Sex, parturition and motherhood without oxytocin?

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Our understanding of the functions of oxytocin in mammals has recently been challenged by findings in transgenic mice in which the oxytocin gene has been knocked out. Mammals generally have only two posterior pituitary nonapeptide genes, for oxytocin and vasopressin, while some marsupials do not express oxytocin but do express a closely related peptide, mesotocin, which acts at the oxytocin receptor (Acher et al. 1995). Birds have arginine vasotocin and mesotocin (Acher et al. 1995), while among other vertebrates a subclass of cartilaginous fishes evidently produces oxytocin (Michel et al. 1993).

Mice homozygous for deletion of exon 1 of the oxytocin gene, containing the oxytocin nonapeptide sequence, were produced by homologous recombination in mouse embryonic stem cells. Offspring from matings of the heterozygotes were in the correct Mendelian frequency, indicating no lethal developmental defects in the homozygotes, which later showed normal sexual maturation, with both males and females showing sexual behaviour and normal fertility (Nishimori et al. 1996). Young and colleagues (1996) used gene targeting to generate a mouse with most of the first intron and the last two of the three exons of the oxytocin gene eliminated. The amount of oxytocin gene transcripts in the supraoptic and paraventricular nuclei of homozygotes was 1% of the wild-type level, with less than 0.4% of the wild-type content of oxytocin in the pituitary gland, and no oxytocin detectable in blood plasma by RIA. In both types of oxytocin gene disablement, the only evident defect was a complete failure of postpartum homozygotes to transfer milk to the suckling young, which consequently did not survive unless the mothers were treated with exogenous oxytocin. Thus parturition and maternal behaviour proceeded without oxytocin, although these processes were not studied in detail.

These findings are surprising in view of the vast literature on a wide range of species suggesting important roles for oxytocin in regulating gonadal function, in expression of sexual behaviour, in parturition and initiation of maternal behaviour as well as in lactation. The amount of oxytocin gene transcripts in the supraoptic and paraventricular nuclei of homozygotes was 1% of the wild-type level, with less than 0.4% of the wild-type content of oxytocin in the pituitary gland, and no oxytocin detectable in blood plasma by RIA. In both types of oxytocin gene disablement, the only evident defect was a complete failure of postpartum homozygotes to transfer milk to the suckling young, which consequently did not survive unless the mothers were treated with exogenous oxytocin. Thus parturition and maternal behaviour proceeded without oxytocin, although these processes were not studied in detail.

Evidence for a role for oxytocin in parturition

Initiation of delivery

One of the first actions of posterior pituitary extracts to be described was the ability to stimulate contractions of the uterus (Dale 1906). Because of this uterotonic action, posterior pituitary extracts were soon used in clinical practice (see Robinson & Amico (1985) for a review). In 1948 a midwife reported her observations of a woman in labour with her second child while still in lactation following the birth of her first child; during each uterine contraction, beads of milk stood out from the nipples, as from a lactating breast during milk let-down (Gunther 1948). The observant midwife speculated that ‘labour proceeds from a series of discharges of pituitrin-like substance which acts on the prepared uterus and which is rapidly rendered inactive’.

Synthetic oxytocin has now been used in clinical and veterinary practice to induce or assist parturition for many years (see Owen & Hanth 1992, Vivrette 1994), and a role for oxytocin is fitted into all models that seek to describe the mechanisms involved in the onset and maintenance of parturition in a range of placental mammals. The classic account of parturition has it that, in late pregnancy, the high circulating concentrations of progesterone induce uterine quiescence while the uterus acquires contractile ability. Shortly before term, plasma progesterone concentrations fall steeply, oxytocin receptor expression in the uterus increases markedly, and uterine contractile activity increases. At term, the contractions rise to a crescendo, resulting in delivery as a result of two interacting positive feedback loops. The first is a local uterine loop: within the uterus itself, prostaglandins and other uterotonic factors are produced and released in response to uterine contractions. The second loop involves the hypothalamus: in response to uterine contractions and vaginal and cervical distension, magnocellular oxytocin neurones in the hypothalamus...
increase their activity resulting in the release of oxytocin from their axon terminals in the posterior pituitary; the released oxytocin acts upon the uterus both to stimulate the further production of prostaglandins and to contribute further to the contractions of the uterus (Fig. 1).

During parturition, elevated oxytocin secretion has been measured in all placental mammals thus far studied, including the rabbit (Fuchs & Dawood 1980), sheep (Glatz et al. 1981), cow (Landgraf et al. 1983), rat (Higuchi et al. 1986), goat (Currie et al. 1988), pony (Haluska & Currie 1988), human (Fuchs et al. 1991), rhesus monkey (Hirst et al. 1993) and pig (Gilbert et al. 1994); representing Australian marsupials, the wallaby shows increased secretion of mesotocin, an oxytocin-like peptide, during parturition (Parry et al. 1996); for the birds, in the chicken the secretion of arginine vasotocin (which is a more potent oxytocic on chicken uterus than the other avian neurohypophysial nonapeptide, mesotocin) increases abruptly at oviposition (Koike et al. 1988).

Again, in all mammals thus far studied, there is a marked increase in uterine responsiveness to oxytocin at term, and oxytocin is the most powerful uterotonic agent identified to date. In the rat, sensitivity to the contractile actions of oxytocin of at least the circular layer of the myometrium greatly increases at the end of pregnancy (Fuchs et al. 1983, Crankshaw 1987), and the sensitivity of mouse and guinea-pig myometrium to oxytocin is increased at the end of gestation (Bell 1941, Suzuki & Kuriyama 1975b), with a similar change and increase in maximal response to oxytocin in the rabbit (Riemer et al. 1986). The sensitivity of the human uterus to oxytocin increases in late pregnancy (Takahashi et al. 1980). Sensitivity to the uterotonic
action of oxytocin increases in late pregnancy in the quokka, a macropodial marsupial (Heller 1973), and in the wallaby (see Renfree 1994), while the endogenous mesotocin can be expected to have similar effects to oxytocin (Bathgate et al. 1995). In the chicken, the sensitivity of the uterus to contractile stimulation by arginine vasotocin, acting via vasopressin-like receptors, peaks at oviposition (Koike et al. 1988, Saito & Koike 1992).

The increased responsiveness of the myometrium to oxytocin in late pregnancy is related to increased oxytocin receptor density, measured as specific radioligand binding. Oxytocin receptor density in the myometrium sharply increases at the end of pregnancy in the rat, and this is localised to myometrial cell membranes, including the longitudinal muscle layer (Soloff et al. 1979, Kaneko et al. 1995, Kawarabayashi et al. 1996). Myometrial oxytocin receptor density increases in the guinea-pig towards the end of pregnancy (Alexandrova & Soloff 1980), and similarly oxytocin receptor density increases greatly at term in the rabbit and sheep myometrium and endometrium (Maggi et al. 1988, 1991, Wathes et al. 1996) and in the human myometrium and decidua (Fuchs et al. 1984). Endometrial oxytocin receptor concentration increases in pregnancy in the cow (Fuchs et al. 1996). The receptor for arginine vasotocin in the chicken uterus is up-regulated a few hours before oviposition (Takahashi et al. 1994).

The increased oxytocin receptor density in the uterus at the end of pregnancy is likely to be a result of stimulated gene expression. Thus there is a large increase in uterine oxytocin receptor mRNA content at the end of pregnancy in the rat (Liu et al. 1996), and in the sheep there is a clear correlation between myometrial and endometrial oxytocin receptor mRNA and protein expression, and with myometrial contractile activity (Wu et al. 1996). Similarly, oxytocin receptor mRNA and protein content in the human myometrium are greatly increased at the end of pregnancy (Kimura et al. 1996). In the cow, oxytocin receptor mRNA expression in the myometrium and endometrium is increased in the third trimester, although there is no evident further increase at term (Ivell et al. 1995). Considering the bulk of the uterus, this enhanced expression must be the most massive increase in peptide receptor production to be seen in the mature mammal.

The temporal pattern of oxytocin receptor expression in the uterus and cervix clearly indicates that endometrial and myometrial oxytocin receptor expression may be a key factor in the timing of parturition in ruminants (Wathes et al. 1996, Wu et al. 1996). In sheep there is a progressive increase in uterine contractile activity toward the end of pregnancy, and, in late pregnancy, infusion of oxytocin antagonists decreases the frequency of uterine contractures (Owiny et al. 1992), involving a blockade of stimulation of uterine prostaglandin production (Jenkin et al. 1994). Similarly in the rhesus monkey and baboon, a nocturnal increase in uterine contractions late in gestation appears to be caused by maternal oxytocin (Hirst et al. 1993), since a selective oxytocin antagonist attenuates the contractions (Honnebier et al. 1989, Wilson et al. 1990). In the rhesus monkey, an increase in nocturnal fetal Δ₁-androstenedione production, entrained by maternal mechanisms, is proposed to increase placental oestrogen secretion, and hence to increase oxytocin secretion, and thereby myometrial contractile activity (Mecenas et al. 1996, Nathanielisz et al. 1995, Giussani et al. 1996), with the further increase in Δ₁-androstenedione at term triggering parturition (Mecenas et al. 1996).

For the rat, as for many species, there is little or no evidence of a rise in oxytocin release before the first delivery, although, since parturition in the rat is preceded by an abrupt and dramatic increase in uterine oxytocin receptor expression, no increase in oxytocin release may be necessary for oxytocin to initiate delivery. Nevertheless, it is generally recognised that, at least for many species, parturition is not necessarily initiated by an increase in oxytocin secretion. Thus, for instance in the guinea-pig, oxytocin antagonist treatment does not delay the start of parturition but does decrease uterine motility at term and prolongs delivery (Schellenberg 1995). In the rat, even very high doses of oxytocin antagonists do not consistently delay the onset of birth in the rat (Chan & Chen 1992), and the delays are relatively short (Antonijevic et al. 1995a). Indeed, in the rat, administration of low doses of oxytocin produces a much more effective delay to subsequent birth than administration of high doses of antagonist (Antonijevic et al. 1995b), possibly reflecting desensitisation of uterine oxytocin receptors, with decreased receptor density on myometrial cells and reduced oxytocin receptor gene expression (Phaneuf et al. 1997), but perhaps also reflecting fatigue of other uterotonin control mechanisms within the uterus. Nonetheless, the onset of parturition in the rat can be advanced by several hours by intravenous infusion of oxytocin, especially if given in pulses (Randolph & Fuchs 1989), and labour is initiated, with variable progression to parturition, at the end of gestation by intermittent electrical stimulation of the neurohypophysis in the rat or rabbit (Lincoln 1971, Boer et al. 1975).

So what other mechanisms are known to be involved in the initiation of parturition? In the pregnant rat, about 24 h before term, circulating progesterone secretion falls dramatically, with the collapse of the corpus luteum (Sanyal 1978). This fall leads to increased intrauterine prostaglandin production (Chan 1983), perhaps partly through the action of local oxytocin on decidal oxytocin receptors (Chan et al. 1993, Larcher et al. 1995), both of which are up-regulated by oestrogen (Lefebvre et al. 1994), and probably involving cytokines (Rozen et al. 1995). Endotoxin given in vivo sensitises the pregnant uterus to oxytocin tested in vitro (Suzuki et al. 1995). The oxytocin gene in human, mouse and rat has an oestrogen response element (also responding to thyroid hormone and retinoic
acid; Richard & Zingg 1990), while the decidua contain oestrogen receptors (Chibbar et al. 1995), but other factors are doubtless involved (Burbach et al. 1995), especially since the equivalent sequence in the sheep and bovine oxytocin genes is mutated such that the oestrogen receptor cannot bind (Ivell et al. 1990, Adan et al. 1991). The human oxytocin receptor gene has half-palindromic oestrogen receptor elements and interleukin-6-binding sequences like the rat and mouse (Inoue et al. 1994, Kubota et al. 1996); however, these half-sites are unlikely to interact with oestrogen receptor in vivo, although the oestrogen antagonist tamoxifen inhibits uterine oxytocin receptor expression at the end of pregnancy in the rat and delays parturition (Fang et al. 1996).

The fall in progesterone will also remove progesterone’s influence on the inhibitory paths within the myometrial cells (Elwardy-Merezak et al. 1994, Cohen-Tannoudji et al. 1995, Tezuka et al. 1995, Orsino et al. 1996), and reduced NO production may also help to activate the myometrium (Izumi & Garfield 1995). In the human, changes in a paracrine network within the fetal membranes and especially the decidua may initiate parturition (Mitchell & Chibbar 1995). Within these tissues a shift to progesterone inactivation and to synthesis of more potent oestrogens will both increase local synthesis of prostaglandins, stimulating contractions, and sensitize the myometrial cells. This sensitisation is achieved by induction of gap junctions between the myometrial cells by oestrogen or relaxin (Tabb et al. 1992, Burghardt et al. 1996), or, in the guinea-pig, blockade of progesterone action thereby increasing gap junction expression (Chwalsz et al. 1991). The marked increase in the expression of the oxytocin receptor gene in the myometrium to greatly increase receptor $B_{\text{max}}$ will also increase sensitivity to oxytocin (Fuchs et al. 1984). In addition, both oxtocin and the oxytocin receptor gene are expressed in the human decidua (Mitchell & Chibbar 1995), and the release of oxytocin from the luminal surface may act in an autocrine or paracrine fashion to stimulate production of prostaglandins (Wilson et al. 1988), which may then act on the underlying myometrium. A further consequence of the decline in local progesterone production is decreased expression of $G_{\text{as}}$ protein in the myometrial cells (Europe-Finner et al. 1994), thus weakening inhibitory pathways acted on by $\beta$-adrenergic agonists, for example. It is also likely that cytokines, produced by cells in the decidua, act on the decidua or the myometrial cells to stimulate prostaglandin production (Mitchell et al. 1990, 1991, Todd et al. 1996). Other mechanisms involving, inter alia, endothelin, NO and relaxin (Goldsmith et al. 1995, Dong & Yallampalli 1996) may also regulate myometrial contractility. In the rat, a decline in luteal relaxin production will contribute to increased excitability, removing relaxin’s inhibitory actions, mediated by protein kinase A, on myometrial cells (Meera et al. 1995); this may involve interference with $G_{\text{uq/11}}$ which mediates oxytocin actions (Sanborn et al. 1995). Thus, while oxytocin may play some role in the initiation of labour, there are certainly other uterine mechanisms that can substitute for it in its absence, and which indeed may play a more dominant role. However, prostaglandins are less effective than oxytocin at inducing parturition in rats at term (Fuchs 1972).

**Progress of delivery**

Thus it is broadly recognised that oxytocin is not generally essential for the initiation of labour, but there is much stronger evidence that it plays an important role in the progress of labour in many species. In the rabbit, oxytocin secretion is maximal with delivery of the first fetus (Fuchs & Dawood 1980). In generally monotocous species, peak oxytocin secretion is seen at the birth of the singleton (sheep (Glatz et al. 1981), cow (Landgraf et al. 1983), rhesus monkey (Frist et al. 1993)). In ruminants, enhanced pulsatile oxytocin secretion is readily demonstrable during parturition (Fuchs 1985, Fuchs et al. 1995). In the pig, a pulse of oxytocin is secreted at the birth of each piglet (Gilbert et al. 1994), while environmental disturbance during parturition both decreases oxytocin secretion and slows down the birth of the piglets (Lawrence et al. 1992). Again though, it is clear that parturition is not necessarily disrupted by the absence of oxytocin: in the rat, oxytocin antagonists have a consistent and marked effect on the progress of parturition when administered after the birth of the first pup (Fig. 2), but, when administered after the birth of the fourth pup, they have no effect in about half of the animals studied (Antonijevic et al. 1995a).

In the human, cystine aminopeptidase from the decidua breaks down oxytocin, and circulates as oxytocinase (Burd et al. 1987), supposedly protecting the myometrium from all but large pulses of oxytocin from the maternal posterior pituitary, and ensuring intermittent stimulation of the myometrium. As in the rat, pulsatile infusion is the most efficient way to stimulate the myometrium with exogenous oxytocin (Cumminskey & Dawood 1990). The power of oxytocin is demonstrated by the association of uterine rupture in multiparae with its inappropriate use to promote labour (Golan et al. 1980). Because of the pulsatile secretion of oxytocin (Fuchs et al. 1991), coupled with the action of the circulating oxytocinase, it has been difficult to consistently show increased oxytocin secretion in women in labour; however, this has been shown in some studies, including early in pre-term labour (Vavra et al. 1993), with a pattern of increasing frequency of pulses (Fuchs et al. 1991), with the most consistent increases measured during the expulsive phase, when the sustained distension of the uterine cervix and vagina leads to strong reflex stimulation of oxytocin secretion (Vasicka et al. 1978, Leake et al. 1981, Thornton et al. 1992). Nonetheless, the importance of oxytocin even in early labour is suggested by the effectiveness in clinical trials of an oxytocin antagonist (Atosiban) in threatened pre-term labour (Goodwin et al. 1996).
In the baboon in labour, intravenous oxytocin antagonist greatly decreases uterine contractile force (Wilson et al. 1990). Since the pituitary oxytocin content is dramatically depleted during parturition in rat, mouse and guinea-pig, the major source of oxytocin release is generally likely to be the maternal pituitary, but it may also be released from the fetal posterior pituitary gland in some species, including the sheep (Dawood et al. 1983), pig (Sander-Richter et al. 1988) and human (Chard 1989), although not the rat (Higuchi et al. 1985). In some species, oxytocin may also be released from the subjacent endometrium or decidua where the gene is strongly expressed at the end of pregnancy, although the amount of peptide produced is very small relative to the posterior pituitary content (human, rat, but not bovine, mouse or sheep: Chibbar et al. 1993, Lefebvre et al. 1993, Ivell et al. 1995, Murphy & Ho 1995, Wathes et al. 1996).

So is the massive release of oxytocin from the maternal pituitary at term an anachronism? A remnant perhaps of an evolutionary history in which the ancestral hormone from which oxytocin evolved played an important role in egg laying, a role played by arginine vasotocin in modern birds (Takahashi et al. 1994). Australian marsupials produce mesotocin rather than oxytocin, and mesotocin in Australian marsupials may not be an ancestor of mammalian oxytocin, but a descendant, since South American marsupials have oxytocin (Acher et al. 1995).

Marsupials such as the tammar wallaby secrete mesotocin in circumstances, including parturition and lactation (see Bathgate et al. 1995, Parry et al. 1996), when eutherian mammals secrete oxytocin, and mesotocin in Australian marsupials may not be an ancestor of mammalian oxytocin, but a descendant, since South American marsupials have oxytocin (Acher et al. 1995).

Figure 2 Progress of parturition in the rat and the slowing actions of oxytocin antagonist or morphine. All rats were fitted with a jugular venous cannula 1 or 2 days before term for injections after birth of the second pup. The control group was given i.v. vehicle, and the differently shaded bars indicate the duration of each subsequent interbirth interval. The i.v. injection of a peptide oxytocin antagonist (Ferring 382; 30 µg/kg) prolonged the intervals between the births of the subsequent four pups, thus doubling the time to deliver five pups after injection. The i.v. injection of morphine sulphate (1 mg/kg) markedly prolonged the interval to the birth of pup 3, and lengthened the next interval, thus also extending the time to deliver five pups after injection. Pulsatile i.v. injection of oxytocin (OXT 1 mU (2 ng; 2 pmoles) every 10 min) after a single injection of morphine after the birth of pup 2 fully restored the progress of normal parturition; continuous infusion (not shown) of the same total dose of oxytocin was ineffective (data from Luckman et al. 1993, Antonijevic et al. 1995a). The prolonged inhibitory action of the oxytocin antagonist clearly indicates an important role for oxytocin in promoting parturition. The inhibitory effects of morphine are a consequence of inhibition of the secretion of oxytocin from the posterior pituitary gland: morphine inhibits the firing of magnocellular oxytocin neurones via µ-opioid receptors on their cell bodies or inputs, and like other opiates it decreases oxytocin secretion in parturition (Russell et al. 1989, Douglas et al. 1993). The restoration of the normal progress of parturition by i.v. pulses of oxytocin supports both a singular action of morphine to inhibit oxytocin secretion and the conclusion that it is secretion of oxytocin from the posterior pituitary in a pulsatile pattern that is important in driving parturition.
immediately after delivery of the neonate, consistent with release during or immediately after delivery (Parry et al. 1996), and again, as in eutherian mammals, uterine sensitivity to oxytocin is maximal at term (see Renfree 1994). In the tammar wallaby, oxytocin antagonists delay the onset of parturition, suggesting that this mechanism is crucially important (Renfree et al. 1996). Parturition in the tammar wallaby (Hearn 1973) and possum (Hinds 1990) is prevented by hypophysectomy, and this is a consequence of loss of posterior rather than anterior pituitary hormones (see Bathgate et al. 1995).

Parturition in the rat

Probably the most complete account of the role of oxytocin during parturition comes from studies in the rat. During pregnancy in the rat, the posterior pituitary content of oxytocin increases by about 50%; during the 90 min or so of normal parturition, this accumulated excess of some 2 µg (1000 mU, sufficient for 1000 to 2000 milk ejections) is secreted into the circulation. Parturition normally begins, and is completed, during the second half of the light cycle on day 21 of gestation (mated on day 0), otherwise the onset will tend to be delayed until the late morning of the following day; for example, meta-analysis of the start times of 293 parturitions in our laboratory (lights off 1900 h, on 0500 h) shows a mode between 1500 and 1700 h on day 21 (n=59) and a second mode between 0900 and 1100 h (n=15) on day 22, with a nadir between 0300 and 0500 h (n=3) on day 22 (Fig. 3).

Figure 3 Diurnal rhythm in time of onset of parturition in the rat. Meta-analysis of observations on parturition in 293 rats, caged singly and housed under controlled conditions, with lights on for 14 h and off for 10 h each day. The time of birth of the first pup shows a clear bimodal distribution across days 21 and 22 of pregnancy, with fewest first births in the lights-off phase. Litter size does not seem to be an important factor in determining time or day of birth. Since the time of birth can be advanced by shifting the lighting cues in mid-pregnancy (Lincoln & Porter 1976), events early in pregnancy do not determine the time of birth. The likely explanation for the diurnal variation in the timing of the first birth is that a maternal signal late in pregnancy triggers the process. In primates, there is a diurnal variation in uterine contractile activity that follows a diurnal rhythm in oxytocin secretion from the maternal posterior pituitary (Hirst et al. 1993, Giussani et al. 1996), and this is proposed as the explanation for the pattern of timing of first births seen in the rat. Oxytocin infusion can advance the onset of parturition by a few hours, and oxytocin antagonist infusion can delay the onset by a few hours (Antonijevic et al. 1995a,b). Infusion of an ineffective dose of oxytocin also delays the onset of parturition (Antonijevic et al. 1995b), probably by desensitising the myometrium. In the mouse, in contrast with the rat, most births occur during the dark phase (around day 19), but a similar explanation may hold (i.e., diurnal variation in maternal oxytocin secretion), although in the oxytocin knockout mouse the precise timing of parturition has not yet been reported (Nishimori et al. 1996).
The mechanisms underlying this timing are not known, but the supraoptic and paraventricular nuclei both receive a direct afferent projection from the suprachiasmatic nucleus (Cui et al. 1997), which is the principal circadian oscillator in mammals (Hastings 1995). Oxytocin secretion is continuously elevated throughout parturition, and superimposed on this elevated baseline secretion are large pulses of oxytocin release (Higuchi et al. 1986). There is clear evidence that the pulsatile pattern of oxytocin secretion is important in driving parturition. The pulsatile secretion derives from the synchronised intense bursting activity of oxytocin neurones in the hypothalamus in the rat and rabbit (Summerlee 1981, O’Byrne et al. 1986), activity similar to that in the rat during reflex milk ejection in response to suckling. The increased electrical activity is accompanied by induction in these neurones of the immediate-early gene c-fos, an indicator of trans-synaptic activation of the neurones, and consequent prominent expression of the protein product Fos in the nuclei of oxytocin cells (Luckman et al. 1993). Furthermore, expression of the oxytocin gene in supraoptic neurones is stimulated at parturition (Douglas et al. 1998), while it is controversial whether expression is increased in pregnancy (Horwitz et al. 1994, Douglas et al. 1998). Fos is also expressed during parturition in brainstem neurones, which project to the oxytocin cells, and these brainstem neurones are activated by uterine contractions even in the absence of parturition itself (Antonijevic et al. 1995b). The secreted oxytocin enters the circulation at a time when uterine responsiveness to oxytocin is maximal as a result of a rapid increase in oxytocin receptor expression in the myometrium at the end of pregnancy (Rozen et al. 1995). Inhibition of oxytocin secretion by exogenous opiates (which act to inhibit the oxytocin neurones directly) slows down the early progress of parturition, and this can be reversed by oxytocin infusion (Russell et al. 1989), with pulsatile delivery of oxytocin being particularly effective (Luckman et al. 1993, Antonijevic et al. 1995b) (Fig. 2). Similarly, mild environmental stress slows down the early progress of parturition and reduces oxytocin secretion, and these effects are fully reversed by the opioid antagonist naloxone, which increases oxytocin secretion and restores the normal progress of parturition (Leng et al. 1988). Blocking the actions of oxytocin by specific antagonists similarly leads to impaired early progress of parturition (Chan & Chen 1992, Antonijevic et al. 1995a).

However, notably, magnocellular vasopressin neurones are also activated during parturition (Lin et al. 1995), and vasopressin release from the posterior pituitary is enhanced during parturition in the rat (Hartman et al. 1986) and in other species (e.g. cow (Landgraf et al. 1983), sheep (Kendrick et al. 1991), pig (Lawrence et al. 1995)). Vasopressin may act weakly at myometrial oxytocin receptors in the rat (Chan et al. 1996), while in the human and rabbit an action on myometrial vasopressin (V1a) receptors is also possible (Bosmar et al. 1994, Maggi et al. 1988, 1990). Since vasopressin can activate oxytocin receptors, its actions would be expected to supplement those of oxytocin during parturition. Is it possible then that, in the absence of oxytocin, vasopressin would be an effective substitute? One might think this unlikely given the much lower potency of vasopressin at the oxytocin receptor (Berde & Boissonas 1968), but vasopressin is quite effective at stimulating prostaglandin F2α (PGF2α) production by the decidua (Chen et al. 1994). Furthermore, if in the absence of endogenous oxytocin the uterine oxytocin receptors are up-regulated, then the actions of vasopressin alone might indeed provide an effective substitute for the absent oxytocin.

In the oxytocin knockout mouse, vasopressin mRNA content in the supraoptic and paraventricular nuclei is normal (Nishimori et al. 1996) or reduced (Young et al. 1996), although this has not been measured in pregnancy.

Parturition in the mouse

Strictly, the outcome of studies on the oxytocin knockout mice ought to be considered in the context of previous experiments on the role of oxytocin in parturition in the mouse. As in the rat and other species, the content of oxytocin in the posterior pituitary decreases during parturition in the mouse (Fuchs 1985), but other than this little is known. Parturition can be interrupted by environmental disturbance, indicating a central mechanism influencing parturition (Newton et al. 1968), but it is not known if this is due to inhibition of oxytocin secretion as it is in the rat. Yet transgenic mice lacking oxytocin have no obvious defects in parturition (Nishimori et al. 1996, Young et al. 1996). In both lines, the expected numbers of live young were delivered, but few details are given on the timing of parturition, although one of the studies (Nishimori et al. 1996) reports that homozygous females mated with homozygous males delivered 18.5–19.5 days post coitum, which is approximately as expected of normal mice.

The sequences of the human, rat, mouse, bovine and sheep oxytocin receptor genes are known (Kimura et al. 1992, Adan et al. 1995, Kubota et al. 1996, Ivell et al. 1995, Riley et al. 1995, Rozen et al. 1995), and there is a striking conservation of the extracellular sequences of the receptor between species so far studied. The mouse oxytocin receptor gene, detected with a specific probe or probe for the rat receptor mRNA, is expressed in the uterus and in particular in the myometrium in pregnancy, peaking just before parturition (Kubota et al. 1996, Mahendroo et al. 1996). Functional receptor is expressed since contractions activity is stimulated by oxytocin, to which the mouse uterus becomes more sensitive by the end of pregnancy (Suzuki & Kuriyama 1975b), and comparison of actions of oxytocin and vasopressin in vitro are consistent with expression of, and action through, the oxytocin receptor (Stepke et al. 1994); sensitivity to vasopressin is also increased at the end of pregnancy (Stepke et al. 1994).
Unlike the rat or human (but like the cow and sheep), the uterus of the pregnant mouse does not express the oxytocin gene (Murphy & Ho 1995).

In other respects, parturition in the mouse involves mechanisms identified or proposed for other mammals. Progesterone secretion falls sharply on the day before parturition, presumably triggering events leading to parturition (Soares & Talamantes 1984). A synthetic gestagen (Org 5933) prolongs pregnancy (Gao & Short 1993). For reasons that are not clear, transgenic mice overexpressing the oestrogen receptor have prolonged gestation and dystocia (Davis et al. 1994). In late gestation, enzymes synthesising the 5α-reduced androgen, 3α-Adiol, (5α-androstan-3α,17β-diol) are induced in the uterus, and transgenic mice with disruption of the gene of the key enzyme 5α-reductase type 1 also have a prolonged pregnancy, then a long labour with dystocia: the patterns of uterine oxytocin receptor gene expression and plasma progesterone and oestrogen profiles are normal and these animals can be rescued by injection of either progesterone antagonist (RU486) or oxytocin (Mahendroo et al. 1996).

Other local uterine mechanisms involved in the onset of parturition in the mouse bear comparison with other species. Uterine cytokines, especially interleukin 1 or 2, probably acting via stimulation of decidual PGE₂ production (Dudley et al. 1993), are implicated in the initiation of parturition in the mouse (Romero et al. 1991, Romero & Tartakovsky 1992, Fidel et al. 1994, Hirsch et al. 1995, Kaga et al. 1996), particularly in pre-term parturition provoked by bacterial endotoxin (Kaga et al. 1996); although not via direct acute actions on the myometrium (Oshiro et al. 1993), there is circumstantial evidence for a role for neutrophils in the uterus in parturition onset (Kasik & Rice 1995). The pregnant mouse uterus contracts in response to PGE₂, and depolarisation sensitivity is much greater in late pregnancy than in early pregnancy (Suzuki & Kuriyama 1975a). PGE₂, histamine and 5-hydroxytryptamine, all potentially from uterine mast cells, together stimulate uterine contractions (Rudolph et al. 1992) and so does endothelin 1, although sensitivity does not change in pregnancy, unlike the large increase in sensitivity to oxytocin (Gong et al. 1994). Uterine histamine content increases prepartum, and is abruptly reduced postpartum (Padilla et al. 1990). Adrenergic agonists inhibit uterine contractions, via the β₂-adrenoreceptor (Chen et al. 1994), becoming less effective near term (Cruz et al. 1990). Relaxin from the corpora lutea inhibits contractions of the mouse uterus via a selective receptor (Fields et al. 1980, Yang et al. 1992, Evans et al. 1993).

An important role for PGE₂ in the initiation of parturition in the mouse is indicated by a study of parturition in mice with knockout of the receptor for PGE₂ (Sugimoto et al. 1997). These homozygous mice are fertile, but do not deliver their young spontaneously, do not show luteolysis at term (and thus have high circulating levels of progesterone) and the uterus is insensitive to oxytocin, not showing the normal increase in oxytocin receptor mRNA expression at term. However, ovariectomy on day 19, precipitating a fall in circulating progesterone, is followed by induction of uterine oxytocin receptor mRNA expression 12 h later, and by parturition after a further 12 h. Clearly any action of PGE₂ on the myometrium is not essential for parturition, provided that oxytocin is secreted to act on up-regulated oxytocin receptors in the myometrium. Instead, PGE₂, or rather its receptor, is essential in mice to induce luteolysis at term, which then allows, through enhanced oxytocin receptor expression, uterine sensitisation to oxytocin. If a myometrial action of PGE₂ compensates for oxytocin in the oxytocin knockout mouse, and oxytocin compensates for lack of any uterine actions of PGE₂ in the PGF receptor knockout mice, then study of crosses between these two knockout strains should provide information about whether there are other redundant mechanisms regulating parturition in mice.

Despite some differences between the mouse and other species studied, it seems unlikely that species differences really explain the lack of apparent effect of the oxytocin knockout on parturition. What is clear though, from work across many species, is that several mechanisms concurrently regulate myometrial contractility in parturition, providing a high degree of redundancy among these mechanisms.

Given this, is there likely to be any single aspect of parturition in which oxytocin plays an essential role which cannot be compensated for by other known systems? Parturition as described above is regulated by both a uterine feedback loop and a hypothalamic feedback loop, of which oxytocin is normally the effector molecule. Clearly the uterine loop alone can effect successful parturition; however, without oxytocin, it seems likely that the progress of parturition may be largely outside the control of the maternal central nervous system. This control seems likely to be important in the circadian timing of parturition, and in regulating the initiation and progress of parturition with regard to environmental circumstances.

In many species the onset and progress of parturition are critically influenced by environmental stress (dog (Bleichert 1962), mouse (Newton et al. 1968), rat (Leng et al. 1988), pig (Lawrence et al. 1992, 1995)). It would certainly be instructive to establish whether the knockout mouse shows any alterations in the pattern or progress of parturition in these respects. However, as suggested above, it is possible that vasopressin may, in the chronic absence of oxytocin, be an effective substitute. It is notable that mice hypophysectomised in mid-pregnancy were observed to start parturition at the appropriate day, but almost half (of sixteen) had a ‘difficult and prolonged parturition’ (Gardner & Allen 1942). Studies of the effects of hypophysectomy in pregnancy on parturition in several other eutherian species have produced similarly variable results (Amoroso & Porter 1966).
Lactation

Clearly, the failure of the oxytocin knockout mice to transfer milk to the suckling young, and the repair of this deficit by oxytocin injection (Nishimori et al. 1996, Young et al. 1996), is powerful evidence for an essential role for oxytocin in effecting the milk-ejection reflex. This finding is entirely consistent with many previous studies in several species. In marsupials, mesotocin has this role (Bathgate et al. 1995), substituting for oxytocin (Lincoln & Renfree 1981b).

In particular, oxytocin is effective in the rat when secreted during suckling in pulses a few minutes apart, and this is a consequence of synchronised burst-firing of the magnocellular oxytocin neurones projecting into the posterior pituitary gland (Belin & Moos 1986). Although bolus injection of vasopressin can elicit milk ejections, it is much less potent than oxytocin (Bisset et al. 1967), and vasopressin neurones do not show synchronised burst-firing, and few otherwise respond, during suckling (Lincoln & Wakerley 1974). For this reason, vasopressin, or rather vasopressin neurones, cannot substitute for oxytocin in lactation. To do so in the oxytocin knockout mouse would require altered ‘wiring’ of vasopressin neurones, and expression of the distinctive and specialised electrophysiological properties normally only seen in oxytocin neurones. Evidently, and not surprisingly, these adaptations do not occur in the oxytocin knockout mice. Furthermore, it is obvious that, at least in the knockout mouse, there are no other mechanisms that can effect milk transfer in the absence of oxytocin. This may reflect the fact that lactation, or specifically milk ejection, is recent in evolution, in contrast with egg laying, the precursor of parturition.

There is evidence from studies in the rat that oxytocin, possibly from the posterior pituitary although it is also secreted from magnocellular axons in the median eminence into the hypothalohypophysial portal blood (Horn et al. 1985), stimulates prolactin secretion in lactation. Thus the consequences of removal or denervation of the posterior pituitary include reduced stimulation of prolactin secretion by suckling (Murai & Ben-Jonathan 1987, Vecseryes et al. 1997). Furthermore, there is a dramatic increase in oxytocin receptor mRNA expression in lactotropes at the end of pregnancy (Breton et al. 1995). Suppression by suckling of the release of dopamine into portal blood and the stimulation of vasoactive intestinal peptide (VIP) or thyrotrophin-releasing hormone secretion are also involved in regulation of prolactin secretion (see Mogg & Samson 1990), so the evident capacity to produce milk in the oxytocin knockout mice (demonstrated by milk transfer when injected with oxytocin) implies adequate prolactin secretion through action of these mechanisms, a clear case of redundancy, and involving differential expression of receptors by subsets of lactotropes, so that the subset of lactotropes expressing oxytocin receptors also expresses VIP receptors (Samson & Schell 1995). However, this has not been studied in detail: reduced prolactin secretion in response to suckling could be a feature of the knockout mouse. This leads to consideration of the usefulness of the oxytocin knockout mice to analyse the importance of other factors in mechanisms in which oxytocin has a role, but in which there is redundancy. Thus, in lactation, appropriate experiments could address the issue of the relative importance of suppression of dopamine secretion or stimulation of VIP secretion in suckling stimulation of prolactin secretion. Similarly, the importance of other factors in parturition, freed from contamination by any effects of oxytocin, can be examined, for example, assessing a role for vasopressin.

Oxytocin and maternal behaviour

Whereas all mammalian species express oxytocin receptors at high levels in the term pregnant uterus (see above), the distribution of oxytocin receptors in the brain shows very wide interspecies variation (Tribollet et al. 1992, Insel et al. 1993), and considerable variation is apparent even between rat and mouse. In the rat, oxytocin, produced and acting in the brain, is important in stimulating the expression of female receptive behaviour (Caldwell et al. 1990) and in penile erection in the male (Melis et al. 1994); in particular, centrally administered oxytocin facilitates lordosis by acting in the ventromedial nucleus of the hypothalamus, through specific oxytocin receptors positively regulated by oestrogen or testosterone (Johnson 1992, McCarthy et al. 1994, Bale & Dorsa 1995). By contrast, in the normal (and homozygous oxytocin knockout) mouse, there is a low density of oxytocin receptors in the ventromedial nucleus (Nishimori et al. 1996), and in the male mouse testosterone negatively regulates oxytocin receptor binding in the ventromedial nucleus, the reverse of the effects of gonadal steroid in the rat (Insel et al. 1993). Also, adult male mouse brain has fewer immunoreactive oxytocin neurones than the female, consistent with a suppressor action of testosterone (Haussler et al. 1990), and again unlike the rat, which shows no sex difference in neuronal oxytocin expression.

Notwithstanding considerable species variability in the suggested central actions of oxytocin, there is evidence across distantly related species of a role for central oxytocin in maternal behaviour. Lactating rats display a range of behaviours, together described as maternal behaviour, which are not normally shown by virgin rats, unless they are exposed to young over many days (Numan 1994). These component behaviors include nest building, gathering young into the nest, and licking and crouching over the young, and lactating rats will readily extend these attentions towards alien foster pups as well as to their own young. Maternal behaviour in the rat appears abruptly
immediately after parturition, and is maintained subsequently throughout lactation. Oxytocin, released within the brain at parturition, is thought to be involved in stimulating the rapid onset of maternal behaviour acting on up-regulated oxytocin receptors (Insel 1986), and i.c.v. injections of oxytocin antagonists are effective in preventing the induction of maternal behaviour (van Leengoed et al. 1987), in particular acting through the ventral tegmental and medial preoptic areas and olfactory bulbs (Pedersen et al. 1994, Yu et al. 1996). In contrast, once maternal behaviour has been induced, i.c.v. injection of oxytocin antagonists has generally been found to be ineffective, although olfactory signals are of key importance (Kolunie & Stern 1995), and antagonist administration into the olfactory bulbs does reduce maternal behaviour (Yu et al. 1995). Similar actions of oxytocin have been demonstrated in sheep, in which the central release of oxytocin is triggered by stimulation of the uterine cervix and vagina (Kendrick et al. 1988). Rapid expression of maternal behaviour can be induced in virgin rats by oestrogen treatment (‘priming’) followed by i.c.v. oxytocin (Pedersen & Prange 1979), provided that the testing is in a novel environment (Fahrbach et al. 1985). In this model, i.c.v. oxytocin antiserum disrupts maternal behaviour (Pedersen et al. 1985).

Against this background, what is already known about the expression of maternal behaviour in the mouse? There is an important difference between the rat and the laboratory mouse. ‘Only a few studies have been directed at examining the hormonal basis of pup-directed maternal behaviour in mice, and this is because the virgin laboratory mouse usually shows spontaneous maternal responsiveness to test pups’ (Numan 1994; our italics), and full maternal behaviour is shown by virtually all late-pregnant laboratory mice (Gandelman 1973). In the light of this, it is less surprising that postpartum maternal behaviour is normal in the oxytocin knockout mouse, since its induction in the laboratory mouse clearly cannot depend on oxytocin release during parturition. Nonetheless, there are detectable quantitative differences in maternal behaviour between virgin and primiparous mice (Laviola et al. 1994). Such subtleties have evidently not been sought in the oxytocin knockout mice. Because lactating mice share their nursing of their litters under natural conditions, unlike rats (Gandelman et al. 1970, see Numan 1994), it is likely that oxytocin deficiency in an individual lactating mouse could be compensated for by this social behaviour. Thus, in the mouse’s natural social context, oxytocin could be considered to be redundant for an individual, even with respect to its role in milk ejection. Disabling natural mutations of the oxytocin gene may be prevalent in such communal nursing species.

In marked contrast with the behaviour of virgin laboratory mice, virgin wild mice usually cannibalise strange pups. Infanticide by pregnant wild mice ceases at the birth of the young, and returns only after the young are weaned. Interestingly, infanticide in virgin wild mice is suppressed after systemic or i.c.v. oxytocin injection (McCarthy et al. 1986, McCarthy 1990). Olfactory processing is again implicated, since prepartum destruction of the noradrenergic input to the olfactory bulbs induces postpartum cannibalism in laboratory mice (Dickinson & Keverne 1988); the mouse olfactory bulb is rich in oxytocin receptors (Insel et al. 1993).

Postpartum mice (including laboratory strains) display aggression toward conspecific intruders. The aggressive behaviour is suckling-dependent (Garland & Svare 1988), but not dependent on prolactin secretion (Mann et al. 1980). Lactational aggressiveness towards intruders is seen also in the rat; in neither rat nor mouse has a role for central oxytocin in this behaviour been tested, but microinjection of oxytocin into the amygdala in the hamster increases aggressiveness of lactating females toward intruder males (Ferris et al. 1992). In the rat, maternal aggression is independent of suckling (Mayer et al. 1987), prolactin secretion (Erskine et al. 1980) and probably oxytocin secretion (Factor et al. 1992), but is dependent on somatosensory inputs to the snout and ventral body surface, and is influenced by volatile odours acting via the olfactory epithelium and olfactory bulb (Ferreira et al. 1987, Stern & Kolunie 1993, Kolunie & Stern 1995). This behaviour in the rat also involves lateral connections of the ventromedial nucleus, a region rich in oxytocin receptors (Bale & Dorsa 1995), which are not involved in pup-directed behaviour (Hansen 1989).

In male oxytocin knockout mice, there is reduced aggressiveness toward intruder males, with no evident sensorimotor deficits in the homozygous knockouts, although reversal by centrally administered oxytocin has not been tested (De Vries et al. 1997).

With regard to this group of actions of oxytocin, in facilitating receptive or copulatory or maternal and related behaviours, for the knockout mice, the fact that the oxytocin knockout is lifelong leaves the possibility for compensation by recruitment of other neuropeptides acting on oxytocin receptors; an obvious candidate is vasopressin. With respect to actions in the brain, matching of oxytocin receptor distribution to sites of vasopressin release, but under appropriate conditions, would be required, while oxytocin receptors in the brain bind vasopressin with a similar affinity for oxytocin (see Barberis & Tribollet 1996). In the rat, centrally administered vasopressin can, like oxytocin, induce maternal behaviour in appropriately primed virgin rats (Pedersen et al. 1982).

In short, the studies to date on normal or oxytocin knockout mice do not allow a critical evaluation of the role of central oxytocin in the expression of maternal behaviour in the mouse. This is made difficult by the facility with which the neural circuitry for this behaviour is activated even in virgin female laboratory mice: in wild mice, and other species, facilitation by release of oxytocin within the brain is likely to be important, with a broad spectrum of...
necessity, while the oxytocin gene product will interact with other neurotransmitters in shaping and evoking maternal behaviour.

Oxytocin and gonadal function

In ruminants, oxytocin from the corpus luteum provides an essential signal to the non-pregnant endometrium to trigger PGF₂α release, which in turn initiates luteolysis and drives further luteal oxytocin secretion in a positive feedback circuit (Flint et al. 1990); in early pregnancy in the sheep, suppression of endometrial oxytocin receptor by interferon τ from the embryo is essential to prevent luteolysis (Lamming et al. 1995). Many species show some evidence of gonadal expression of either vasopressin or oxytocin; in the human, oxytocin and oxytocin receptors are expressed in cumulus cells around the oocytes (Furuya et al. 1995), and oxytocin is expressed in Sertoli cells of the bovine testis (Ang et al. 1991). However, there are considerable interspecies differences in the regulation of luteolysis, and there is no evidence that gonadal oxytocin plays a role in rodents similar to that documented for ruminants. Previous studies in the mouse on the role of oxytocin in gonadal function indicate a capacity to stimulate ovulation (Robinson et al. 1985) and weak expression in granulosa cells; at the anterior pituitary, oxytocin can advance the luteinizing hormone preovulatory surge in the rat (Robinson & Evans 1990). Evidence has also been put forward that oxytocin can stimulate blastocyst development in vitro (Furuya et al. 1995), act on seminiferous tubule motility (Nicholson et al. 1986) and possibly stimulate, via vasopressin receptors, testosterone production (Tahri-Joutei & Pointis 1989), although the oxytocin gene is not naturally expressed in the mouse testis (Ang et al. 1991).

Reconciliation

If the above accounts of studies on the roles of oxytocin in mice and other mammals appear to be inconsistent with observations on oxytocin knockout mice, the inconsistency may be more apparent than real. Specifically, with regard to parturition, it is perfectly reasonable to affirm that oxytocin is released from the pituitary in large pulses, which act upon a uterus that is expressing an abundance of oxytocin receptors, and thereby influences uterine contractility and the progress of parturition, while at the same time affirming that, in the absence of oxytocin, other mechanisms may be substituted to ensure ultimately successful parturition. Indeed, it would have been brave to deny the likely truth of any part of this, for there have been many previous well-described examples of normal labour in humans and experimental animals with apparently complete posterior pituitary dysfunction (Amoroso & Porter 1966). Even in birds, egg laying has been observed to occur after acute neurohypophysectomy (Nakada et al. 1993). With regard to other reproductive roles of oxytocin, it seems clear that species differences are considerable and obscure the interpretation of results from the knockout mouse.

Conclusion

Despite the reservations expressed in this review, there is much that can be learned about the roles of oxytocin in the oxytocin knockout mouse. In particular, even if the results from the knockout mouse can tell us little about the role played by oxytocin in reproductive functions, they can certainly be invaluable in telling us how these are fulfilled in the absence of oxytocin.

If nothing else, it is now evident that oxytocin has physiological actions that are strictly redundant. Natural selection is harsh in culling features that are counter-adaptive, but clearly the actions of oxytocin on the uterus at term, which involve co-ordinated up-regulation of uterine oxytocin receptor expression, if not essential are not counteradaptive. Indeed, we may expect that any local uterine, neural or endocrine mechanism that will tend to favour successful delivery at term will be preserved by selection, provided that the costs of maintaining a redundant mechanism do not exceed the costs of eliminating it. The neurohypophysial system regulates the delivery of progeny in all vertebrates, and, during its long evolutionary history, other mechanisms may have evolved convergent roles simply by a process of exclusion. When everything that opposes the actions of oxytocin in parturition is excluded, the things that remain are neutral, assist oxytocin or, in dogging the footsteps of oxytocin, can substitute for it. What these key substitutes are in the oxytocin knockout mouse are at present not known.

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