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Central oxytocin and reproductive behaviours

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Oxytocin is a neurohypophyseal hormone that has long been associated with uterine contraction during parturition and milk ejection during nursing. Recent studies have suggested that oxytocin is also a neurotransmitter that has central effects important for reproduction, including the initiation of parental and sexual behaviours. This review describes oxytocin pathways in the brain and examines their regulation by gonadal steroids. Brain oxytocin receptors are remarkable for their plasticity and for striking species differences in their distribution. The molecular characterization of this receptor has provided several clues to the regulation of its expression. Comparative and transgenic studies suggest that central oxytocin release may influence reproductive behaviours, but the importance of these central effects depends on the pattern of expression of the receptor – a pattern that is species-specific.

Early in this century, Sir Henry Dale reported that a substance in posterior pituitary extracts induced uterine contractions (Dale, 1909). In 1953, du Vigneaud and his colleagues published the sequence of this 'oxytocic' substance, demonstrating a nine amino acid peptide with a disulfide bond creating a ring structure (du Vigneaud *et al.*, 1953). In time, oxytocin was shown to be the phylogenetically newest member of an old family of nine amino acid peptides of similar structure. Although ancestral forms were found in various vertebrates (Archer, 1974) and even invertebrates (van Kesteren *et al.*, 1992), oxytocin was shown to be almost exclusively mammalian. Perhaps it is not simply coincidence that oxytocin has been implicated in two prototypically mammalian aspects of reproduction: uterine contraction during labour and milk ejection during nursing.

Until recently, a typical textbook description of oxytocin would note its synthesis in the hypothalamus, transport to the posterior pituitary, and release in response to cervical dilatation (uterine contraction) or suckling (milk ejection). While this description is basically correct, it does not explain why males have oxytocin, why increased oxytocin is often not detected during labour, or how oxytocin affects memory, stress responses and renal function. We now know that oxytocin is made in several peripheral tissues, including testis, ovary, uterus and placenta (reviewed in Gainer and Wray, 1994). Indeed, at parturition, the concentration of oxytocin mRNA in the rat uterus may be 70 times higher than that in the hypothalamus (Lefebvre *et al.*, 1992). At parturition the uterus may be influenced by local paracrine release of oxytocin even in the absence of increased circulating concentrations of the hormone from the pituitary. In addition to this potential paracrine role within the uterus, the presence of oxytocin receptors in a great number of tissues, including the brain, kidney, thymus, as well as male and female reproductive tracts, suggests that the traditional description of oxytocin as an endocrine hormone for labour and nursing will need to be revised to include several new physiological roles (Barberis and Tribollet, 1996).

This paper will review the role of oxytocin within the brain as a neurotransmitter, focusing on its effects on reproductive behaviour. Four general points will be emphasized. First, the effects of oxytocin depend not only on synthesis and release of the peptide in the brain, but also on the distribution and regulation of central receptors. Second, both oxytocin and oxytocin receptors in the brain, like receptors in uterine and mammary tissue, are regulated by gonadal steroids. Third, both the distribution and the regulation of oxytocin receptors vary depending on the species studied. Finally, oxytocin within the CNS (central nervous system) regulates the onset of reproductive behaviours, but these effects are species-specific and dependent on gonadal condition.

Oxytocin as a neurotransmitter

Oxytocin is synthesized primarily in two hypothalamic nuclei: the paraventricular (PVN) and the supraoptic (SON) nuclei, where it is transcribed as part of a precursor molecule, neurophysin (reviewed in Gainer and Wray, 1994). In addition to neurosecretory projections to the posterior pituitary, oxytocin cells in the parvocellular regions of the PVN project to a broad array of central targets: rostrally to the olfactory bulb, diffusely through the telencephalon, and caudally into the brainstem and spinal cord (Buijs, 1978; Swanson and Kuypers, 1980; Sofroniew and Weindl, 1981). Oxytocin may be the predominant PVN peptide with autonomic projections – with terminals evident in both sympathetic and parasympathetic centres (Sawchencko and Swanson, 1982). Ultrastructural studies have demonstrated that these 'extra-hypothalamic' oxytocin projections make classic synaptic contacts (Voorn and Buijs, 1983) from which oxytocin is released after potassium or veratridine induced depolarization (Buijs and van Heerikhuize, 1982). This central oxytocin pool may be considered independent of neurohypophyseal oxytocin release, at least in the sense that CSF (cerebrospinal fluid) and plasma oxytocin responses to various stimuli are not correlated (Perlow *et al.*, 1982; Jones *et al.*, 1983; Kendrick *et al.*, 1986).

Oxytocin receptors in the brain

A receptor for oxytocin has been cloned and sequenced, initially from human uterus (Kimura *et al.*, 1992) and more recently

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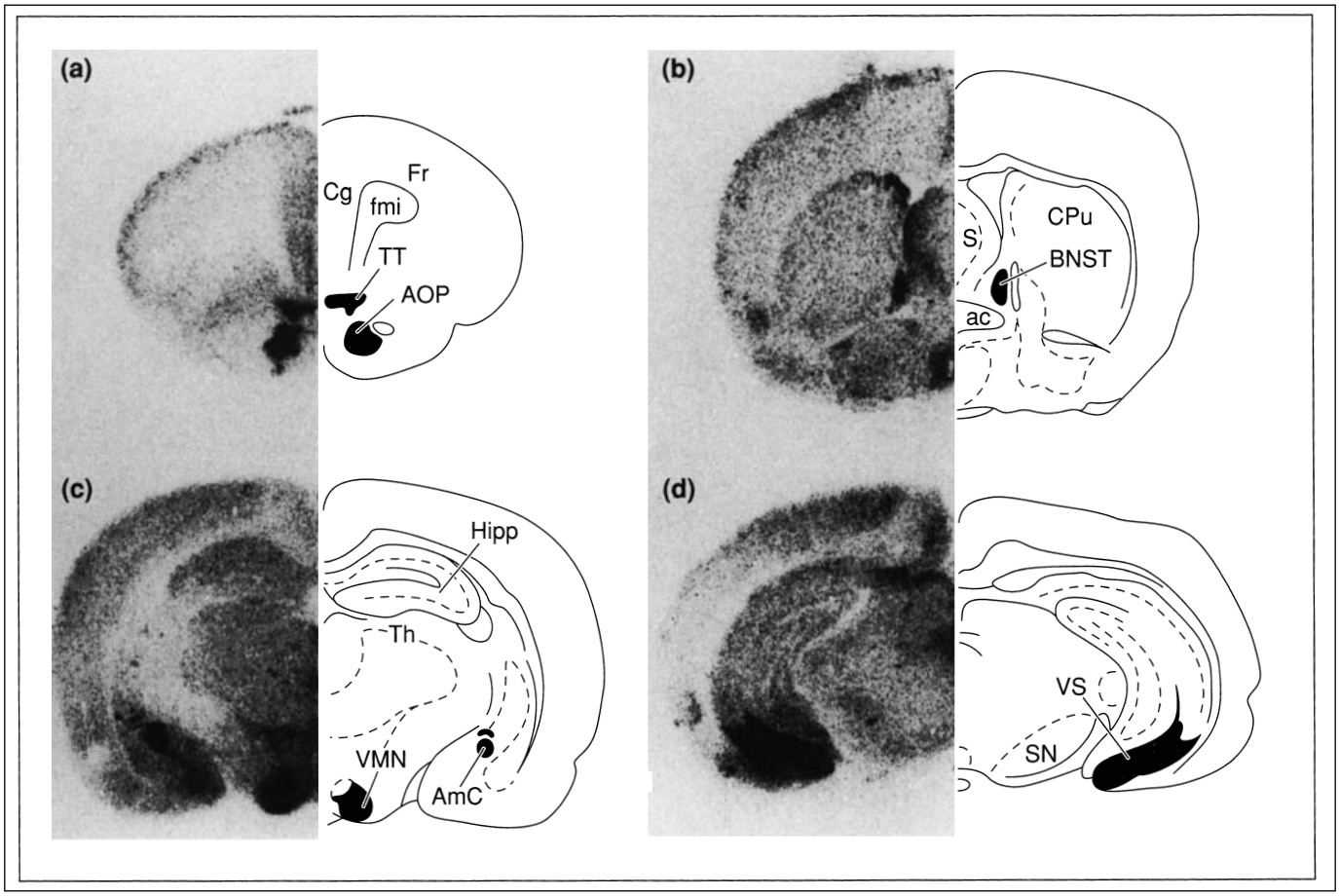

Fig. 1. Brightfield autoradiograms of [^{125}I]-labelled oxytocin antagonist (OTA) binding show oxytocin receptor localization in the rat brain coronal sections from most rostral (a) to most caudal (d). Binding is highest in the anterior olfactory nucleus (AOP), tenia tecta (TT), bed nucleus of the stria terminalis (BNST), ventromedial nucleus of the hypothalamus (VMN), central nucleus of the amygdala (AmC), and ventral subiculum (VS). Other abbreviations: Fr (frontal cortex), Cg (cingulate), S (septum), CPu (caudate-putamen), Hipp (hippocampus), Th (thalamus), SN (substantia nigra), fmi (fornix major), ac (anterior commissure).

from the parturient uteri of rats (Rozen *et al.*, 1995), cows (Ivell *et al.*, 1995) and voles (Young *et al.*, 1996a). The cDNA for the human oxytocin receptor encodes a 389 amino acid protein which is structurally within the rhodopsin family of G protein-coupled, seven transmembrane-spanning domain receptors. Although this receptor is coupled to a G protein, we still know relatively little about the intracellular consequences of receptor binding.

The human oxytocin receptor gene is located close to 3p26.2 of chromosome 3 (Kimura, 1995). Studies of gene structure demonstrate four exons in humans and three exons in other species, with the coding region represented completely in the last two exons, separated by a 12kb intron. The coding region shares more than 90% homology across species, with roughly 40–50% homology with other nonapeptide receptors, such as V1a and V2.

The distribution and regulation of the brain oxytocin receptor has been studied most extensively in rats (Barberis and Tribollet, 1996). Initially using [^{3}H]oxytocin and later with a more selective iodinated oxytocin antagonist, (d(CH_2)$_5$-[Tyr(Me)$_2$,Tyr-NH_2^9]OVT) (denoted [^{125}I]OTA), several investigators have described binding in the olfactory nucleus, bed nucleus of the stria terminalis, amygdala, ventromedial nucleus of the hypothalamus and subiculum (see Fig. 1).

Although some of these regions also stain for oxytocin terminals, the match between terminals and receptors is imperfect. Presumably, oxytocin release in the brain can affect receptors in a paracrine fashion, as postulated in peripheral organs. There is no evidence of a difference in receptor binding between males and females, but there is a curious pattern of receptor binding in development that shows little resemblance to the distribution of receptors in the adult brain (Shapiro and Insel, 1989; Tribollet *et al.*, 1989).

Regulation of oxytocin in the brain

Although it is generally assumed that oxytocin synthesis is regulated by gonadal steroids, the evidence is surprisingly confusing. The promoter sequence of the rat oxytocin gene has a putative (although imperfect) oestrogen-response element around position -160, and promoter constructs inserted into heterologous systems demonstrate very marked oestrogen responsiveness (Burbach *et al.*, 1990; Richard and Zingg, 1990). In the rat hypothalamus, oxytocin mRNA is increased during oestrus and gestation (van Tol *et al.*, 1988; Zingg and Lefebvre, 1988). However, oestrogen given to an ovariectomized female

has proven conspicuously weak (Chung *et al.*, 1991) or ineffective (Burbach *et al.*, 1990) for inducing oxytocin mRNA. In fact, a closer look at oxytocin mRNA through gestation and lactation demonstrates a decrease not an increase in the early days postpartum, in spite of the high circulating oestrogen measured at this time (Crowley *et al.*, 1993).

This confusing picture may be partly clarified by recent results using gonadal steroid treatments that more closely mimic the physiological changes occurring during gestation in rats. If oestrogen and progesterone are given for 14 days, and then progesterone is withdrawn and oestrogen is continued for another 2 days (to model the end of gestation), oxytocin mRNA increases (Crowley *et al.*, 1995). Progesterone alone is insufficient, and prolonged treatment with oestrogen and progesterone together without progesterone withdrawal fails to induce oxytocin mRNA. Apparently oestrogen priming and progesterone withdrawal are the keys, although the mechanism is undoubtedly indirect, as would be expected since oxytocin neurones are not enriched with oestrogen receptors (Burbach *et al.*, 1990).

In sheep, oxytocin mRNA increases at parturition, not only in the paraventricular nucleus but also in the medial preoptic area and bed nucleus of the stria terminalis (Broad *et al.*, 1993). In contrast to results in rat studies, treatment of ovariectomized ewes with progesterone or oestrogen alone increases oxytocin mRNA in each of these regions. In the PVN, progesterone appears to have a greater effect than oestrogen, and the combination of progesterone and oestrogen increases oxytocin mRNA less than either steroid alone. As progesterone decreases oxytocin release in the rostral hypothalamus (Broad *et al.*, 1993), the steroid effects on oxytocin mRNA in sheep may reflect not only changes in synthesis but also alterations in release, feedback and transcript degradation.

Regulation of oxytocin receptors in the brain

Soloff and colleagues demonstrated exponential increases in [^{3}H]oxytocin binding in rat uterine and mammary tissue at parturition (Soloff *et al.*, 1979). The rat brain shows a similar effect, but the increase in binding in the brain at parturition is restricted to the ventromedial nucleus of the hypothalamus and the bed nucleus of stria terminalis (Insel, 1990). Of the various regions with oxytocin receptors in the rat brain, these have the highest concentrations of oestrogen receptors (Pfaff and Keiner, 1973). Treating ovariectomized rats with oestrogen increases oxytocin receptor binding by as much as fourfold in the ventromedial nucleus of the hypothalamus, with more modest effects in the bed nucleus of the stria terminalis and the amygdala (Johnson *et al.*, 1989). Within these regions, it is not yet clear whether the same cells that synthesize oxytocin receptors also have oestrogen receptors, but the absence of oestrogen receptors in the subiculum and olfactory nucleus accounts for the lack of response to oestrogen in these regions. The mechanism by which oestrogen increases oxytocin receptor binding is still unclear. As oestrogen induces hypothalamic oxytocin receptor mRNA *in vivo* (Bale and Dorsa, 1995) but not *in vitro* (Zingg *et al.*, 1995), there may be an important intermediate coupling oestrogen to receptor gene expression. As with the induction of the oxytocin gene, the regulation of oxytocin receptor binding appears to be affected by the interaction of oestrogen and progesterone, although the effects of progesterone in the hypothalamus may be conferred upon the distribution of receptors rather than the number of binding sites (Schumacher *et al.*, 1989).

In summary, gonadal steroids have robust effects on oxytocin and oxytocin receptor expression in the rat brain. The dose–response relationship, the progesterone effect, and the temporal course may differ for induction of the peptide and induction of its receptor. There may also be important variations within specific oxytocin synthesizing cells, with subpopulations of parvocellular and magnocellular neurones responding with different sensitivities. It is also likely that steroids influence post-receptor transduction events (Vallet *et al.*, 1990). Certainly the results listed above provide compelling evidence that receptors in different brain areas respond differentially to gonadal steroids. Recent data suggest that receptors in the subiculum, which do not respond to oestrogen, are sensitive to adrenal steroids (Liberzon *et al.*, 1994). One model for the action of oxytocin in the brain rests on this neuroanatomically discrete pattern of regulation, permitting physiological changes in gonadal and adrenal steroids to induce specific targets for increased oxytocin neurotransmission (Fig. 2).

Species differences

Although species differences in oxytocin cells and fibres are relatively subtle, the differences in the distribution of brain receptors between mouse and rat and monkey are remarkable (Insel *et al.*, 1993). Indeed, even closely related species, such as the prairie vole and the montane vole, show few common areas of receptor binding (Insel and Shapiro, 1992). Species differences in binding are supported by differences in mRNA distribution, suggesting that changes in binding maps reflect differences in regional synthesis and are not due to species differences in receptor affinity or selectivity (Young *et al.*, 1996a). In addition, species differ not only in the distribution of oxytocin receptors but also in their regulation (Insel *et al.*, 1993). For instance, as noted above, gonadal steroids increase binding in the ventromedial nucleus of the rat hypothalamus. In the same region of the mouse brain, gonadal steroids appear to slightly decrease [^{125}I]OTA binding (Fig. 3).

What is the molecular basis for these species differences in expression and regulation? Although there are few species differences in the coding region for the oxytocin receptor, comparative analysis of the 5′ flanking region of the oxytocin receptor gene suggests considerable variation in the sequences flanking the start site of the gene (Fig. 4).

To investigate how sequence variation in the promoter region of this gene might determine species-specific patterns of expression, we have developed transgenic mice carrying a construct composed of the vole oxytocin receptor promoter coupled to a β-galactosidase reporter gene (Young *et al.*, 1996b). The resulting mice show a neuroanatomically discrete pattern of expression of the transgene including regions normally found in the vole but not the mouse brain, consistent with the notion that the promoter sequences confer tissue-specific expression.

In summary, oxytocin receptors show marked differences in brain distribution across species. The importance of these differences for function can hardly be overstated. If the peptide is activating an entirely different network of targets within the

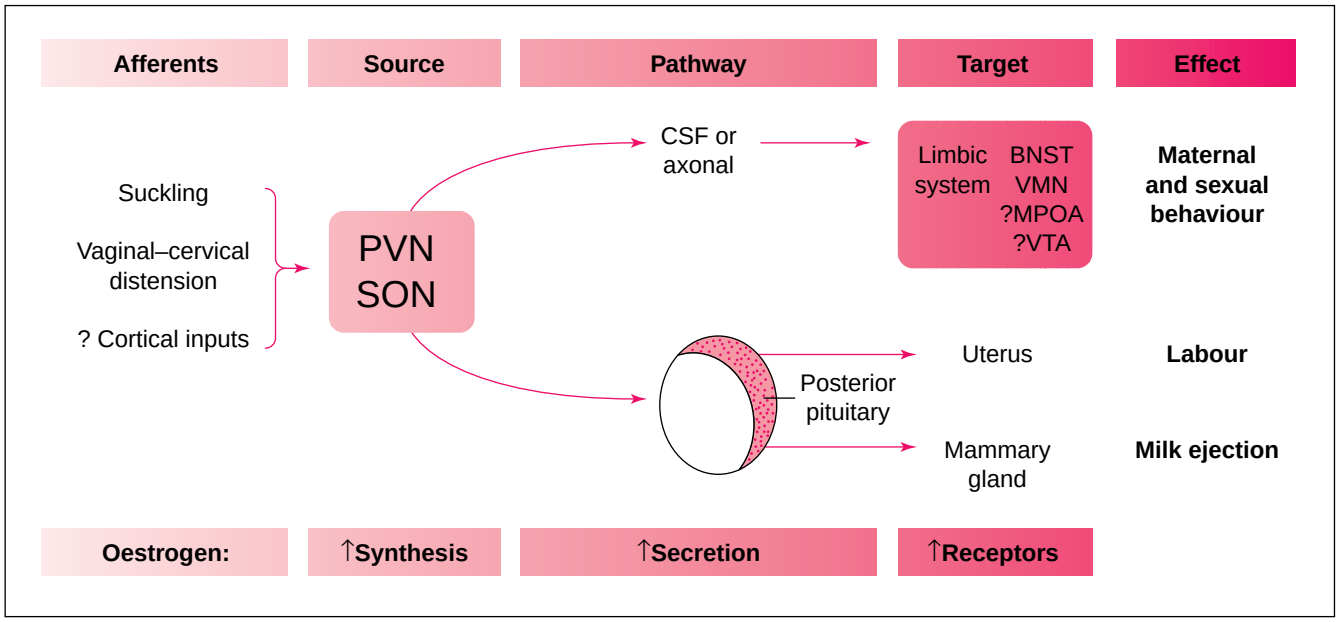

Fig. 2. Schematic diagram of oxytocin neurotransmission showing parallel central and peripheral pathways. Oxytocin is synthesized in paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus and is transported to the posterior pituitary for release into the general circulation where it influences labour and milk ejection. Within the rat brain, oxytocin via either cerebrospinal fluid (CSF) or axonal transport, affects several limbic nuclei (BNST, bed nucleus of the stria terminalis; VMN, ventromedial nucleus of the hypothalamus; and possibly the MPOA, medial preoptic area and the VTA, ventral tegmental area) where it facilitates the initiation of maternal and sexual behaviours. Oestrogen increases synthesis, release, and receptor binding, but probably via an indirect mechanism that includes one or more intermediates.

brain, it is functionally a different peptide. It follows that one cannot generalize accurately from effects in one species to another without knowing that receptors are in the same regions in both species. In addition, the receptor structure may change across species, such that [^{125}I]OTA may not label the oxytocin receptor. For instance, in the sheep olfactory bulb, where oxytocin has physiological effects, [^{125}I]OTA binding cannot be detected (Levy *et al.*, 1992). In rhesus monkeys, [^{125}I]OTA appears to bind to a V1a receptor and selective oxytocin receptor binding has not yet been detected (Toloczko *et al.*, in press). Labelled cRNA probes that are species-specific will be important tools for mapping oxytocin receptors in the future.

Reproductive Behaviours

The remainder of this review will focus on the evidence that central oxytocin influences reproductive behaviours. Oxytocin has been implicated in a diverse range of physiological functions, including food intake, stress responses, temperature regulation and social memory (reviewed in de Wied *et al.*, 1993). The effects of oxytocin on reproductive behaviours need to be considered in this broader physiological context.

Sexual behaviour

Both male and female sexual behaviours are associated with oxytocin release. Studies in female rats, rabbits, sheep and humans demonstrate that plasma oxytocin concentrations increase following vaginocervical dilatation (reviewed in Carter, 1992). In female sheep (Kendrick *et al.*, 1988a) and rats (Steinman *et al.*, 1992), vaginocervical stimulation has also been associated with central release of oxytocin, but only if the female has received oestrogen. Plasma oxytocin increases in human males immediately after ejaculation (Murphy *et al.*, 1987), possibly facilitating seminal transport.

Several studies have indicated that oxytocin has an important function within the rat CNS for regulating either sexual motivation or sexual performance. After low amounts of gonadal steroid priming (0.1 µg oestradiol benzoate and 100 µg progesterone), ovariectomized females usually show little proceptive or receptive behaviour and central administration of oxytocin (500 ng) induces a threefold increase in the time spent in physical contact with the male. At moderate doses of oestradiol benzoate (10 µg), oxytocin clearly increases receptive behaviour (measured as the amount of lordosis) but only if progesterone is available (Arletti and Bertolini, 1985; Gorzalka and Lester, 1987; Schumacher *et al.*, 1989). After prolonged priming with oestradiol benzoate (3 days), lordosis increases after oxytocin (800 ng) even in the absence of progesterone (Caldwell *et al.*, 1986).

Although these various experiments indicate that exogenous oxytocin can increase female sexual behaviour, none of these results demonstrate that oxytocin is physiologically involved in either proceptive or receptive processes. A physiological role of oxytocin was first investigated by administering the selective antagonist d(CH_2)$_5$[Tyr(Me)$_2$,Tyr-NH_2^9]OVT), or OTA, intracerebroventricularly to rats primed with oestradiol and progesterone. When OTA was given at the same time as progesterone, before the onset of receptivity, lordosis was decreased in a dose-dependent fashion (Witt and Insel, 1991). The identical

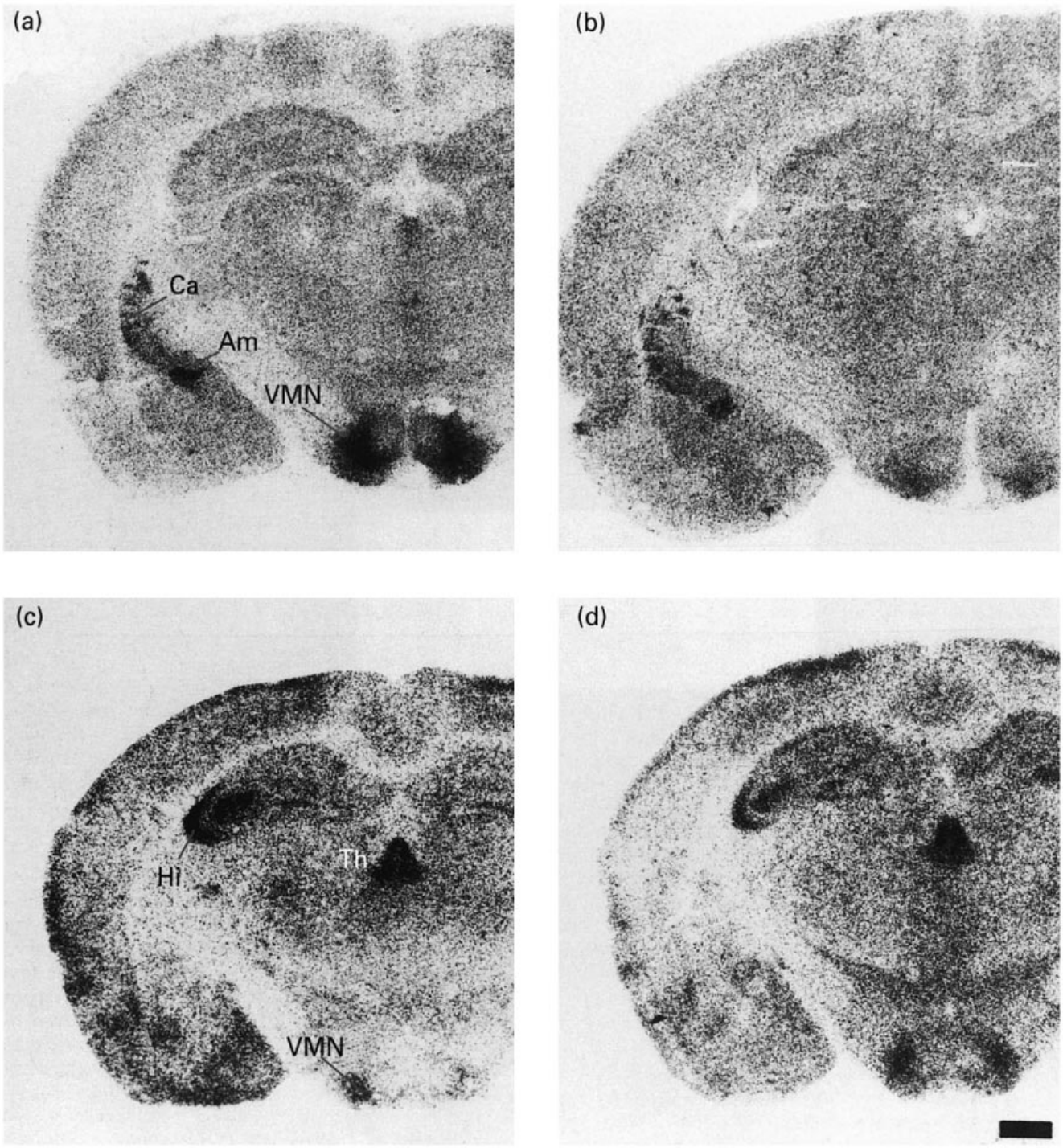

Fig. 3. Brain oxytocin receptors, localized by [^{125}I]-labelled OTA binding, in (a) an intact male rat, are localized in ventromedial nucleus of the hypothalamus (VMN), amygdala (Am), and caudate (Ca). (b) Three weeks after castration, binding is reduced in the VMN (but not other regions), consistent with receptors in this region being dependent on gonadal steroids. At the same level of the mouse brain, oxytocin receptors are found in different regions. As seen in an intact male mouse (c), binding is most intense in the midline thalamus (Th) and hippocampus (Hi), with little evidence for receptors in the VMN. In contrast to the castrated rat, in a castrated mouse (d), binding increases in the VMN, but decreases slightly in the hippocampus. It appears that these species differ not only in the distribution of oxytocin receptors but also in their regulation by gonadal steroids. (Adapted from Insel *et al.,* 1993)

dose of antagonist was ineffective for decreasing lordosis when administered after the onset of sexual receptivity. These results suggest that endogenous oxytocin is important for the initiation but not the expression of sexual receptivity in female rats, which is consistent with the observations of increased plasma oxytocin just before the onset of receptivity (Sarkar and Gibbs,

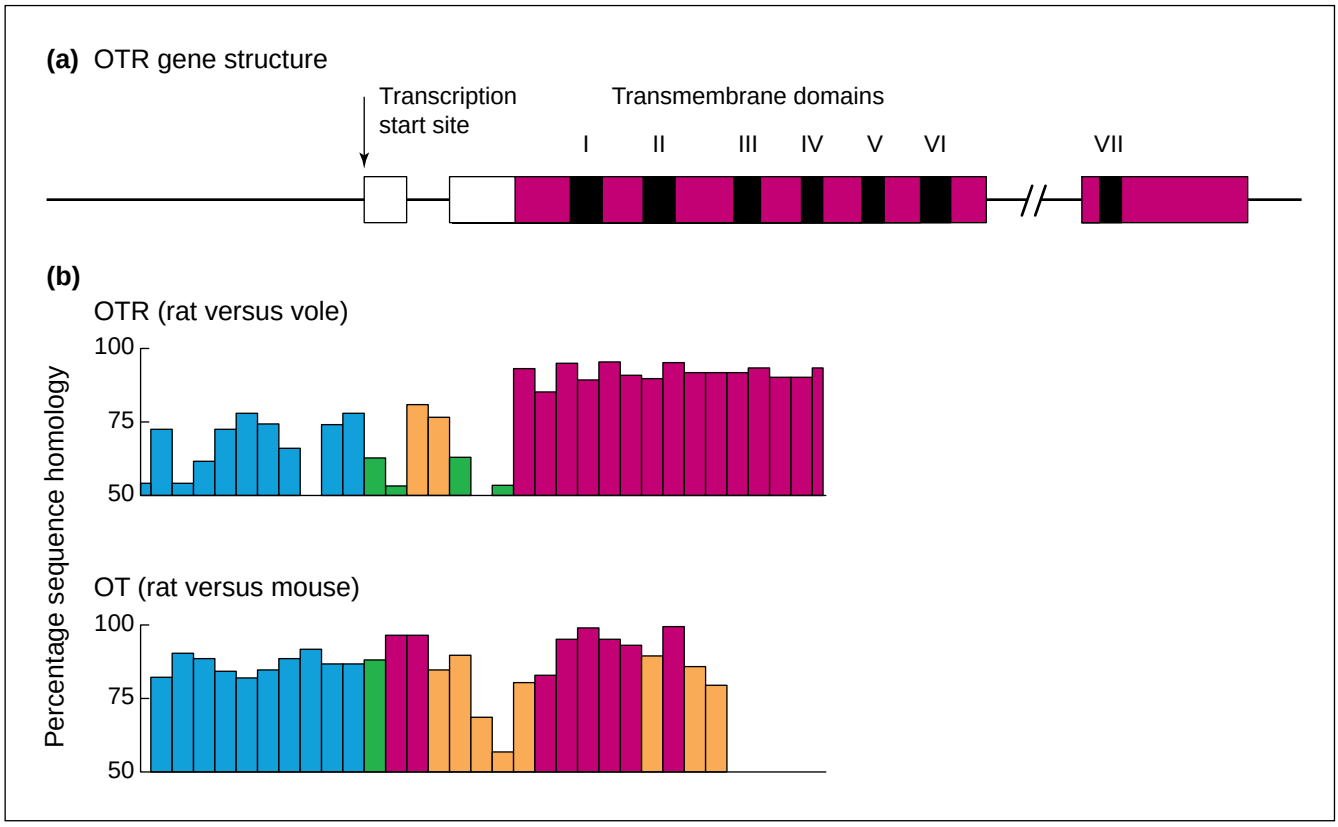

Fig. 4. (a) The oxytocin receptor (OTR) gene is in the rhodopsin family of G protein-coupled receptors with seven hydrophobic domains. In rats, this receptor gene has three exons with a 12kb intron separating exons II and III. (b) Comparing the oxytocin receptor gene in 50 base intervals in rats with that in voles demonstrates at least 90% homology throughout the coding region, but a marked lack of conservation in the 5′ flanking region upstream of the translation start site. In contrast to this lack of homology for the oxytocin receptor gene, (c) the gene for oxytocin shows relatively high conservation throughout the 5′ flanking region as well as within the coding sequence. Coding sequences (red); intronic sequences (orange); 5′ untranslated region (green); 5′ flanking region (blue).

1984) and the peripheral release of oxytocin with sexual behaviour (Fuchs *et al.*, 1981). The neural sites required for the effects of oxytocin on reproductive behaviour appear to include the ventromedial nucleus (a hypothalamic area previously implicated in the integration of the lordosis reflex)(McCarthy *et al.*, 1994) and the medial preoptic area (an area showing increased immunoreactive oxytocin after sexual behaviour) (Caldwell *et al.*, 1989, 1994). It should be noted that these effects have not been observed in other species. Indeed, injection of oxytocin into the ventromedial nucleus of a steroid-primed ewe appears to decrease rather than increase sexual receptivity (Kendrick and Keverne, 1992).

Central oxytocin pathways have also been implicated in the mediation of male sexual behaviour. Penile erection in rats can be induced with intracerebroventricular oxytocin doses as low as 5 ng (Argiolas *et al.*, 1985) and even lower doses (3 ng) can elicit this effect with site-specific injections into the PVN (Melis *et al.*, 1986). As with female sexual behaviour, central administration of an oxytocin antagonist, d$(CH_2)_5$Tyr(Me)-[Orn^8]vasotocin, greatly reduces male sexual interest and performance as measured by declines in frequency of mounts, intromissions, and ejaculations, even though effects on nonsexual behaviours such as locomotor activity were not evident in these same animals (Argiolas *et al.*, 1988). In one study, oxytocin injected centrally prolonged the post-ejaculatory refractory period, consistent with a role in male sexual satiety (Stoneham *et al.*, 1985). In a study using the Fos protein to map cellular activity, a group of oxytocin cells in the posterior PVN were activated after ejaculation (Witt and Insel, 1994). This same region appears to project directly to the sexually dimorphic bulbocavernosus nucleus in the lumbar cord (Wagner and Clemens, 1991, 1993), suggesting that a specific monosynaptic oxytocin pathway may influence ejaculation in male rats.

Parental behaviour

One of the first attempts to investigate the effects of oxytocin within the CNS tested the hypothesis that oxytocin in the brain would induce maternal behaviour that coordinated with the effects of the hormone on labour and lactation. Several studies have reported that oxytocin given centrally (but not peripherally) to virgin female rats induces full maternal behaviour within minutes (reviewed in Insel, 1992). It is important to realise that virgin female rats display little interest in infants and when presented with foster young will either avoid or cannibalise them (Rosenblatt and Siegel, 1981). Just before parturition (or

after specific steroid regimens that mimic the physiological changes of parturition), there is a rapid, dramatic shift in motivation from a lack of interest to a driven, relentless pursuit of nestbuilding, retrieval, licking, grouping and protection of pups. No other peptide or drug has been shown to induce maternal behaviour so quickly in virgin females.

However, oxytocin does not act alone. In all the studies demonstrating an induction of maternal behaviour after central oxytocin administration, the response appears dependent on priming with gonadal steroids – no effects of oxytocin are observed in ovariectomized females unless they are treated with oestradiol. The sites at which oxytocin functions to induce maternal behaviour remain incompletely defined, although results from site-specific injections implicate the medial preoptic area, the ventral tegmental area, and the olfactory bulb, paradoxically regions with very low receptor expression (Fahrbach *et al.*, 1985a; Pedersen *et al.*, 1994; Yu *et al.*, 1996).

Does oxytocin have a physiological role in the induction of maternal behaviour? This question can be answered by blocking central oxytocin pathways using centrally administered antagonists or antisera, or by performing lesions. Studies with all of these methods demonstrate that after either experimentally simulated (Fahrbach *et al.*, 1985b; Pedersen *et al.*, 1985) or natural parturition (van Leengoed *et al.*, 1987; Insel and Harbaugh, 1989), the onset of maternal behaviour can be blocked by oxytocin antagonism. One key feature of these studies is that oxytocin antagonists do not appear to disrupt maternal behaviour *per se*; they block its initiation. The same intervention after parturition, when maternal behaviour is established, is without effects.

It appears from these studies in rats that the effects of oxytocin within the CNS influence the initiation of maternal behaviour consistent with the role of this peptide in peripheral tissues for the induction of labour and milk ejection. Further evidence for this apparent effect of oxytocin on maternal 'motivation' comes from studies in virgin sheep, which resemble nulliparous rats in their normal absence of maternal interest. Vaginocervical stimulation, a potent stimulus for both central and peripheral oxytocin release (Kendrick *et al.*, 1988a), induces the rapid onset of maternal behaviour in steroid-primed ewes (Keverne *et al.*, 1983). More important, central but not peripheral oxytocin administration increases maternal interest in nulliparous ewes, changing their behaviour towards newborn lambs from avoidance to exploration and caretaking (Kendrick *et al.*, 1987). Injections of oxytocin into the medial preoptic area reduce aggressive behaviours towards lambs (Kendick *et al.*, 1993) and injections into the paraventricular nucleus induce full maternal behaviour in nonparturient ewes (Da Costa *et al.*, 1996). At parturition, the concentration of oxytocin in the CSF increases to concentrations approximating those found in plasma (Kendrick *et al.*, 1986). Concurrent increases in the substantia nigra and the olfactory bulb approximate 60% and 30%, respectively (Kendrick *et al.*, 1988b).

Pair bond formation

One of the most complex forms of sociosexual behaviour, pair bond formation, can only be studied in species that make selective, enduring relationships. Prairie voles, *Microtus ochrogaster*, are North American monogamous rodents, similar to field mice but distinct in their social organization. Both field and laboratory studies have demonstrated that prairie voles form selective and enduring partner preferences as a consequence of mating (Carter *et al.*, 1995). In the field, prairie voles generally nest in multigenerational communal burrows with a single reproductively active male and female. If the breeding male or female is removed, the remaining vole rarely accepts a new mate (Getz *et al.*, 1993). In the laboratory, partner preference has been defined operationally using a three-way choice test in which the female can choose to sit by herself or, via a tunnel, can access a cage with a tethered familiar male (mate) or a different cage with a tethered novel male (stranger). The mate and stranger are tethered to ensure that the choice of social contact resides with the test female and not the stimulus male. In these tests, a partner preference is defined as increased time spent with one male relative to time in either the neutral cage or time with the other male. After mating, female prairie voles reliably choose to sit with their mates (Williams *et al.*, 1992). This preference is still evident three weeks after mating, even in the absence of further social experience (Insel and Hulihan, 1995).

Females exposed to a male for 24 h but not given an opportunity to mate generally do not form a partner preference. As mating appears essential for the rapid induction of a partner preference and mating has been associated with oxytocin release in various mammals, the hormone might be important for partner preference formation in prairie voles. Indeed, in female prairie voles, central but not peripheral oxytocin administration facilitates partner preference formation even in the absence of mating (Williams *et al.*, 1994). Conversely, central administration of a selective oxytocin antagonist does not appear to influence mating, but prevents formation of a partner preference (Insel and Hulihan, 1995). Oxytocin, therefore, appears to be both necessary and sufficient for partner preference formation, the first step in the development of a pair bond in this monogamous species.

The unique pattern of oxytocin receptor distribution in prairie voles may reveal a mechanism for these behavioural effects. Oxytocin receptors in this species are expressed in the midline prefrontal cortex and nucleus accumbens, two regions that have been implicated in reinforcement (Insel and Shapiro, 1992). Presumably, activation of these pathways by oxytocin released during mating could establish a positive association with the mate. In non-monogamous montane voles, which are closely related to prairie voles, oxytocin receptors are not found in these regions and mating is not associated with the formation of a partner preference (Insel and Shapiro, 1992).

Conclusion

Any description of the behavioural role of oxytocin must consider the species in question (Table 1). The evidence that oxytocin can facilitate the onset of maternal and sexual behaviours in rats and maternal behaviour in sheep is excellent. In both these species, maternal behaviour is normally absent until parturition and, at least in rats, the initiation of maternal behaviour is associated with an increase in brain oxytocin receptors. In many mammals, nonparturient females show maternal behaviour. In these species, there is no evidence that oxytocin is either necessary or sufficient for maternal behaviour. Indeed,

Table 1. Central oxytocin and reproductive behaviours

Behaviour	Injections of oxytocin	Injections of oxytocin antagonist	Associated CNS release	Probable brain site
Female sexual behaviour				
Rat	Increased	Decreased	Yes	VMN, MPOA
Sheep	Decreased	?	Yes	VMN
Male sexual behaviour				
Rat	Increased	Decreased	?	PVN
Maternal behaviour				
Rat	Increased	Decreased	?	VTA, OB
Sheep	Increased	?	Yes	PVN
Mouse	?	No effect	?	?
Pair bonding				
Prairie vole	Increased	Decreased	?	?

?, no data available; VMN, ventromedial nucleus of the hypothalamus; MPOA, medial preoptic area; PVN, paraventricular nucleus; VTA, ventral tegmental area; OB, olfactory bulb.
References provided in text.

an oxytocin knockout mouse shows ostensibly normal sexual and maternal behaviours (Young *et al.*, in press). These mice, which lack oxytocin, exhibit normal parturition but fail to lactate (Nishimori *et al.*, 1996). Unlike rats and sheep, maternal behaviour in these mice is not restricted to the postpartum period and does not appear to be dependent on oestrogen stimulation.

A role for oxytocin in human reproductive behaviour remains speculative. Oxytocin receptors have been described in the human brain, but their distribution does not conform to distributions in rat, sheep, or mouse and their function remains unknown (Loup *et al.*, 1991). There is great intuitive appeal to the hypothesis that abnormalities in central oxytocin neurotransmission lead to inadequate human maternal or sexual behaviour, analagous to oxytocin antagonist-treated rats. A clinical syndrome of selective oxytocin deficiency has not been described. Results with the oxytocin knockout mouse suggest that this syndrome might present as an inability to nurse, but it is possible that various deficits in socio-sexual behaviours may also be associated with an oxytocin deficiency in humans. Without *in vivo* assays for oxytocin neurotransmission in the brain or selective pharmacological probes that cross the blood–brain barrier, a role for oxytocin in the human brain will be difficult to demonstrate.

At least in animal studies, we now have the tools to investigate oxytocin from the molecular to the behavioural levels. This neuropeptide, which is phylogenetically one of the youngest, was ironically among the first to be synthesized. In the past decade, we have learned that the brain is a target organ for oxytocin and that there are striking species differences in both the brain receptor distribution and the behavioural effects of this neuropeptide. The challenge now is to understand how oxytocin alters the integration of neural processing to influence complex behaviours.

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