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# Oxytocin: cellular and molecular approaches in medicine and research

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In May 1995 the third Hanseatic Endocrine Conference at Stade, Germany, attracted 140 scientists from all over the world to summarize the current knowledge on one hormone – oxytocin. This article presents the major findings of the meeting with the realisation that oxytocin provides major model systems with which to elaborate a whole series of novel endocrinological paradigms, as well as being the example of choice for establishing revolutionary new techniques, which will no doubt spread to studies of other hormone systems. The papers from this symposium will be published in full*.

For many biomedical scientists and clinicians, oxytocin is still the small peptide hormone released from the posterior pituitary at the end of pregnancy, which is involved in causing uterine contractions at birth, and is subsequently the prime agent in mediating the milk let-down reflex in response to suckling. This concept is exploited all over the world when an oxytocin infusion is applied to accelerate a slowly progressing birth, and occasionally to assist lactation. It is, however, now clear that oxytocin has other functions in reproduction, being produced peripherally as well as by the neurohypophysis, and it has important actions within the brain, evident as specific behaviours.

## Molecular biology and evolution

Oxytocin belongs to a very old family of molecules, with representatives throughout the animal kingdom: from worms and insects to vertebrates. Early in vertebrate evolution the single representative of the family, probably vasotocin, which is still found today in cyclostomes, underwent a gene duplication giving rise to the two subfamilies of oxytocic and pressor peptides found in all higher vertebrates (R. Acher, Paris). One of the major questions here is what duplicated first – the hormone, the receptor, or the function? The cloning and analysis of genes for the peptides and their receptors from numerous organisms will help us understand how such endocrine systems evolve and develop. An interesting highlight of the symposium was the description of the entire gene locus encoding both isotocin and vasotocin in the Japanese pufferfish, *Fugu rubripes* (B. Venkatesh, Singapore). Unlike the situation in higher vertebrates, here the two genes are in the form of tandem repeats, with two other genes in between. It was suggested that the inverse arrangement in mammals may have evolved by a subsequent inversion of one of the genes encoding the hormones, bringing the two related genes even closer together in the genome, and thus possibly encouraging the gene conversion events that appear to be so characteristic of the mammalian vasopressin and oxytocin genes. The combined gene locus in mice was described in detail by A. Ratty (Singapore) and H. Gainer (Bethesda).

Although we now have a linear description of the chromosome sequence, very little is known about what the sequence means, and how this genetic information is interpreted by cells expressing oxytocin. Several reports were presented describing potential control elements in the upstream, promoter region of the oxytocin gene (H. Zingg, Montreal; P. Burbach, Utrecht; N. Walther, Hamburg). The clear message was that, although such elements may respond to oestrogen receptors, or thyroid hormone receptors, or retinoic acid receptors under heterologous conditions *in vitro*, there is little evidence that these control elements respond to such nuclear receptors *in vivo*. Even in the one case where it was proven that nuclear orphan receptors like the steroidogenic factor 1 (SF-1) bind to the oxytocin gene promoter *in vivo*, in luteinizing ovarian granulosa cells (N. Walther, Hamburg), this factor alone is insufficient to explain the massive upregulation of the oxytocin gene in this tissue. Some insight into the problem was provided by results from painstaking studies in transgenic mice (D. Murphy, Singapore; H. Gainer, Bethesda), which suggest that a combination of elements involving both the oxytocin and the vasopressin genes may be involved, with interest being focussed on a region downstream of the oxytocin gene.

One of the enigmas in oxytocin and vasopressin research has always been the role of neurophysin, which is co-produced with the nonapeptide hormone within the same polypeptide precursor in all species so far examined. Now a plausible explanation has been offered (P. Burbach, Utrecht), with neurophysin taking on the role of a molecular chaperone. Mutations in the neurophysin moiety appear to disrupt the normal secretory pathway for the peptide, and may be decisive in determining a constitutive or a secretory route for peptide production.

One aspect which illustrates that things may not be quite as they seem on superficial inspection was the observation that oxytocin immunoreactivity in rat magnocellular neurones need not mean that there is normal oxytocin gene expression in those neurones. It has been elegantly shown that homologous recombination can take place *in vivo* between the vasopressin and the oxytocin genes in solitary neurones to give rise to chimaeric molecules that are translated, and appear to be accompanied

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by curious ultrastructural phenotypes (J. Morris, Oxford). This should not, however, be regarded only as a curiosity, since it seems to illustrate a very fundamental aspect of somatic gene repair and modification which may turn out to reflect one of the critical fail-safe systems in all cells to maintain normal function in the face of mutational pressure.

## Regulation of oxytocin production

Although the genetic information provides no easy answers, it is also evident from a number of studies that oxytocin is regulated by the sex steroids. The site of action of these steroids is far from clear, except that in the rat hypothalamus oestrogen accompanied by progesterone withdrawal, mimicking the situation at parturition and lactation, causes a marked increase in mRNA encoding oxytocin (J. Amico, Pittsburgh). Since the hypothalamic magnocellular neurones in the rat do not contain oestrogen receptors, this effect must be mediated by other neuronal systems. This may not be the case for oxytocin in the rat uterus, where a similar effect of steroids is observed (H. Zingg, Montreal), and where, at least in some cells, oxytocin expression and oestrogen receptors do colocalize. In an attempt to bridge this gap between sensory input to the magnocellular neurones and activation of oxytocin gene transcription, it has been shown that one of the first events upon stimulating oxytocin cells is upregulation of the Fos protein, an early response transcription factor (S. Luckman, Cambridge), although it is not known whether this is directly linked to the regulation of the oxytocin gene.

It is now accepted that the control of oxytocin production in the hypothalamus and its secretion from the posterior pituitary is a complex process that is regulated by neuronal input from a variety of central sources. The regulation of oxytocin secretion from the posterior pituitary gland has been extensively studied, and the influence of stimuli from the nipples or contracting uterus and stretched uterine cervix are well known. In the rat, systemic cholecystokinin, acting via the vagus and central pathways, and hyperosmolarity, acting via the anterior hypothalamus, are strong stimuli to oxytocin secretion, reflecting the role of oxytocin in natriuresis. In the chick, where vasotocin appears to subserve both osmoregulatory and oxytocic functions, it could be shown that, even before hatching, osmotic stress causes an upregulation of the vasotocin gene and an increase in responsiveness of the magnocellular neurones (R. Grossmann, Celle).

One of the most interesting features, and one which makes oxytocin secretion an ideal model system to study, is its pronounced pulsatility. An intermittent burst-firing activity in magnocellular oxytocin neurones leads to the pulsatile oxytocin secretion underlying the milk-ejection reflex, and probably also parturition (J. Wakerley, Bristol). This ability of magnocellular oxytocin neurones to discharge intermittently at high frequency simultaneously with each other during a steady afferent barrage from the suckled nipples remains incompletely understood. The mechanism develops towards the end of pregnancy (J. Wakerley, Bristol), with morphological changes in the magnocellular nuclei, and increased GABA and glutamate synaptic contacts onto oxytocin neurones (D. Theodosis, Bordeaux). However, the special feature of the milk-ejection bursts of the oxytocin neurones is the positive feedback action of oxytocin itself. This occurs at two sites at least, one on the magnocellular oxytocin neurones themselves (M. J. Freund-Mercier, Strasbourg; I. Neumann, Munich) with a disinhibitory action on GABA-mediated input or actions (A. Brussaard, Amsterdam), and the other in the bed nucleus of the stria terminalis, a part of the facilitatory network impinging on the magnocellular oxytocin neurones (J. Wakerley, Bristol). This action appears to be modulated by sex steroids (J. Wakerley, Bristol). Of particular interest is that the pulsatile secretion of oxytocin from the posterior pituitary gland that can be demonstrated in sheep at luteolysis is also facilitated by oestradiol, and suppressed by progesterone (J. McCracken, Shrewsbury).

Another developing area of oxytocin research is the role that oxytocin plays in controlling the anterior pituitary (D. Samson, North Forks). Oxytocin is transferred via the hypophyseal portal system from the hypothalamus and has significant effects either directly or synergistically on prolactin, corticotrophin and gonadotrophin production, thus modulating anterior pituitary dependent functions.

## The molecular biology of the oxytocin receptor

The biggest single breakthrough in recent years has been the cloning of the oxytocin receptor (T. Kimura, Osaka). This achievement has at last provided not only a detailed structure of the receptor, but also tools in the form of DNA probes, antibodies, transfected cell systems and antisense techniques, which have opened up many new avenues for research.

The oxytocin receptor cDNA or gene structure is now known for the human (T. Kimura, Osaka), pig (F. Fahrenholz, Frankfurt), rat (H. Zingg, Montreal), sheep (A. Flint, Sutton Bonington) and cow (R. Ivell, Hamburg). The encoded protein sequences are very highly conserved and conform to the typical seven transmembrane, G protein-coupled receptor family (Fig. 1). A comparison of the primary protein sequences shows that some regions are more highly conserved than others. For example, apart from the transmembrane domains, the first and second extracellular loops are absolutely conserved across all mammals so far examined, whereas there is substantial substitution possible in other regions, which apparently does not jeopardize oxytocin binding or signal transduction (R. Ivell, Hamburg).

The production of cell lines transfected with wild-type or mutated receptor gene constructs has already allowed a preliminary analysis of the residues essential for oxytocin receptor ligand-binding and activation (B. Chini, Milan; C. Barberis, Montpellier; F. Fahrenholz, Frankfurt; N. Yarwood and M. Wheatley, Manchester). These preliminary results appear to support the contention based on the evolutionary comparison of sequences. This research will lead to a three-dimensional structure of the oxytocin receptor complex and should provide an important molecular basis for the future design of oxytocin agonists and antagonists. A very important point was raised by F. Fahrenholz (Frankfurt), who showed that the affinity and properties of the oxytocin receptor can be modulated by the lipid content of the membrane in which the receptors are expressed. This means that even though there is only a single gene for the oxytocin receptor in the mammalian genome, there may still be apparently different pharmacological receptor subtypes, depending upon the nature of the cells in which the receptor gene is expressed.

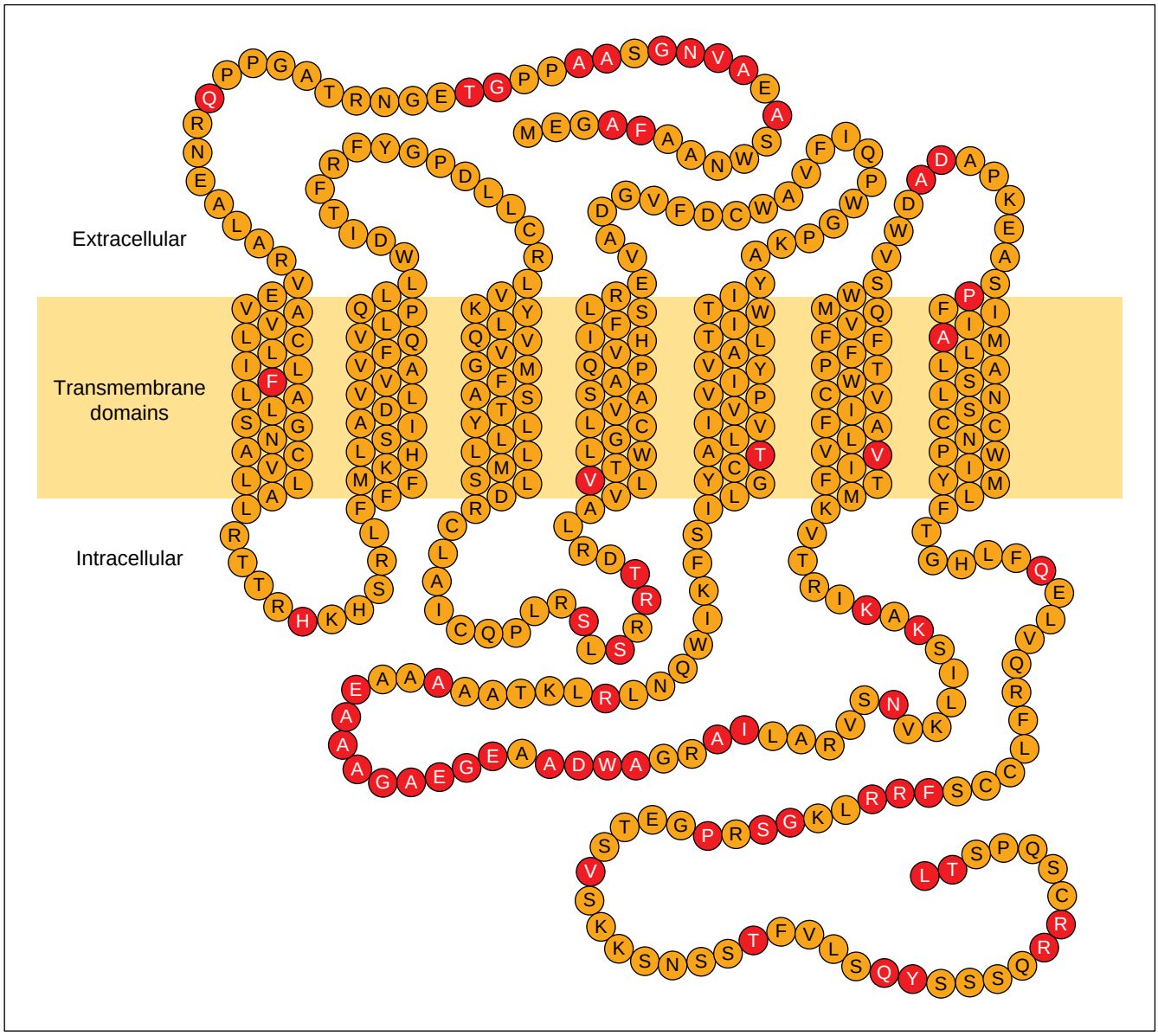


**Fig. 1.** Schematic structure of the bovine oxytocin receptor. Amino acids that are not conserved through mammalian evolution are shown in red. (Modified after Ivell and Russell, 1995.)

The use of receptor-specific nucleic acid probes and antibodies has led to a wealth of new observations. In the uterus of the rat, human, sheep and cow, it has been shown that the massive upregulation of oxytocin binding observed at the end of pregnancy in the endometrium and myometrium is due largely to an increase in gene transcription (T. Kimura, Osaka; H. Zingg, Montreal; A. Flint, Sutton Bonington; A. R. Fuchs, Hamburg). The same is true for the ruminant during luteolysis (A. Flint, Sutton Bonington; A. R. Fuchs, Hamburg). The value of the ruminant model for examining the regulation of the oxytocin receptor gene was emphasized, particularly the role of the blastocyst product, interferon-τ, which appears specifically to switch off the oxytocin receptor gene in the endometrium, thus preventing oxytocin-induced prostaglandin production and luteolysis, and hence signalling pregnancy (A. Flint, Sutton Bonington).

A suprising finding was that, using an anti-receptor antibody, T. Kimura (Osaka) was unable to locate receptors in the myoepithelial cells of the human breast where they might have been anticipated; instead they appeared to be on stromal and glandular epithelial cells. In the rat kidney, mRNA encoding the oxytocin receptor is found in the pars recta of the proximal tubule and in the macula densa, but this expression is oestrogen dependent and, in pregnancy, expression is very low (N. Ostrowski, Bethesda). Could this, considering the natriuretic actions of oxytocin, explain the dramatic retention of fluid and electrolytes in pregnancy? In the brain, particularly in the ventromedial nucleus, the content of mRNA encoding the

oxytocin receptor is increased by oestrogen, consonant with the known importance of oxytocin for female sexual receptivity in this region (T. Bale, Seattle). These two studies also illustrate the very tissue-specific nature of oestrogen effects, and emphasize the indirect mode of oestrogen action, stimulating in one tissue, and inhibiting in another.

## Oxytocin in the brain and behaviour

It is now evident that oxytocin is not only produced by the magnocellular nuclei with the sole purpose of being exported via the posterior pituitary into the bloodstream for peripheral functions. Already alluded to is the finding that oxytocin is released within the magnocellular nuclei themselves and appears to function by establishing a positive feedback loop on oxytocinergic neurones, orchestrating and amplifying the oxytocin response (M. J. Freund-Mercier, Strasbourg; I. Neumann, Munich). Introducing antisense oligonucleotides against the mRNA encoding oxytocin peptide into the supraoptic nuclei acutely depresses the milk-ejection reflex. The interpretation, however, is not simple, since the neurones are also rendered unresponsive to afferent stimuli (I. Neumann, Munich). The use of antisense oligonucleotides, now widely used in the brain to influence behaviour, was critically appraised by G. Jirikowski (Jena), who was able to show that triple helix formation also occurred, as well as the assumed binding to the specific mRNA, and thus that sense oligonucleotides may also inhibit function and be unsuitable as negative controls.

In the brain, plasticity of oxytocin actions on behaviour appears to be partly a consequence of changes in oxytocin receptor expression, and antisense oligonucleotides against the oxytocin receptor can attenuate sexual and affiliative behaviour. Oestrogen may enhance the central anxiolytic action of oxytocin in the mouse also by this means (M. McCarthy, Baltimore). A combination of oestrogen and oxytocin significantly reduced anxiety. In two closely related species of American vole, there is a striking contrast in pair-bonding behaviour, being marked in the prairie vole (*Microtus ochrogaster*) and completely absent in the montane vole (*M. montanus*). This difference correlates with distinct patterns of oxytocin receptor distribution in these two species, with fewer receptors in the latter (T. Insel, Atlanta). Molecular analysis of the basis of these differences in social behaviour is potentially important in the context of human behavioural and psychiatric disorders. In one study it was shown that severely depressed patients had significantly reduced serum concentrations of oxytocin (G. Jirikowski, Jena).

Regarding male sexual behaviour, the central transmitters involved in regulating the oxytocin neurones in the paraventricular nucleus responsible for activating the neural circuits leading to penile erection in the rat have been well characterized. Nitric oxide has a key role in mediating the actions of such transmitters on oxytocin neurones, and hence of oxytocin itself in the brain (A. Argiolas, Cagliari).

There are complementary actions of oxytocin in the brain to its renal natriuretic action. Evidence from a centrally administered oxytocin antagonist, or a conjugate of ricin-A and oxytocin, which is taken up by neurones that have oxytocin receptors and evidently disables them, shows that oxytocin restrains salt appetite when it is stimulated. Ethanol has similar effects, possibly because it inihibits central oxytocin release (J. Verbalis, Washington). There are implications here for the sequelae, and perhaps causes of excessive alcohol intake!

## Oxytocin and the regulation of uterine function

Magnocellular oxytocin neurones are activated during parturition in the rat, thereby secreting pulses of oxytocin. This activation is partly reflex, via a pathway from the uterus or birth canal, probably relayed by neurones in the nucleus of the tractus solitarius (A. Douglas, Edinburgh). This may be the same pathway activated by systemic cholecystokinin, but notably, stimulation of oxytocin neurones by this noradrenergic pathway is particularly sensitive to inhibition by μ-opioids. These can act on the neurones in the brainstem, but more importantly on their terminals in the magnocellular nuclei, and on the oxytocin neurones themselves (G. Leng, Edinburgh; A. Douglas, Edinburgh). This central opioid mechanism becomes tonically active in pregnancy, perhaps leading to withdrawal excitation like that which follows morphine withdrawal in dependent rats (G. Leng, Edinburgh). These changes, together with those leading to activation of the milk-ejection reflex, typify the striking plasticity of the oxytocin neurones.

It is beyond question that the myometrium at the end of pregnancy is a target for oxytocin. Questionable, however, has been how important oxytocin is in parturition, what the source of that oxytocin is, and the role played by the endometrium. Although in most species plasma oxytocin is increased during parturition, with pulses overlying a steady increase (A. R. Fuchs, Hamburg; A. Douglas, Edinburgh), this has been difficult to demonstrate in humans except in the final stages of labour, even taking precautions to inactivate the circulating aminopeptidase. There is continuing debate about maternal versus fetal posterior pituitary oxytocin (P. Mitchell, Edmonton; Y. Dawood, Houston). In addition, the gene encoding oxytocin may also be expressed in the ovary, in particular in the corpus luteum, in several species, and in the endometrium or decidua in others. The discovery of a large increase in decidual mRNA encoding the oxytocin peptide mRNA content at the end of pregnancy in the human (P. Mitchell, Edmonton), and in the rat (H. Zingg, Montreal), but not in all species, may provide a paracrine answer to this problem. Unfortunately for this solution, the concentration of oxytocin peptide for these two species in the decidua is not greater than that in the circulation (P. Mitchell, Edmonton) and it would, if released, probably act on the endometrium rather than on the myometrium. Indeed, in cyclic ruminants, prostaglandin production by the endometrium in response to oxytocin is the signal precipitating luteolysis (J. McCracken, Shrewsbury), unless a blastocyst is present to block oxytocin receptor expression (A. Flint, Sutton Bonington). The list of possible interactions between different sources of oxytocin and the uterus is finally completed with the cow, in which at parturition the corpus luteum resumes its production of oxytocin, which has been silent throughout pregnancy, and thus supplements the oxytocin from the pituitary. There appears to be a negligible contribution of oxytocin from the uterus in this species (A. R. Fuchs and R. Ivell, Hamburg). In the rat, the ovary is not a source of oxytocin, but the endometrium at the end of pregnancy has a high content of mRNA

encoding oxytocin, although it contains little peptide (H. Zingg, Montreal).

Whereas the relative importance of the uterus and ovary as sources of oxytocin may differ between species, all placental mammals secrete oxytocin from the posterior pituitary gland, and even in humans what can be interpreted as stimulation of oxytocin secretion by the Ferguson reflex can be measured, at least at the end of the second stage of delivery. However, careful study of oxytocin secretion at the end of pregnancy in the cow suggests that oxytocin may indeed be important in the initiation of parturition as well as in its continuation (A. R. Fuchs, Hamburg), and the effectiveness of oxytocin antagonists in the treatment of pre-term labour strengthens this view also for humans (M. Akerlund, Lund).

In summary, these data suggest that there may be a variety of mechanisms invoked to initiate parturition, one of which may involve local oxytocin production, possibly within the uterus linked to local prostaglandin release. But it is also conceivable that pituitary oxytocin could be part of this initiation cascade. Once the cascade is set into action, however, pituitary oxytocin acting directly on the myometrium is a major inducer of birth contractions in the so-called second phase.

It is generally thought that changing secretion of oestrogen and progesterone towards the end of pregnancy is important in the regulation of oxytocin peptide and receptor gene expression. The content of oxytocin mRNA in human decidua increases *in vitro* in response to oestrogen, with no effect of progesterone (P. Mitchell, Edmonton), while in the rat endometrium *in vivo* the reported stimulatory action of oestrogen is enhanced by progesterone (H. Zingg, Montreal). The issue of how these steroids may regulate oxytocin peptide gene expression has been referred to already, as have sex steroid influences on hypothalamic expression of this gene (J. Amico, Pittsburgh). At the end of pregnancy, progesterone may inhibit the release of oxytocin from the posterior pituitary gland (A. Douglas, Edinburgh), although it stimulates release in the nonpregnant sheep and pig (J. McCracken, Shrewsbury; M. Mirando, Pullman).

In the human, there does not appear to be a change in oxytocin metabolism in the decidua at the end of pregnancy (P. Mitchell, Edmonton). Whereas oxytocin receptor density in the myometrium increases greatly at the end of pregnancy (A. R. Fuchs, Hamburg; A. Lopez-Bernal, Oxford), this may not be due to an increase in the mRNA encoding the specific receptor (S. Thornton, Cambridge). In contrast, during pregnancy in the rat, there is increased receptor gene expression in the uterus (H. Zingg, Montreal), and in the cow, in the myometrium, endometrium and cervix (A. R. Fuchs, Hamburg). In the rat, mRNA encoding the oxytocin receptor is induced by oestrogen, with no effect of progesterone, except that it decreases oxytocin receptor binding, indicating post-transcriptional modification (H. Zingg, Montreal). This oestrogen effect would appear again to be indirect, since no functional oestrogen response element has yet been located in the promoter of the receptor gene.

A particularly interesting new model with which to look at pregnancy and perinatal physiology is the marsupial (R. Bathgate and L. Parry, Hamburg). There appears to be a mesotocin-dependent physiology in marsupials very similar to that regulated by oxytocin in eutherian mammals. Moreover, marsupials have only a very short intrauterine pregnancy, such that many stress and osmotic effects due to the large fetal volume in higher mammals do not obscure the endocrinology.

## The oxytocin receptor and intracellular signal transduction

Both *in vivo* studies, mostly using uterine myometrial cells (A. Lopez-Bernal and S. Phaneuf, Oxford; B. Sanborn, Houston), and transfection studies with cloned oxytocin receptors (P. Riley and R. Abayasekara, London) show that the oxytocin receptor functions primarily via a phospholipase C route leading to inositol trisphosphate ($IP_3$) generation. In myometrial cells, the receptor is coupled by $G_q$ and possibly $G_i$ proteins to the phospholipase C, which via $IP_3$ causes an increase in intracellular $Ca^{2+}$ leading to muscle contraction. Production of $IP_3$ parallels the increase in oxytocin receptor density, but $G_q$ expression does not change; instead $G_s$a content, which is increased in pregnancy, mediating inhibition of contraction via cAMP and protein kinase A, falls at parturition. Thus removal of inhibitory intracellular mechanisms may be more important in increasing myometrial sensitivity to oxytocin than is upregulating excitatory mechanisms (A. Lopez-Bernal, Oxford; B. Sanborn, Houston).

## The role of oxytocin within the ovary

It has been mentioned on several occasions in this summary that oxytocin is produced locally within the ovary. Usually this has been with reference to the ruminant corpus luteum, where very large amounts of oxytocin are produced, which clearly influence the endocrinology of circulating oxytocin. However, ruminants are special in that evolution has selectively amplified a local oxytocin system in the ovary, which can be found in many other species, but with only a low level of expression. However, very little is known about this local ovarian system. In the porcine ovary, oxytocin is made in granulosa/luteal cells and W. Wuttke (Göttingen) suggested that is has a dual role, being involved in the early cycle in luteinization and in the late cycle in luteolysis. In the latter context, oxytocin is effective only on luteal cells previously exposed to tumour necrosis factor $\alpha$.

In the marmoset monkey, oxytocin has been convincingly shown to be a product of the granulosa cells within preantral follicles (A. Einspanier, Göttingen). Also the receptor can be detected, but in a different layer of granulosa cells from the peptide ligand. After a gonadotrophin stimulus, there is an increase in both oxytocin and oxytocin receptors, which now overlap spatially and thus establish an autocrine/paracrine loop within the preovulatory follicle. As oxytocin can stimulate progesterone production by these granulosa cells (A. Einspanier, Göttingen), also in the baboon (F. Khan-Dawood, Houston), human and pig (W. Wüttke, Göttingen), this points to an elegant mechanism whereby, through the action of the gonadotrophin stimulus, the oxytocin system can integrate and amplify the luteinization process and formation of the progesterone-producing corpus luteum. Another factor in this differentiation step may be the induction by oxytocin of tight junctions between the granulosa/luteal cells, as witnessed by the expression of connexin-45 in these cells (F. Khan-Dawood, Houston). Thus oxytocin may be a key factor in the regulation of the follicle in the periovulatory period.

What is often forgotten is that even after ovulation, granulosa cells still accompany the oocyte into the oviduct. Now called cumulus cells, these continue to produce oxytocin. In a study using human and mouse oocytes and cumulus complexes, oxytocin appeared to transfer to the zona surface (K. Furuya, Saitama), and may play a role in implantation. Moreover, treatment of mouse blastocysts with oxytocin in culture improved their rate of development (K. Furuya, Saitama).

## The role of oxytocin in the male

It is inevitable that most emphasis in oxytocin research is on the female. However, oxytocin may have important functions also in the male. In addition to being made in the hypothalamus, oxytocin is produced by the Leydig cells of the testis and appears to influence Leydig cell steroidogenesis in a paracrine or autocrine manner (H. Nicholson, Bristol). It is now emerging that oxytocin may also be produced in other parts of the male tract, including the prostate, where it appears to have an effect on 5α-reductase, responsible for converting testosterone to dihydrotestosterone (H. Nicholson, Bristol), and oxytocin concentrations appear to be increased in hyperplastic prostates.

## Oxytocin agonists and antagonists

Throughout the symposium, an important feature was the use made of novel ligand agonists and antagonists. These are finding application not only in research to probe oxytocin-dependent physiology, but also in the case of the Ferring antagonist, Atosiban, in advanced clinical trials, where this substance very effectively inhibits preterm contractions, and also appears to be effective in controlling dysmenorrhoea (P. Melin, Malmö; M. Akerlund, Lund). It should be noted, however, that this compound also has anti-pressor activity, being a moderate $V_{1a}$ antagonist, so that caution is required in interpreting results. These classically designed ligands may one day be ousted by orally compatible non-peptide compounds. However, the currently developed molecules of this type, though extremely interesting, are not yet clinically acceptable (D. Pettibone, West Point). The massive contribution made by Maurice Manning (Toledo) in making available almost unlimited amounts of novel agonists and antagonists to fellow workers in the field was honoured by a special lecture (The Dr Frederik Paulsen Lecture).

## The way ahead

Although the numerous new molecular tools described at this symposium will undoubtedly give rise to a flood of new and challenging research literature, a number of very open questions still remain. One of these relates to the sensitivity of the techniques. In the days of the polymerase chain reaction, when do we decide that the concentration of a molecule is simply too low for it to be functionally relevant? On the other hand, low level, presumably paracrine systems seem to abound in apparent redundancy of effect. Does this mean that the hormone systems are truly redundant, i.e. functionless? Or does this mean that our old-fashioned endocrine paradigms are simply inappropriate, and that we must develop new ones to comprehend these systems.

One intriguing aspect that emerged in several quite independent physiological areas was the involvement of oxytocin in positive feedback systems, which through continued stimulation lead to catastrophe-type events, for example, the autocrine effect of oxytocin in the magnocellular nuclei of the hypothalamus to concert and amplify the input until a burst of firing results. Another example is the uterus of the cyclic ruminant, where luteal oxytocin, in a positive feedback loop to the uterus, induces prostaglandin $F_{2\alpha}$ release from the endometrium, which in turn leads to more luteal oxytocin secretion and finally to luteolysis. Probably a similar positive feedback via pituitary oxytocin, uterine contraction and the Ferguson reflex leads ultimately to the expulsion of the fetus. Within the marmoset preovulatory follicle, we see how establishment of an autocrine or paracrine system in the granulosa cells leads to an increase in progesterone release, with ovulation and the formation of the corpus luteum as irreversible results. For most other peptide–receptor systems, desensitization of the receptor is a regular accompanying phenomenon, and is an important element in typical negative feedback inhibition. The disparity of the mechanisms that involve oxytocin in positive feedback effects would imply that there is a feature in the molecular structure of the receptor itself that encourages this behaviour. It is predicted that the oxytocin receptor will not desensitize in the same way as do other peptide receptors. Indeed, in a preliminary study, desensitization occurred in myometrial cells, but at the post-receptor level, with no change in ligand binding (G. Asboth, Oxford). In addition, treatment of rat brain with an oxytocin antagonist increased the effective ligand-binding capacity of oxytocin receptors (M. J. Freund-Mercier, Strasbourg). The molecular basis for this phenomenon is awaited with interest.

Finally, no-one seemed to be aware of any specifically oxytocin-associated pathologies. Could this mean that all such defects are lethal, and therefore never extant, or that the symptoms are subtle and pre-empted by nonspecific clinical treatment? For example, could women who experience protracted labour have an oxytocin abnormality? Or could certain cases of infertility involve an oxytocin-related defect, or an inability to breast-feed, or disturbances in penile erection or ejaculation, or certain psychiatric disorders? Once one begins this possible list, then symptoms become obvious, but have usually been assessed and treated without consideration of possible oxytocin pathophysiology. In this context, it will be very interesting to follow the results of the several ongoing attempts to produce transgenic 'knock-out' mice, either for the peptide or for the receptor.

A particularly exciting development has been the finding that oxytocin may be involved in prostatic hyperplasia (H. Nicholson, Bristol), and also in the growth of breast cancer cells (G. Bussolati, Palermo; Y. Ito and T. Kimura, Osaka). The diagnostic and therapeutic opportunities in this field will certainly be rapidly explored.

The symposium in Stade was the first comprehensive meeting on oxytocin for several years. The participants of the meeting were all agreed that it made an excellent account of the current status of our knowledge and ignorance.