt6-oxytocin(4-9) (n = 5) (0.3 nM) and 4. Cyt6-oxytocin(3-7) (0.3 nM) (n = 5). Statistical evaluation was performed by a one-way ANOVA followed by Fisher LSD test for post-hoc comparisons. **$p < 0.01$, *$p < 0.05$.

The parameters recorded are defined as follows: *Locomotor activity* (all horizontal activity registered by photocells), *Forward locomotion* (horizontal activity, when the animal is moving in the same direction), and *Peripheral activity* (horizontal activity within 25 mm from the wall) [51].

Results. (Cyt6-oxytocin2-9) and (cyt6-oxytocin4-9) decreased locomotor activity ($p < 0.05$), (cyt6-oxytocin3-7) increased forward locomotion ($p < 0.05$) and (cyt6-oxytocin(4-9)) tended to increase peripheral activity ($p = 0.08$), when compared to saline treated controls (Fig. 1).

Oxytocin(1-8), oxytocin(1-7) and (cyt6-oxytocin3-9) did not influence spontaneous motor activity.

In summary, i.c.v. administration of fragments of oxytocin, in which the cyclic part of the oxytocin molecule was intact, but in which the length of the three-peptide-long extension was modified, did not influence spontaneous motor activity in comparison to controls. Oxytocin, in which the cyclic structure of oxytocin had been opened up (i.e., the linear oxytocin cyt6-oxytocin2-9 and the linear fragment of oxytocin cyt6-oxytocin4-9) and in which the carboxy-terminal end of the molecule (pro-leu-gly) was retained, decreased motor activity compared to controls. This effect is consistent with sedation or an anti-stress effect, and it can, therefore, be assumed that these linear fragments of oxytocin activate the alpha 2-adrenoreceptor. In previous experiments, an amnesic effect has been demonstrated by C-terminal

fragments, such as cyt6-oxytocin4-9) [46–48].

By contrast, a smaller, linear oxytocin fragment (cyt6-oxytocin3-7) containing the middle-6 amino acids of oxytocin stimulated forward locomotor activity significantly more compared to the control group. These data suggest that the mid portion of the oxytocin molecule stimulates spontaneous forward motor activity. As forward locomotor activity has been linked to activation of the dopamine system, the mid portion of the oxytocin molecule might stimulate dopaminergic function.

Conclusion B2-3

Administration of cyclic oxytocin and of a short linear fragment of oxytocin (cyt6-oxytocin3-7) stimulated spontaneous forward locomotor activity, whereas linear fragments of oxytocin with a retained carboxy-terminal decreased spontaneous motor activity. The stimulation of forward locomotor behavior may be linked to an increase in dopaminergic function [52]. The inhibitory effect is likely to be mediated by alpha 2-adrenoreceptors and is a part of the anti-stress pattern exerted by linear oxytocin fragments with an intact carboxy terminal.

The role of cysteine residues in linear fragments

Many different mechanisms may exert the anti-inflammatory effects linked to oxytocin. Some of them are exerted in the brain by inhibiting the HPA axis and the sympathetic nervous system and may in part be mediated by changes in alpha 2-adrenoreceptor activity.

Oxytocin, however, also exerts powerful anti-inflammatory and antioxidant actions by local effects in central and peripheral tissues. Oxytocin reduces inflammation caused by carrageenan and decreases levels of interleukins, peroxidase formation, and increases glutathione levels [22,23,53]. In addition, it stimulates growth and differentiation of normal cells but inhibits the growth of some cancer cell lines obtained from breast, ovary, bone, and colon [54]. All these effects of oxytocin may contribute to its health-promoting effects.

A clue to the mechanism by which oxytocin exerts its local anti-inflammatory, and antioxidant effects can be obtained by comparing the anti-inflammatory/antioxidant effects of oxytocin with those that are obtained after administration of N-acetyl-cysteine (NAC). NAC consists of an acetylated-cysteine molecule and inhibits inflammation, promotes growth, and induces differentiation of cells. The effect of NAC is supposed to be mediated by the thiol group of cysteine and, in addition, NAC is an important precursor to glutathione [55,56].

When linear oxytocin fragments are formed, the oxytocin molecule opens up between the amino acid residues, tyrosine, and cysteine. As a consequence, a cysteine residue will form an extension from the linear peptide cyt6-oxytocin2-9 and in this way a cysteine residue becomes available. It is, therefore, likely that the principal hormone oxytocin is metabolized to linear fragments before it exerts these types of anti-inflammatory and antioxidant effects. NAC may be formed as a metabolite of oxytocin, showing that the cysteine residue may be split off from the linear fragments of oxytocin to exert effects by itself or after acetylation in the liver to form NAC.

Conclusion B4

When the cyclical oxytocin molecule is opened up between the amino acids, tyrosine, and cysteine, a linear variant of oxytocin is formed containing the same amino acids as the cyclical form of oxytocin. One cysteine residue (one of the two cysteines being linked together by a disulfide bond within the oxytocin molecule) now extends from the linear oxytocin at position 6. The cysteine, and more specifically, the thiol groups, are likely to induce some of the anti-inflammatory and antioxidant effects of oxytocin. The inhibitory effect of oxytocin on certain cancer cell lines could also be linked to this mechanism.

Conclusions regarding the existence of active oxytocin fragments based on observations following stimulation of sensory nerves in animal experiments and breastfeeding and skin-to-skin contact between mother and infant

Stimulation of low-threshold (non-noxious) cutaneous sensory nerves in rats induces immediate oxytocin-mediated increase of pain threshold and anti-stress effects

Stimulation of non-noxious cutaneous nerves in rats induces, in addition to a release of oxytocin into the cerebrospinal fluid and circulation, increased pain threshold, as well as powerful and immediate anti-stress effects, such as lowering of blood pressure and cortisol levels [57–60]. These data are in line with the results from the previously reported experiments in which oxytocin administered to rats, via central mechanisms, increased pain threshold and decreased stress levels, such as blood pressure and cortisol levels [15,17,18].

In addition, the increase in pain threshold induced by nervous stimulation was blocked by administration of an oxytocin antagonist, suggesting that the effect was mediated via the classical oxytocin receptor [58]. This is in line with the results reported above regarding administration of oxytocin. The decrease in blood pressure caused by nervous stimulation was, however, not blocked by administration of an oxytocin receptor antagonist, suggesting the involvement of another type of receptor [57]. As reported previously, the anti-stress effects observed after administration of oxytocin in rats were not blocked by the oxytocin receptor antagonist [17,18] indicating that the anti-stress effects were not induced by the principal oxytocin molecule itself, but rather by a fragment of oxytocin. In fact, the data from the previously reported results suggest that the anti-stress effects of oxytocin might be mediated by linear oxytocin, or linear C-terminal fragments linked to activation of alpha 2-adrenoreceptors, and not by the principal hormone oxytocin itself. It is likely that the same fragments induce the anti-stress effects observed also after nervous stimulation.

Surprisingly, the anti-stress effects occurred almost immediately after onset of the nervous stimulation, and not after a delay, as in the experiments in which oxytocin was administered. To allow for the immediate anti-stress effects, either one or several preformed oxytocin fragments with anti-stress effects should be released from nerves in the brain, or oxytocin, released from nerves, should be immediately degraded to one or several active fragments.

Breastfeeding and skin-to-skin contact between mother and infant after birth induce immediate anti-stress effects

Some further information as to the physiological role of oxytocin and its active fragments can be deduced by studying effects, which are induced in the mother and the baby during breastfeeding and skin-to-skin contact after birth. Both breastfeeding and skin-to-skin contact between mother and infant immediately after birth are linked to a release of oxytocin and to some physiological adaptations, including an increased pain threshold and powerful anti-stress effects [9,61].

In the experiments reported above (C 1), low-intensity stimulation of nerves from the skin were associated with immediate anti-stress effects. Similarly, the anti-stress effects induced by breastfeeding and skin-to-skin contact occurred almost immediately after the onset of the stimulation. It is, therefore, possible that the immediate anti-stress effects observed in response to breastfeeding or skin to skin are not only induced by oxytocin but also by oxytocin fragment(s), which might correspond to linear oxytocin or linear C-terminal fragments linked to activation of alpha 2-adrenoreceptors [9,61,62].

Conclusion C1-2

The rapid onset of the anti-stress effects caused by non-noxious stimulation of nerves from the skin in rats, as well as by breastfeeding and, in particular, skin-to-skin contact between mothers and babies, speaks in favor of a direct release of preformed fragment(s) of oxytocin with anti-stress properties, or an immediate degradation to such fragments of oxytocin secreted from the neurons in areas of the brain involved in stress regulation.

Suckling and skin-to-skin contact between mother and baby decrease stress levels via different oxytocin linked mechanisms; the HPA axis and the sympathetic nervous system respectively

As mentioned in the previous section, both breastfeeding and skin-to-skin contact increase pain threshold and induce powerful and instant anti-stress effects [61,63,64], effects that we suggest are induced by the principal hormone oxytocin and/or linear fragment(s) of oxytocin within the brain.

Despite these similarities, there are some important differences between the effect patterns caused by suckling and skin-to-skin contact regarding oxytocin levels and the mechanism by which cortisol levels controlled indicating a more important role for oxytocin fragments in skin-to-skin contact.

Suckling during breastfeeding stimulates the release of oxytocin from the oxytocin-producing cells in the SON and PVN in the hypothalamus, which is reflected by a pulsatile pattern of oxytocin in the circulation. At the same time, oxytocin released within the brain decreases the activity in the HPA axis and thereby the levels of ACTH, the pituitary hormone that stimulates cortisol secretion as well as cortisol levels [9,12,27,61,62]. Skin-to-skin contact does not give rise to a pulsatile oxytocin pattern and surprisingly, skin-to-skin contact in spite of giving rise to profound and rapid anti-stress effects, including decreased cortisol levels, is not associated with decreased activity in the HPA axis and levels of ACTH [61,62]. Cortisol secretion can, however, be influenced without the involvement of the HPA axis. Increased activity within the sympathetic nervous system increases cortisol levels by increasing the affinity of ACTH to its receptors in the adrenal cortex. Consequently, a decreased sympathetic-nerve activity (or increased parasympathetic-nerve activity) will decrease the binding of the ACTH receptors in the adrenal cortex, thereby lowering cortisol levels [61,62,65].

The two different stress regulatory systems; the one involving the hypothalamus and the HPA axis and the one involving the sympathetic nervous system may be activated in different situations; suckling involves the HPA axis, whereas skin to skin contact, via stimulation of cutaneous nerves, preferentially involves the autonomic nervous system. The two ways of regulating cortisol secretion can be demonstrated in cows, where suckling regulates cortisol levels via the HPA axis, whereas stroking of the skin in front of the udder decreases the activity in the sympathetic nervous system [9,61,62,66]. The skin mediated inhibitory effect on cortisol levels is most likely mediated by the linear oxytocin fragments with a retained carboxy-terminal, which give rise to anti-stress effects by activation of alpha-2-adrenoceptors, in brain areas involved in the control of the autonomic nervous system.

Not only cortisol secretion is modulated in this way in response to skin-to-skin contact. Also, blood pressure and heart rate would be decreased in response to a decreased activity of the sympathetic nervous system caused by such linear oxytocin fragments.

Are oxytocin fragments produced and released outside the brain?

It has recently been shown that oxytocin is not only produced in the hypothalamus, but also peripheral tissues, such as the gastrointestinal tract, cardiovascular system, uterus, ovaries, skin, and mucosa [8]. It would be reasonable to assume that fragments of oxytocin, (e.g., with stimulatory or anti-stress properties) are also produced outside the brain.

Oxytocin is produced in blood vessels, and it is, therefore, reasonable to assume that also oxytocin fragments are produced or that oxytocin is metabolized to linear fragments in the blood vessels. The blood vessels of the skin could, therefore, be an additional source of oxytocin fragments in response to suckling and skin-to-skin contact. In these situations, skin temperature increases in both mother and baby because of increased flow in the blood vessels of the skin [67]. The increased blood flow occurs as a consequence of decreased tone in the muscular sphincters regulating blood flow. Linear oxytocin, or linear fragments, which stimulate alpha 2-adrenoreceptors, should have this relaxing action on the muscles in the blood vessels and should thereby promote blood flow.

Subtle stimulation of the skin during skin-to-skin contact (e.g., by touch, warmth, light pressure, and stroking) induces vasodilation in the skin via a decreased activity in the sympathetic nerves. This decrease in sympathetic tone may trigger the release of oxytocin fragments with alpha-2-adrenoceptor activity from the blood vessels and thereby induce vasodilation, increased blood flow, and increased skin temperature [9,61].

Fragments of oxytocin may also be released from other parts of the body in response to enhanced parasympathetic or decreased sympathetic nervous tone (e.g., from the gastrointestinal tract during feeding and digestion).

Oxytocin fragments released from peripheral compartments may influence brain function

As suggested above, fragments of oxytocin may be produced or released from the blood vessels and other peripheral compartments. As reported previously, in addition to linear fragments with a retained carboxy-terminal, which presumably activates alpha 2-adrenoreceptors, smaller linear mid fragments of the principal, cyclic oxytocin molecule, which promote motor activity, possibly via increased dopaminergic activity exist and such fragments may also be produced outside the brain. Skin-to-skin contact may induce a release also of such small activating oxytocin fragments from blood vessels or other peripheral sites.

The oxytocin molecule itself does not pass the blood-brain barrier. By contrast smaller, linear fragments of oxytocin may more easily pass the blood-brain barrier and, therefore, such fragments might when released from peripheral sites, influence brain function. As the smaller linear mid fragments of the principal, cyclic oxytocin molecules influence dopamine activity, they may stimulate not only social interaction but also promote other dopamine linked action such as wellbeing in the mother and baby, and bonding between the two after birth, and later during breastfeeding.

It is, of course, also possible that similar fragments with anti-stress or activating properties are released from the gastrointestinal tract not only during feeding but also in other situations when vagal nerve activity is stimulated.

Conclusion C 3–5

Oxytocin and oxytocin fragments are produced and released from peripheral compartments, such as the blood vessels and gastrointestinal tract. Such fragments, with anti-stress or activating effects, may contribute to the effects observed during skin-to-skin contact and other situations of closeness. Smaller fragments may even pass the blood-brain barrier and influence brain function. The peripheral pools of oxytocin are under control of the autonomic nervous system. A decrease of sympathetic nervous activity is linked to a release of oxytocin and fragments from the blood vessels, and the parasympathetic/vagal nerves stimulate the release of oxytocin from the gastrointestinal tract. Mild and pleasant stimulation of the skin, such as light pressure, stroking, and warmth, induces activation of non-noxious sensory fibres in the skin, which decreases sympathetic nervous activity and gives rise to increased parasympathetic activity and hence a release of oxytocin and its fragments from peripheral sites.

Different oxytocin levels/profiles with RIA and ELISA support the existence of linear oxytocin fragments in the circulation

An interesting, and at first confusing, observation is that different patterns of oxytocin levels are observed in response to skin-to-skin contact when radioimmunoassay (RIA) or enzyme-linked immune assay (ELISA) are used to measure oxytocin levels. With RIA, a rise of oxytocin levels is observed during skin-to-skin contact, only in response to the newborn's hand massage of the mother's breast [68]. No rise in oxytocin levels is observed in the newborn. By contrast, a protracted rise of oxytocin levels in response to skin-to-skin contact is observed in mothers, and in fathers and newborns, when oxytocin is measured with ELISA [69]. These differences may be linked to different characteristics

of the two methods used. The antibodies used in the RIA are known only to recognize the intact cyclic oxytocin molecule [58]. The antibodies used in the commercially available ELISA-kit may be less specific regarding its binding properties and may, therefore, also detect linear oxytocin fragments in addition to the principal hormone oxytocin itself [70]. These data (i.e., the different response patterns obtained by RIA and ELISA) are consistent with the suggestion that linear oxytocin fragments are released in response to skin-to-skin contact. The RIA does not record them because they lack the cyclical structure of the principal hormone oxytocin, but they are with the less specific ELISA, that can probably also detect linear fragments [70,71].

Consequences of the hypothesis and discussion

In the present article, we have hypothesized that some of the effects by the principal hormone oxytocin are, in fact, exerted by active fragments of oxytocin. It must be pointed out that this is a hypothesis, and further experiments must be performed to prove that this assumption is correct. Other mechanisms may of course contribute, and it is, for example, known that the oxytocin receptor (sometimes called the promiscuous receptor) can activate different intracellular pathways, which can induce different and sometimes opposite effects [10]. Another mechanism that might influence the effects of oxytocin are the polymorphisms that exist in the oxytocin and oxytocin-receptor genes. The role of such genetic variations is presently being evaluated, and the single nucleotide polymorphism rs53576 in the oxytocin receptor gene has been associated with a multitude of effects [72,73].

However, the oxytocin antagonist does not block the delayed and sustained effects that we have attributed to active oxytocin fragments. We, therefore, consider it more likely that these fragments of oxytocin are indeed active and may exert more specific effects than the principal cyclic hormone oxytocin itself. The oxytocin fragments may interact directly with other receptors, such as opioid receptors and alpha 2-adrenoreceptors, and also directly or indirectly with several other types of signaling pathways including dopaminergic, serotonergic, and cholinergic pathways [9].

Alpha 2-adrenoreceptors and the system of energy conservation

The active oxytocin fragments seem to be involved in different clusters of effects of oxytocin. For example, the anti-stress effects seem to be mediated through alpha 2-adrenoreceptors by linear fragments, with an intact carboxy-terminal, which has been demonstrated regarding the decrease of cortisol levels and blood pressure and, in fact, any effect that is regulated by central noradrenergic transmission. Also, the positive effect on the release of gastrointestinal hormones of oxytocin involves alpha 2-adrenoreceptors. The effects of oxytocin on the levels of glucocorticoid and mineralocorticoid receptors in the brain, and on thyroid hormones, may also be mediated by alpha 2-adrenoreceptors. All these effects tend to reduce stress levels and stimulate growth and restoration. The alpha 2-adrenoreceptor system has been labelled the system of energy conservation. This system can be activated in different situations when the need for energy is of extra importance, such as during female reproduction [61] or in any situation, when conservation of energy is important, e.g., as a response to the shortage of food.

Other effect patterns of oxytocin may, of course, be linked to activation of other signalling systems.

A behavioural sequence giving rise to health-promoting effects

It's important to note that oxytocin may be involved in several types of interaction, by promoting interaction and approach behaviors (the principal hormone oxytocin and possibly small linear fragments), which is then followed by sedation and relaxation (the linear fragments of oxytocin with an intact carboxy-terminal region). All types of positive

interaction, not only between mother and child, might involve these sequential components. The release of oxytocin and its fragments will also be linked to several types of health-promoting effects, by the anti-stress effects as well as by the anti-inflammatory and antioxidant actions and restorative functions. Together, these effects may explain why positive relationships, which involve repeated interaction and oxytocin release, exert such positive effects on health [9]. From this perspective, it is of particular interest that several of the effects of oxytocin become long-lasting in response to repeated administration of oxytocin.

Clinical applications

Recently the literature concerning the psychological effects of oxytocin has exploded. A role of oxytocin for different aspects of social behaviors, interaction, bonding etc. has been demonstrated, using observations, questionnaires and brain-imaging techniques. In addition, its role in different pathological states, such as depression, schizophrenia, anxiety disorders, and in particular autism have also been discussed [74,75].

Intravenous administration of oxytocin is not suitable for long-term treatments, nor is oral administration as oxytocin is broken down in the gastrointestinal tract, and is not absorbed from the gastrointestinal tract in any significant amounts. Oxytocin sprays for nasal administration have been developed for the promotion of the milk let-down reflex in breastfeeding women. This spray has also been administered in clinical studies at higher dose levels. Although the results are sometimes conflicting several studies show positive effects on several aspects of social interaction, such as duration of eye contact [76], social salience [77] and brain functioning [78,79]. Positive effects have also been demonstrated in the treatment of autism in schizophrenia, in particular in response to high doses of oxytocin [80,81]. In addition, some studies indicate very strong effects in the treatment of anxiety disorders and posttraumatic stress disorders (PTSD) [29,82].

As some of the effects of oxytocin seem to be exerted by active fragments, the use of different oxytocin fragments as pharmaceutical agents would be an attractive way to induce specific effects. These suggestions are supported by the finding of positive effects of oxytocin fragments in an animal model for autism [47]. Nothing is, of course, known regarding the potential effects of the oxytocin fragments described in this study regarding psychiatric disorders. The fact that the fragments differently influenced social behavior and stress levels opens up the possibility of more specific effects in the treatment of e.g., autism spectrum disorders, and stress- and anxiety-related disorders. The smaller mid fragments may be advantageous in the treatment of, for example, autism spectrum disorders. The mid fragment might also be linked to a release of dopamine and reward.

The longer linear fragments with an intact carboxyterminal may be of greater advantage in the treatment of anxiety and stress-related disorders. Such fragments might also be of importance in the treatment of some type of cardiovascular disease, such as high blood pressure, and other stress-related diseases, such as metabolic syndrome and polycystic ovarian syndrome (PCOS). The linear and smaller fragments might also be easier to administer because they would presumably pass biological membranes more easily than the principal cyclical form of oxytocin. Also, fragments in which the cysteine residue is exposed may be of therapeutic importance since they may decrease inflammation and induce antioxidant effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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