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REVIEW

# Possible actions of gonadal oxytocin and vasopressin*

D. Claire Wathes

*Department of Anatomy, The Medical School, Bristol BS8 1TD, U.K.*

## Introduction

The hypothalamo–neurohypophysial system is involved in the synthesis and release of the two posterior pituitary hormones, oxytocin and vasopressin. The involvement of these hormones in lactation, parturition, vasoconstriction and the regulation of diuresis is well established, and will not be covered in this review. It has been suspected for some time that they may also be involved in other factors concerned with reproduction such as sperm transport and luteolysis (see Harris, 1947; Cross, 1955; Armstrong & Hansel, 1959). Many early studies used pharmacological doses of these hormones when testing their effects on reproduction and this led to questions concerning the physiological validity of the results. However, it has now been demonstrated that the gonads of a number of mammals contain large amounts of oxytocin and somewhat lower concentrations of vasopressin which they probably synthesize (Wathes & Swann, 1982; Wathes *et al.*, 1982, 1983b; Flint & Sheldrick, 1982, 1983; Wathes, Swann, Birkett, Porter & Pickering, 1983a). The subject therefore merits reconsideration and in compiling this review I hoped that a reappraisal of the earlier data from a new viewpoint might help to suggest what functions these gonadal hormones could be having.

Hormone values given in this review are presented in international units or weights. They have not been standardized because of unknown variations in the purity of preparations used by different authors. An approximate conversion figure for oxytocin and vasopressin is 1 i.u. = 2 μg.

## Characterization of luteal oxytocin and vasopressin

### *Historical survey*

It is surprising now to realize that the oxytocic action of the corpus luteum was first discovered by Ott & Scott in 1910 (a, b). These workers used a goat with a cannulated mammary gland as their model and measured the amount of milk released before and after the injection of various tissue extracts. An extract of infundibulum increased the secretion of milk by a factor of about 100 in 5 min (Ott & Scott, 1910a), while "the corpus luteum, pineal body and thymus increased the quantity of milk fourfold in five minutes. The ovary minus the corpus luteum had no effect" (Ott & Scott, 1910b). The species from which the glands were obtained was not stated but the oxytocic action of the corpora lutea was less marked than that of the pineal or thymus (Ott & Scott, 1911). Ott & Scott (1912) also showed that an extract of ovary minus the corpus luteum inhibited the oxytocic action of the infundibulum.

* This work is dedicated to the memory of Professor E. C. Amoroso who suggested a number of years ago that I should carry out some studies on oxytocin.

These results were confirmed by Schäfer & Mackenzie (1911) and Mackenzie (1911) who used the anaesthetized lactating cat and found that an aqueous extract of ovine corpus luteum could induce milk let down routinely. They also showed that, whereas treatment with infundibular extract also led to an increase in blood pressure, treatment with corpus luteum extract did not. This is in accord with recent findings that the ratio of oxytocin to vasopressin is only 2000:1 in the bovine corpus luteum whereas the two hormones are present in comparable amounts in the infundibulum (Wathes *et al.*, 1983a).

Work in other species was less successful. Corpus luteum extract did not induce milk let down in women (Schäfer, 1913) or cows (Gavin, 1913). These failures, coupled with the confusion between the progestagenic and oxytocic actions of the corpus luteum on the mammary gland (see Hammond, 1913), may have led to the subsequent cessation of work on this topic.

*Recent studies*

The oxytocic action of the corpus luteum has now been 'rediscovered'. Fields, Fields, Castro-Hernandez & Larkin (1980) identified a factor in bovine corpora lutea which stimulated contractions in a mouse uterus bioassay. We obtained similar bioassay results in Bristol when testing extracts of ovine corpus luteum (Wathes & Swann, 1982). As this material showed parallelism in a radioimmunoassay for oxytocin using two different and specific antisera we attempted to characterize it in more detail. As well as stimulating uterine contractions, the extract caused intramammary pressure changes in the rat which were indistinguishable from those induced by Syntocinon (Wathes & Swann, 1982; Wathes *et al.*, 1983b). It eluted at the same position as oxytocin on Sephadex G-50 and when tested by high-performance liquid chromatography (HPLC) a single peak of immunoreactive material eluted in the same position as the oxytocin standard (Wathes & Swann, 1982). The latter test is a good discriminator for oxytocin as related peptides such as arginine vasotocin, vasopressin (AVP) and mesotocin do not have the same elution time.

Further studies in cattle revealed that bovine corpora lutea also contained an oxytocin-like peptide with similar bioactivity and the same elution position as oxytocin on Sephadex G-50, G-75 and HPLC (Wathes *et al.*, 1983a, b). In addition we found much smaller quantities of vasopressin-like material. This showed parallelism in a radioimmunoassay for AVP and eluted at the same position as AVP on HPLC.

Our identification of the main peptide as oxytocin has been supported by studies from two other laboratories. Flint & Sheldrick (1983) used a different extraction procedure and antiserum to estimate luteal oxytocin concentrations in the non-pregnant ewe and obtained similar results (Flint & Sheldrick, 1983: 1·7 μg/g; Wathes & Swann, 1982: 2·6 μg/g). Fields, Eldridge, Fuchs, Roberts & Fields (1983) confirmed that their bovine luteal extract stimulated contractions in the mouse uterus which could be prevented by preincubation of the extract with a monoclonal antibody to oxytocin. The bioactivity also eluted at the same position as oxytocin on HPLC.

Amino acid sequence analysis is needed before it can finally be confirmed that the peptide is oxytocin. Nevertheless, it has been identified on the basis of its chemistry, immunoreactivity and bioactivity and any differences between the luteal compound and oxytocin can only be minor.

## Measurements of oxytocin and vasopressin concentrations

*During the oestrous cycle*

*Sheep.* Measurements of oxytocin during the oestrous cycle of the ewe show that the circulating levels increase and decrease in synchrony with changes in the progesterone concentration, falling to a minimum at oestrus (Webb, Mitchell, Falconer & Robinson, 1981; Sheldrick & Flint, 1981; Mitchell, Kraemer, Brennecke & Webb, 1982; Schams, Lahlou-Kassi & Glatzel, 1982a; Flint & Sheldrick, 1983). However, the oxytocin levels drop before the progesterone levels, indicating that

the amount of production or release is not exactly parallel (Schams, Prokopp & Barth, 1983). In the pregnant ewe oxytocin levels fall at about Day 15 although the progesterone level is maintained (Webb *et al.*, 1981; Sheldrick & Flint, 1981, 1983a). I am unaware of any measurements of circulating vasopressin levels during the oestrous cycle.

The ovine corpus luteum is a rich source of oxytocin at a concentration of about 2 μg/g (Wathes & Swan, 1982; Flint & Sheldrick, 1983; Wathes *et al.*, 1983b). It now seems likely that it is the corpus luteum rather than the neurohypophysis which supplies much of the circulating oxytocin during the oestrous cycle. This idea is supported by the work of Flint & Sheldrick (1982) who measured an arterial venous difference in oxytocin concentration across the ovary but not across the head during the luteal phase. Treatment of such ewes with cloprostenol (an analogue of PGF-2α) led to a rapid increase in the oxytocin concentration in the ovarian but not the jugular vein, and this was accompanied by a fall in the luteal oxytocin content (Flint & Sheldrick, 1982, 1983). After ovariectomy of cyclic ewes circulating oxytocin concentrations fell to the limit of detection of the assay (Schams *et al.*, 1982a). The luteal oxytocin concentration decreases during pregnancy (Wathes & Swan, 1982; Sheldrick & Flint, 1983a) and after hysterectomy (Sheldrick & Flint, 1983b) and this change is reflected in blood levels of oxytocin. Rodgers, O'Shea, Findlay, Flint & Sheldrick (1983) have demonstrated in-vitro synthesis of oxytocin by the large luteal cells of the ovine corpus luteum.

*Cow.* Circulating oxytocin concentrations during the oestrous cycle of the cow are lower than in the ewe but follow the same trend, with the highest values found in the early and mid-luteal phases (Schams, 1983). The bovine corpus luteum also contains microgram quantities of oxytocin (Wathes & Swan, 1982; Wathes *et al.*, 1983a, b; Fields *et al.*, 1983) and the amount of oxytocin in the corpus luteum increases from a low value just after ovulation to a peak in the mid-luteal phase (D. C. Wathes, unpublished observations). D. L. Walters, E. Schallenberger & D. Schams (personal communication) have shown that the oxytocin concentration in the vena cava is higher than that in the jugular vein during the mid-luteal phase. The corpus luteum also contains bovine neurophysin I which is synthesized as part of the same precursor molecule as oxytocin in the hypothalamus (Wathes *et al.*, 1983a). Its presence also suggests that the changing blood levels during the cycle reflect ovarian biosynthesis. Another similarity with the ewe is that the ovarian oxytocin content is greatly reduced in pregnancy in the cow (Wathes *et al.*, 1983a).

Wathes *et al.* (1983a) also showed that the bovine corpus luteum contained picogram amounts of vasopressin. Although these values were lower than for oxytocin, they were nevertheless about 2 orders of magnitude higher than those reported in the circulation of the lactating cow (Landgraf, Wehowsky, Schulz, Schulze & Bothur, 1982). Luteal vasopressin concentrations also appear to follow a cyclic pattern similar to that for oxytocin (D. C. Wathes, unpublished observations) but variations in circulating levels during the oestrous cycle have not been reported.

*Primates.* Measurements of oxytocin or its associated neurophysin (oestrogen-stimulated neurophysin) in women and rhesus monkeys are in general agreement in that there is an increase in mid-cycle lasting from about 2 days before until 2 days after the LH peak (human: Legros, Franchimont & Burger, 1975; Mitchell, Haynes, Anderson & Turnbull, 1980; Amico, Seif & Robinson, 1981a; monkey: Robinson, Ferin & Zimmerman, 1976; Falconer, Mitchell, Mountford & Robinson, 1980). This increase occurred soon after the preovulatory rise in circulating oestradiol levels but remained elevated for longer. The two events may be related as the administration of exogenous oestrogen is known to stimulate the release of neurophysin (Robinson *et al.*, 1976; Amico *et al.*, 1981b). A second minor increase in neurophysin or oxytocin levels occurred during the luteal phase (Legros *et al.*, 1975; Mitchell *et al.*, 1980; Falconer *et al.*, 1980).

Studies on the circulating vasopressin levels during the menstrual cycle do not show any major alterations. Forsling, Åkerlund & Strömberg (1981) found a small increase at the time of ovulation with lowest values at the onset of menstruation. Punnonen, Viinamäki & Multamäki (1983) observed a slight rise in plasma vasopressin at the time of the preovulatory oestradiol surge, but this increase was not significant. In the rhesus monkey there was no significant alteration in the concentration of vasopressin in 24-h urine samples at different stages of the cycle (Miller & Wilks, 1973). In menopausal women oestrogen treatment alone augmented vasopressin release whereas

combined treatment with medroxyprogesterone and oestrogen led to a fall in circulating levels (Forsling, Strömberg & Åkerlund, 1982).

In our own studies we have measured the oxytocin and vasopressin levels of human ovaries removed at surgery (Wathes *et al.*, 1982; D. C. Wathes & M. G. R. Hull, unpublished observations). Concentrations of oxytocin in individual corpora lutea ranged from 0·03 to 37·7 ng/g and of vasopressin from undetectable values (< 30 pg/g) to 15·4 ng/g. We could not measure vasopressin in 7 samples of follicular fluid and oxytocin levels were at the limit of detection of the assay. We have as yet been unable to observe any consistent relationship in ovarian content with the stage of the cycle. However, this may be a reflection of the quality of the material as most of the samples were obtained from women experiencing irregular cycles. Similar quantities of immunoreactive oxytocin and vasopressin have also been detected in human ovarian tissue by J. M. Schaeffer, A. J. W. Hsueh, J. Liu & S. S. C. Yen (personal communication).

In primates, therefore, the main elevation in circulating oxytocin occurs at ovulation, in contrast to the situation in the sheep and cow in which it is found in the luteal phase. It seems unlikely that this ovulatory rise in oxytocin is from an ovarian source as the concentration in the ovary is too low and does not appear to increase at an appropriate time. It has also been shown that an oestrogen-stimulated rise in neurophysin secretion still occurs in ovariectomized monkeys (Robinson *et al.*, 1976). Nevertheless, the obvious difficulty in obtaining ovaries from 'normal' cyclic women means that it would be unwise to draw firm conclusions at present.

*Rat.* Heller (1957, 1959) first reported that the posterior pituitary content of oxytocin and vasopressin altered during the oestrous cycle of the rat. He found that the levels of both hormones changed in parallel and were highest during pro-oestrus and oestrus and lowest during metoestrus. Similar results were obtained by Swaab & Jongkind (1970) and Swaab, Jongkind & de Rijke-Arkenbout (1970) who estimated the synthetic activity of the supra-optic and paraventricular nuclei (SON and PVN) at different stages of the cycle and by Crowley, O'Donohue, George & Jacobowitz (1978) who measured the hormone content of the pituitary gland by RIA. The amount in the pituitary gland appears to mirror that in the blood because the highest concentration of circulating vasopressin was found on the morning of pro-oestrus (Skowsky, Swan & Smith, 1979) and the ability of the posterior lobe to release oxytocin *in vitro* was greater during oestrus than dioestrus (Pitzel, Bischoff & Konig, 1981).

There is disagreement about whether these changes are regulated by steroids or gonadotrophins. In preliminary experiments Heller (1959) showed that the glandular concentration of both oxytocin and vasopressin in ovariectomized rats could be increased by oestrogen treatment. This work was confirmed by Crowley *et al.* (1978) and Skowsky *et al.* (1979) and is also supported by the fact that the changing concentrations in the neurohypophysis during the oestrous cycle parallel the known alterations in circulating oestradiol-17β. However, Swaab & Jongkind (1970, 1971) found that the synthetic activity of the SON and PVN had increased by 2 weeks after ovariectomy and that this activity could be decreased by oestradiol benzoate. They argued that the changing steroid concentration during the cycle influenced gonadotrophin secretion which in turn affected the activity of the magnocellular hypothalamic neurones. In support of this they showed that treatment of oestrogen-primed ovariectomized rats with HMG, hCG, LH or FSH but not prolactin increased the synthetic activity of the SON and PVN (Swaab & Jongkind, 1970, 1971). This discrepancy may have been caused by differences in technique because Swaab & Jongkind measured the presence of TPP-ase, an enzyme marker for Golgi activity, in the hypothalamus, whereas the other authors measured the hormone content of the neurohypophysis. Studies on oxytocin and vasopressin concentrations in the ovary of the rat are lacking.

*Horse.* Measurements of circulating oxytocin concentrations in the mare on 4 days of the oestrous cycle showed significantly higher concentrations on Day 2 of oestrus and Day 5 after ovulation than on Days 10 and 15 when the animals would have been in the mid-luteal phase (Burns, Kumaresan & Douglas, 1981). Although these data are limited, and do not indicate the source of the oxytocin, they suggest that the pattern is more similar to that in the human than to that in the cow or sheep.

*Oxytocin and vasopressin concentrations in the male*

Oxytocin is present in the neurohypophysis in similar amounts in animals of both sexes (Heller, 1959; Fitzpatrick, 1966a). Circulating levels in the male are generally near or below the limit of detection of current radioimmunoassays although, as discussed below, both oxytocin and vasopressin may be released during sexual stimulation.

We have recently shown that testicular material from rats (Wathes *et al.*, 1983b; H. D. Nicholson & R. W. Swann, unpublished observations), men (Nicholson *et al.*, 1983) and bulls (D. C. Wathes & J. F. Foulkes, unpublished observations) contains small amounts of oxytocin, vasopressin, and their respective neurophysins, suggesting local biosynthesis. The concentrations measured were as follows: oxytocin: rat 1–6 ng/g; man 0·3–3 ng/g, bull 0·2 ng/g; vasopressin: rat 0·7–1·6 ng/g, man 0·01–0·7 ng/g, bull at limit of detection of assay. However, Skowsky *et al.* (1979) showed that levels of vasopressin in plasma had increased by 2 weeks after castration in the male rat, and that this rise could be prevented by testosterone administration.

*Effect of mating on oxytocin release in the female*

A number of early studies provided indirect evidence that oxytocin and vasopressin are released at coitus because increased uterine activity (cow, dog, rat, guinea-pig), milk ejection (man, horse, cow) and antidiuresis (rat, human) may occur at this time (see review by Fitzpatrick, 1966b). Measurements on oxytocic activity in blood are more equivocal.

Hawker, Roberts & Walmsley (1959) could not detect any plasma oxytocin in ewes bled before and after mating but their bioassay would have been inadequate to detect low levels. Other workers have reported oxytocin release during coitus, but not in all individual animals. Such a response was found in 1/4 mares (Walmsley, 1963), 2/2 women (Fox & Knaggs, 1969) and 8/36 goats (McNeilly & Ducker, 1972). Fuchs (1972) estimated the oxytocin release in mated rabbits by examining the pattern of intrauterine activity and concluded that it occurred rarely and was of little significance. However, oxytocin was almost always detectable around the time of oestrus in goats (McNeilly & Ducker, 1972). A rise in concentration frequently occurred before mating, and could be triggered by various stimuli such as the sight or smell of a male goat. It appears therefore that the strength of this response varies both between species and perhaps between individuals within a species.

*Effects of mating on oxytocin release in the male*

An elegant experiment by Debackere, Peeters & Tuyttens (1961) involved cross-circulating blood between a lactating ewe and a ram. Massaging the seminal vesicles of the ram caused a sharp rise in intramammary pressure in the ewe. Peeters & Debackere (1963) followed this by demonstrating an inhibition of water diuresis in hydrated rams after massage of the seminal vesicles or coitus, and on the basis of these two experiments they estimated that seminal vesicle massage caused the release of between 40–100 mU oxytocin and 3–4 mU of arginine vasopressin (AVP). These findings were supported by measurements of oxytocic activity in plasma after ejaculation in the boar (bioassay; Ewy, Wojcik, Barowicz, Kolcak & Wierzchos, 1972), bull (bioassay: Bereznev, 1963; Sharma & Hays, 1968, 1973) and ram (bioassay and RIA: Sharma, Fitzpatrick & Ward, 1972). In all cases oxytocin was undetectable before coitus. In the ram, levels had returned to baseline within 5 min whereas in the bull oxytocin values peaked 4–6 min after service. An increase in oxytocin levels following sexual arousal has been found in Pony stallions (Burns *et al.*, 1981). Sharma *et al.* (1972) also reported an increase in vasopressin levels in the ram as measured by RIA but no details were given.

On the other hand, Sybesma (1968) could find no oxytocic activity in bull plasma after mating and Schams, Baumann & Leidl (1982b), using a sensitive RIA to monitor oxytocin levels in 5 bulls during natural mating, were likewise unable to detect any immunoreactivity, although they found a rise in oxytocin 3–5 min after electroejaculation. Further work is required to resolve these differences in results.

*Effect of genital stimulation on oxytocin release in the female*

Ferguson (1941) showed that stretching the cervix caused uterine contractions in the rabbit which could be abolished by pituitary stalk section. Distension of the cervix or vagina also caused milk ejection in goats, cows and ewes (Andersson, 1951; Hays & VanDemark, 1953a; Debackere & Peeters, 1960; Debackere *et al.*, 1961) which was accompanied by changes in cistern pressure equivalent to that caused by an injection of 20–50 mU oxytocin in the ewe (Debackere *et al.*, 1961). Further evidence that oxytocin is released after vaginal distension was provided by bioassay data for cows (Fitzpatrick, 1957), ewes (Roberts & Share, 1968) and goats (Roberts & Share, 1970) and by radioimmunoassay data for ewes (Flint, Forsling, Mitchell & Turnbull, 1975), goats (Blank & DeBias, 1977), and heifers (Schams *et al.*, 1982b). The reflex release of oxytocin was enhanced by concurrent treatment with oestradiol dipropionate (ewes: Roberts & Share, 1969) but inhibited by administration of progesterone whether given intramuscularly (ewes: Roberts & Share, 1969), intravenously (goats: Roberts & Share, 1970; Blank & DeBias, 1977) or directly into the lateral cerebral ventricle (goats: Roberts, 1971a). Some goats failed to release oxytocin in response to vaginal stimulation during the summer (Roberts, 1971b; Blank & DeBias, 1977). This seasonal variation did not appear to coincide precisely with the breeding season, although accurate records were not kept.

*Conclusions*

High levels of oxytocin (μg/g) have been identified in the corpora lutea of cows and sheep. In the human ovary and the rat and human testis concentrations of oxytocin are measured in ng/g. Vasopressin has been found in small amounts (generally pg/g) in ovaries of women and cows and testes of men and rats. Other species have not yet been studied in detail although preliminary observations from our own and other laboratories suggest that oxytocin is probably present in the ovaries of the sow, goat, rat and rabbit.

In cows and sheep both peripheral and ovarian oxytocin levels reflect the activity of the corpus luteum during the oestrous cycle. They increase during the early luteal phase and start to decline again shortly before progesterone levels decrease at luteolysis. However, the two hormones do not follow the same trend during pregnancy when progesterone levels are maintained but oxytocin levels fall. In contrast, maximum circulating concentrations of oxytocin and vasopressin in women are found at the time of ovulation, and the same probably applies to rats. There is again a lack of information from other species.

Many of the data available on oxytocin and vasopressin release during mating and other sexual stimulation are based on bioassays and are not particularly reliable. It appears that both hormones can be released during mating in both sexes, but there is considerable variation between animals and not all studies are in agreement. It is therefore unlikely that either hormone is invariably released into the peripheral circulation at coitus, but local changes in concentration within the gonads have not been investigated.

## Actions of oxytocin and vasopressin on the motility of the reproductive tract

*Ovary*

Ovaries from a number of mammals exhibit spontaneous rhythmic contractions both *in vivo* and *in vitro* which originate primarily from the hilar and medullary portions of the ovary (for review, see Espey, 1978). Several workers have shown that treatment with oxytocin can increase the resting tension as well as the frequency and amplitude of the contractions (rat: Gimeno, Borda, Rettori, Borda & Gimeno, 1973; Roca *et al.*, 1976; Sterin-Borda, Borda, Gimeno & Gimeno, 1976; Roca, Garófalo, Piriz, Martino & Rieppi, 1977; guinea-pig: Gimeno *et al.*, 1973; rabbit: Virutamasen, Smitasiri & Fuchs, 1976; monkey: Virutamasen, Wright & Wallach, 1973). Although Roca *et al.*

(1976) originally found no difference in the sensitivity to oxytocin at different stages of the oestrous cycle they, like Sterin-Borda *et al.* (1976), subsequently (Roca *et al.*, 1977) reported that a greater response occurred from ovaries of rats in pro-oestrus. The minimum concentration of hormone tested in these studies was roughly between 0·4 and 6·0 ng/ml. However, Soloff (1975) could not find any specific receptors for oxytocin in the ovaries of rats that had been treated with oestrogen for 2 days.

It is possible that contractions of the ovarian follicle wall are involved in the process of ovulation, although considerable controversy surrounds this subject (see Espey, 1978; Martin & Talbot, 1981a, b). O'Shea & Phillips (1974) examined the contractility *in vitro* of strips of ovine follicle wall. In 8 strips large doses of oxytocin (minimum tested 8 μU/ml) increased the tone whereas in 3 other strips, all from luteinized follicles, oxytocin either had no effect or decreased the tone. Roca *et al.* (1978) induced ovulation in rabbits with hCG and found that concurrent treatment with antiserum to oxytocin significantly reduced the number of ovulations per animal. However, they did not prove that this action was directly on the ovary. It is therefore clear that oxytocin can influence ovarian motility, but whether or not this is important physiologically remains to be confirmed.

*Oviduct*

The motility of the oviduct varies during the reproductive cycle, with a peak in contractile activity generally present around the time of ovulation (e.g. cow: Ruckebusch & Bayard, 1975; rabbit: Suzuki & Tsutsumi, 1981; pig: Zerobin & Sporri, 1972; Rodriguez-Martinez, Einarsson & Larsson, 1982; man: Helm, Owman, Sjöberg & Walles, 1982; review: Pauerstein & Eddy, 1979). Experiments *in vitro* showed that human oviducts responded to oxytocin concentrations of 8 μU/ml by an increase in tone and a less pronounced increase in the frequency and amplitude of contractions (Rorie & Newton, 1965). Strips from the isthmic portions of ovine oviducts also showed a marked stimulation following oxytocin treatment (Edqvist, Einarsson, Gustafsson, Linde & Lindell, 1975). A similar type of response was demonstrated *in vivo* in women (Guiloff, Andres, Ibarro-Polo & Gomez-Rogers, 1974), sheep (Noonan, Adair, Halbert, Ringo & Reeves, 1978), cows (Ruckebusch & Bayard, 1975) and pigs (Zerobin & Sporri, 1972). In women, the sensitivity of the oviducts to oxytocin appeared greatest during the proliferative phase of the cycle, and in cows a response was only seen around the time of oestrus (Guiloff *et al.*, 1974; Ruckebusch & Bayard, 1975). The majority of these effects were achieved with doses of hormone which probably gave blood levels within the physiological range. Soloff, Rees, Sar & Stumpf (1975) localized oxytocin binding sites in the smooth muscle cells of the rat oviduct by autoradiography and Soloff (1975) compared the binding sites for oxytocin in the uterus with those in the oviduct of the rat. He found that they were similar in terms of affinity, ligand specificity and number per mg particulate protein, and that at both sites there was an increase in receptor concentration in response to oestrogen.

*Uterus (non-gravid)*

A discussion of the role of oxytocin in controlling uterine activity at parturition is outside the scope of the present review. The reader is referred to reviews by Chard (1972), Porter (1975) and Fuchs (1978) and to the papers of Alexandrova & Soloff (1980a, b, c), Glatz *et al.* (1981), Sellers *et al.* (1981) and Fuchs, Fuchs & Husslein (1982). Most mammals show considerable variations in the motility of the uterus during the oestrous cycle. It is possible that this activity is influenced by the release of endogenous oxytocin or vasopressin and the relevant data from several species have been examined.

*Sheep, cow and pig.* These have been considered together because the observed pattern of uterine activity as assessed by intrauterine pressure recordings, electromyographs, or isolated uterine strips is essentially similar. The greatest activity occurs during pro-oestrus and oestrus when regular large amplitude contractions are observed, whereas in the luteal phase there may be small non-

propagated contractions or almost complete quiescence (ewe: Croker & Shelton, 1973; Naaktgeboren *et al.*, 1973; Ruckebusch & Bueno, 1976; Harding *et al.*, 1982; cow: Evans & Miller, 1936; Cupps & Asdell, 1944; Hays & VanDemark, 1953b; Döcke, 1962; Zerobin & Sporri, 1972; Ruckebusch & Bayard, 1975; Brand *et al.*, 1976; sow: Zerobin & Sporri, 1972). For cows and sheep there is some evidence that contractions propagate from the cervix towards the oviduct during oestrus, with a reversal in direction after oestrus, although the precise details of this switch vary between authors (ewe: Croker & Shelton, 1973; Naaktgeboren *et al.*, 1973; Hawk, 1975; Rexroad, 1980; cow: Döcke, 1962). The alteration in activity during the cycle is due in part to the changing steroid environment: activity is abolished by ovariectomy and can be restored by oestrogen treatment, with or without progesterone, in ewes (Lye & Porter, 1978; Porter & Lye, 1983) and cows (Hays & VanDemark 1953b; Ruckebusch & Bayard, 1975).

In the ewe the sensitivity of the myometrium to oxytocin varies during the cycle with little responsiveness during the luteal phase (Roberts & McCracken, 1976). Fitzpatrick (1960) used cows in the luteal phase to obtain a dose–response curve for the action of oxytocin on uterine contractions and found an effect with doses in excess of 3 units. This explains why early work on the cow, using large doses of oxytocin, showed that the uterus was capable of responding with intense contractions at all stages of the cycle (Hays & VanDemark, 1953c: 15 units *in vivo*; Cupps & Asdell, 1944: 0·1–0·2 units *in vitro*). Other workers, who presumably used lower doses, found that the uterus became refractory to oxytocin during the luteal phase (Evans & Miller, 1936; Zerobin & Sporri, 1972; Ruckebusch & Bayard, 1975). The observed variation in response during the cycle is related to the changing steroid environment because it has been shown for sheep, cows and goats that oestrogen treatment enhances and progesterone treatment depresses the action of oxytocin on the myometrium (sheep: Carrick & Cupps, 1976; Lye & Porter, 1978; Porter & Lye, 1983; cow: Hays & VanDemark, 1953c; Fitzpatrick, 1960; goat: Jones & Knifton, 1975). Fitzpatrick (1960) examined the action of vasopressin in the cow and found that this hormone also induced uterine contractions in a dose-related manner. Its relative potency was only about 17% that of oxytocin, but the uterine response to vasopressin was also enhanced by oestrogen treatment.

*Man.* The literature on the variations of uterine activity during the menstrual cycle has been well reviewed by Porter (1975). He concluded that much of the earlier work on this subject was suspect because of poor recording technique and that the most reliable data were those provided by Csapo (1970a, b). These showed frequent, low-amplitude contractions during the follicular phase and mid-cycle. During the luteal phase the amplitude remained low but the frequency decreased. With the onset of menstruation a pattern of low-frequency, high-amplitude pressure cycles developed.

The non-pregnant human uterus is of interest in that it is unresponsive to oxytocin stimulation during most of the cycle. Coutinho & Lopes (1968) found that exogenous oxytocin had little effect from early in the follicular phase until late in the secretory phase, whereas Hendricks (1966) reported that the only clear response to oxytocin occurred in one subject on Day 9 of the cycle. Doses up to 10 i.u. given i.m. and 2 i.u. given i.v. were tested. However, the uterus appears to show a biphasic response to vasopressin. During menstruation vasopressin will stimulate uterine contractions at a much lower dose than that required for oxytocin (Coutinho & Lopes, 1968; Strömberg, Åkerlund, Forsling & Kindahl, 1983). In the follicular phase there is a gradual reversal in action, and by mid-cycle vasopressin will inhibit uterine activity (Coutinho & Lopes, 1968). Fuchs & Coutinho (1971) showed that the administration of plasma volume expanders during menstruation (which would be expected to decrease pituitary vasopressin release) resulted in a marked suppression of uterine activity which could be restored by vasopressin treatment. This evidence supported the ideas that vasopressin may be a principal factor stimulating uterine contractions at menstruation. Women with primary dysmenorrhoea also have significantly higher circulating vasopressin levels than normal controls (Strömberg, Forsling & Åkerlund, 1981). However, as reported above, circulating vasopressin levels are actually lowest at the onset of menstruation in normal women (Forsling *et al.*, 1981).

The uterus is most sensitive to vasopressin during the luteal phase when progesterone levels are high, and in the menopausal woman progesterone treatment is required to get a stimulatory response to vasopressin (Coutinho & Lopes, 1968). The switch from the inhibitory to the stimulatory action of vasopressin is also said to occur at the beginning of the luteal phase, and this can be blocked by oestrogen treatment (Coutinho & Lopes, 1968). This would suggest that the high oestrogen levels during the follicular phase promote uterine inhibition, whereas high progesterone levels promote uterine stimulation. A poor response to oxytocin occurs during the follicular and luteal phases and cannot therefore be attributed to a progesterone block.

*Rat.* Studies on the uterine activity of the rat during the oestrous cycle using electrical or pressure recordings have shown that activity is minimal during pro-oestrus. At oestrus the frequency of contractions rises to a maximum but bursts of electrical activity are of variable amplitude. Metoestrus is characterized by regular, high-frequency bursts, with activity decreasing again in late dioestrus (Fuchs, 1976; Talo & Kärki, 1976; Ishikawa & Fuchs, 1978; Downing, Porter & Redstone, 1981). The low activity at pro-oestrus is associated with high circulating oestrogen levels and this is in accord with studies of the ovariectomized rat that show that oestrogen treatment causes periods of uterine quiescence, which can be overcome by progesterone administration (Burns, 1972; Fuchs, 1976; Downing *et al.*, 1981).

The sensitivity of the uterus to oxytocin stimulation varies during the cycle, being maximal at pro-oestrus and oestrus (Chan, O'Connell & Pomeroy, 1963) and can be increased by oestrogen treatment (Follett & Bentley, 1964). This is probably because oestrogen increases the affinity of oxytocin binding to its receptors in the rat uterus (Soloff, 1975). The rat myometrium will contract in response to vasopressin, but it is less sensitive to vasopressin than to oxytocin throughout the cycle (Heller, 1974).

*Rabbit.* In anoestrus the uterus is quiescent but as the rabbit comes into oestrus spontaneous rhythmic contractions develop (Harris, 1947; Aref & Hafez, 1973). After copulation there is an initial rapid increase in activity followed by a gradual decline to quiescence by about Day 4 of pregnancy (Aref & Hafez, 1973). In the non-pregnant rabbit oxytocin and vasopressin both stimulate uterine activity (Harris, 1947; Laudanski, Åkerlund & Batra, 1977; Nissenson, Flouret & Hechter, 1978). The response to oxytocin is increased by oestrogen treatment and abolished by progesterone (Harris, 1947; Nissenson *et al.*, 1978).

### *Male tract*

The known effects of oxytocin and vasopressin on the female reproductive tract led a number of workers to investigate their influence in the male. The earlier experiments (1916–1940, cited by Fitzpatrick, 1966a, and Melin, 1970) did not provide good evidence for an action of posterior pituitary hormones on the vas deferens and epididymis but, because the experimental techniques were insensitive and the posterior pituitary preparations impure, no firm conclusions can be drawn from the results reported. Since then, Setchell & Linzell (1968) found no effect of oxytocin on the flow of fluid through the rete testis of the ram and Jaakkola & Talo (1981) reported that oxytocin had no significant effects on the electrical and mechanical activity of the caput and cauda epididymidis of the rat. However, the majority of papers indicate that both hormones can affect the motility of several parts of the male tract.

Niemi & Kormano (1965) observed the motility of rat seminiferous tubules *in vitro*: lower doses of oxytocin (10 mU/ml) decreased the interval between successive peristaltic contractions whereas higher doses (100 μU/ml) produced tonic contractions. Both effects occurred only in rats more than 13 days old. Oxytocin is also said to increase the contractile activity of the tunica propria and albuginea of the testis (Cross, 1955; Bereznev, 1963). Several workers have shown that oxytocin can stimulate contractions of the epididymis and vas deferens in the rabbit (Cross, 1955; Bereznev, 1963; Melin, 1970), mouse (Hib, 1974a, b) and ram (Knight, 1972, 1974a). Similar effects were reported for vasopressin (rabbit: Melin, 1970); rat: Jaakkola & Talo, 1981; mouse: Hib, 1974b;

ram: Knight, 1974a). Melin (1970) noted that the epididymis and vas deferens were more responsive to the actions of both hormones in the regions proximal to the testis. Neither hormone affected the motility of the seminal vesicles in the rabbit (Cross & Glover, 1958; Melin, 1970). In the in-vitro experiments effects were obtained with lowest doses in the range 168–700 pg/ml. In the in-vivo experiments injections were given intravenously and effects were observed with blood levels which probably reached 100–500 pg/ml. These doses are therefore 1–2 orders of magnitude higher than levels found in the peripheral circulation, but are in the same range as concentrations measured within the testis.

*Effects on semen*

Milovanov, Bereznev & Gorohov (1962) found that oxytocin administered immediately before copulation increased the volume of semen from bulls and Milovanov *et al.* (1962) and Kihlström & Melin (1963) obtained similar results in rabbits. Ewy, Bielanski & Zapletal (1963) cannulated the vas deferens of rams and measured the continuous rate of sperm discharge. An intravenous injection of 2 i.u. oxytocin increased the rate of discharge 7–50 times between 10 and 40 min after treatment. The output then decreased to a value below normal so that the total daily emission remained unaffected. These data led Cross (1966) to suggest that oxytocin released during copulation could accelerate sperm transport to the epididymis in readiness for the next ejaculation.

This short-term effect of oxytocin on sperm output has been confirmed in the rabbit (Fjellström, Kihlström & Melin, 1968), ram (Knight & Lindsay, 1970; Knight, 1974b; Voglmayr, 1975) and rat (Ågmo, Andersson & Johansson, 1978). In the majority of these experiments the circulating oxytocin level probably reached the ng/ml range. Sharma & Hays (1976) found that treatment of rabbits with methallibure, which is thought to block oxytocin release at the hypothalamic level, reduced the number of spermatozoa per ejaculate by 45%. This loss could be overcome by simultaneous oxytocin treatment.

In all cases reported above, oxytocin caused an increase in the volume of seminal plasma which was sometimes accompanied by an increased sperm output, suggesting that the effect was due to increased contractility of the upper genital tract. However, there is some discrepancy over the time course of this response. Milovanov *et al.* (1962), Kihlström & Melin (1963) and Knight & Lindsay (1970) all found a response when oxytocin was administered immediately ($< 5$ min) before mating. However, Ewy *et al.* (1963) found that oxytocin exerted its maximum effect on sperm output after 10–40 min and Voglmayr (1975) found a biphasic increase in sperm output to the rete testis between 40–60 and 80–140 min after treatment. In addition Knight & Lindsay (1970) found that, if oxytocin treatment (7 i.u./day) was continued over a longer period, it eventually (after 6 weeks) caused a decrease in the volume of semen and the number of spermatozoa per ejaculate, with an increased percentage of abnormal spermatozoa. They therefore suggested that oxytocin could inhibit the early stages of spermatogenesis. However, in the rabbit, Armstrong & Hansel (1958) found that long-term treatment with a higher dose of oxytocin (10 i.u./day for 11 weeks) stimulated the development of the seminiferous tubules and the Leydig cells. These experiments clearly indicate that oxytocin, and possibly vasopressin, can influence sperm transport in the male if administered exogenously. However, it is not yet known whether this effect occurs naturally.

*Conclusions*

It is clear that oxytocin and vasopressin can influence the motility of most parts of the reproductive tract in both sexes. In the female the uterus and oviduct are most sensitive to exogenous oxytocin around the time of oestrus and oxytocin could therefore play a role in gamete transport. For this effect to be of importance oxytocin must always be released around the time of ovulation or be released as a reflex response to mating or other sexual stimulation. Whilst there is

considerable indirect evidence that the latter occurs, these findings have not generally been confirmed by the relatively insensitive bioassays used on blood plasma samples and there have been insufficient studies using more sensitive radioimmunoassays. In the two farm species which have been studied (sheep and cow) circulating oxytocin concentrations are actually lowest at oestrus. In women there is an elevation in oxytocin in mid-cycle, but the human tract appears relatively insensitive to oxytocin stimulation. Vasopressin may be involved in uterine contractions at menstruation, but again circulating levels appear lowest at this time. An involvement of ovarian oxytocin and vasopressin in controlling the motility of the female tract seems unlikely, as the main source of the peptides is the corpus luteum rather than the follicle, and the luteal phase is characterized by little spontaneous uterine activity and poor sensitivity to oxytocin stimulation. An involvement of luteal oxytocin in parturition is also unlikely, because luteal concentrations in the cow and sheep decrease markedly in early pregnancy.

In the male both oxytocin and vasopressin can affect the motility of the epididymis and vas deferens, particularly in regions near the testis. Oxytocin has also been shown to increase peristaltic contractions of the seminiferous tubules, and it has a well documented ability to cause a short-term increase in semen output. This is probably achieved by increasing the rate of transport of the spermatozoa from the testis to the epididymis. In-vitro effects on the male tract were observed with hormone concentrations as low as 168 pg/ml in the mouse (Hib 1974a, b) while intravenous injections in rabbits and rams produced noticeable changes in activity with blood levels which probably did not exceed 100–500 pg/ml (Melin, 1970; Knight, 1972, 1974a). These hormone levels are greater than those found in the peripheral circulation but measured concentrations of oxytocin and vasopressin within the rat testis have reached 6 ng/g and 1600 pg/g respectively. These levels should be sufficient to activate the seminiferous tubules. An effect on the epididymis would probably depend on a local transport system, which could be via blood, lymph or the tubules. It is therefore possible that oxytocin and vasopressin from the testis could be involved in semen transport, but as yet this hypothesis is purely speculative.

## The luteolytic action of oxytocin

### *Oestrous cycle length*

Interest in the luteolytic action of oxytocin was initiated by Armstrong & Hansel (1959) when they demonstrated that oxytocin administered during the first week of the bovine oestrous cycle led to premature luteolysis between 8 and 12 days after ovulation. The corpora lutea of treated animals failed to attain a normal size and contained a marked increase in connective tissue (Armstrong & Hansel, 1959; Hansel & Wagner, 1960; Staples, McEntee & Hansel, 1961). Labhsetwar, Collins, Tyler & Casida (1964) reported that corpora lutea subjected to daily oxytocin treatment from the day of oestrus contained cavities filled with blood clots on Day 7. Large fluid-filled cystic corpora lutea were also found quite frequently after oxytocin treatment (Hansel & Wagner, 1960; Staples *et al.*, 1961). In all cases few normal luteal cells were present and the progesterone content was usually lower than in control heifers. The decrease in progesterone content showed a significant dose–response relationship to the amount of oxytocin administered (Donaldson & Takken, 1968). Corpora lutea removed on Day 8 after oxytocin treatment on Days 3–6 showed a decreased ability to synthesize progesterone *in vitro* (Carlson & Black, 1969).

Further work showed that oxytocin only caused a premature return to oestrus when given on Days 3–6 of the cycle. Treatments on Days 0–4, 7–13 and 15–22 were ineffective (Hansel & Wagner, 1960). Indeed, Mares & Casida (1963) reported that oxytocin treatment of heifers on Days 12 and 13 of the cycle actually increased the progesterone content of corpora lutea on Day 14. Harms, Niswender & Malven (1969) showed that when oxytocin was given from Day 2 a difference in the progesterone content of treated and control corpora lutea could first be detected on Day 5. Similarly Donaldson, Hansel & van Vleck (1965) found that treatment with oxytocin on Days 2–3

produced no significant alteration of the progesterone content by Day 4 whereas treatment on Days 2–6 significantly reduced the progesterone level by Day 7. Taken together these data indicate that corpora lutea are only affected by exogenous oxytocin during their initial growth and that excess oxytocin at this time can impair their normal development severely, both in terms of histological structure and ability to synthesize progesterone.

Similar experiments have been performed on sheep and goats. In the goat, Cooke & Knifton (1981) showed that treatment with daily injections of oxytocin between Days 3 and 6 of the cycle caused a premature return to oestrus on Day 6 or 7 compared with a normal cycle length of about 20 days. Individual oxytocin injections on Day 3 or 6 were ineffective. However data from the ewe are more equivocal. Milne (1963) was unable to cause a premature return to oestrus in Merino ewes despite trying three different doses (15–50 i.u.) on various days of the cycle. The maximum dose of 50 i.u. per day is luteolytic in goats (Cooke & Knifton, 1981). Using the same dose given daily for the first 7 days of the cycle in a different breed of sheep (Karagouniko) Hatjiminaoglou, Alifakiotis & Zervas (1979) shortened the length of the cycle to 6 days in 2 out of 8 animals. Treatment on Days 8–14 did not have this effect. However, Hatjiminaoglou *et al.* (1979) did find a more uniform effect of treatment on corpus luteum weight which was significantly lower on Day 14 of the cycle in both treatment groups compared with control animals. Similarly, Milne (1963) reported fewer normal luteal cells in treated than control ewes but, as he only examined the ovaries from 3 animals, this finding is inconclusive.

Two groups have also investigated the effect of immunization against oxytocin on ovarian cyclicity in ewes. Sheldrick, Mitchell & Flint (1980) found that after active immunization about one third of the cycles were prolonged, increasing the length by an average of 3·7 days. Also using active immunization Schams *et al.* (1983) found that some cycles were prolonged up to 64 days in 4 out of 5 ewes. In both studies there was a correlation between cycle length and antibody titre, but all the variability between animals could not be explained in this way. Using passive immunization with oxytocin antiserum given on Days 3–16, Schams *et al.* (1983) prolonged the cycle length in 4 out of 8 ewes. In the non-reacting animals the plasma progesterone levels were the same as in the previous control cycle, whereas in the reacting ewes they remained at the mid-luteal level for a longer time. Schams *et al.* (1983) also showed that immunization significantly reduced the free oxytocin levels in the jugular vein, but the concentration within the ovary was not measured.

In several other species oxytocin has been shown not to have a luteolytic action. In the mare, Neely, Stabenfeldt & Sauter (1979) were unable to alter plasma progestagen concentrations by daily injections of 150 units oxytocin (a dose which is effective in the cow) on Days 4–8 of the cycle. In the pig daily injections of various doses of oxytocin for the first 7 days after oestrus did not alter the interval to next oestrus (Duncan, Bowerman, Anderson, Hearn & Melampy, 1961). In the rat, Brinkley & Nalbandov (1963) reported that injections of 20 units oxytocin per day for the first 5 days of pseudopregnancy (a massive dose) did not alter the length of pseudopregnancy. Daily or 3 times injections of 2 units oxytocin to a series of guinea-pigs, starting on each day of the cycle and continuing for 3 days, did not alter the cycle length (Donovan, 1961).

### *The uterine involvement in the luteolytic action of oxytocin*

*Cow, sheep and goat.* In their original paper Armstrong & Hansel (1959) showed that oxytocin failed to induce luteolysis in 2 heifers that had been hysterectomized on the day after oestrus. This finding was confirmed by Anderson, Bowerman & Melampy (1965) and Ginther, Woody, Mahajan, Janakiraman & Casida (1967). Anderson *et al.* (1965) were, however, able to induce luteolysis in partly hysterectomized heifers by treatment with oxytocin and Ginther *et al.* (1967) found that in unilaterally hysterectomized heifers the uterine horn ipsilateral but not contralateral to the corpus luteum was required. These experiments provide compelling evidence that the luteolytic action of oxytocin is mediated by the uterus.

It was postulated that uterine motility might be important in this response and drugs known to

affect this were therefore administered concurrently with oxytocin. It was found that the luteolytic action in heifers could be blocked by atropine (Armstrong & Hansel, 1959; Black & Duby, 1965), reserpine (Armstrong & Hansel, 1959), adrenaline (Black & Duby, 1965) and noradrenaline (Auletta, Currie & Black, 1972) but not by isoproterenol (Auletta *et al.*, 1972). These data suggest that alpha but not beta adrenergic stimulants will block the action of oxytocin but the way in which these drugs act is unclear.

In 1972 McCracken *et al.* proposed that prostaglandin (PG) F-2α was the uterine luteolytic hormone in sheep. It was then demonstrated that mechanical stimulation of the uterus released PGF-2α (Roberts, Barcikowski, Wilson, Skarnes & McCracken, 1975). As mechanical stimulation of the tract was also known to release oxytocin by the Ferguson reflex, Roberts *et al.* (1975) reasoned that the luteolytic action of oxytocin might be mediated by the release of PGF-2α from the uterus.

This led to the hypothesis (described by McCracken, 1980, and McCracken, Schramm, Barcikowski & Wilson, 1981) that luteolysis in the ewe is caused by an interaction of oxytocin with its receptor in the late luteal phase stimulating the synthesis and release of PGF-2α by the uterine endometrium. The evidence for this can be summarized as follows. Mechanical stimulation (which causes oxytocin release) or oxytocin itself, both stimulate the release of PGF-2α by the ovine uterine endometrium with a marked variation in response during the oestrous cycle (Roberts *et al.*, 1975; Roberts & McCracken, 1976). There is a low release in the early cycle, no effect in the mid-luteal phase, and a pronounced release in the late luteal phase. This variation in response can be correlated with the number of endometrial oxytocin receptors which are almost undetectable on Days 5 and 13 of the cycle, increase on Day 15 and peak at oestrus (Roberts, McCracken, Gavagan & Soloff, 1976). The appearance of these receptors in the ewe is apparently controlled by circulating steroid levels. McCracken, Gammal, Glew & Underwood (1978) used the ability of an arterial infusion of oxytocin to evoke PGF-2α secretion from the autotransplanted uterus of an ovariectomized sheep as an in-vivo marker for the appearance of the oxytocin receptor. Oestradiol-17β infused alone for 12 h consistently caused a significant increase in oxytocin-induced PGF-2α release whereas progesterone alone had no effect. When a 12-h infusion of oestradiol-17β was superimposed at different times during a 10-day progesterone infusion, progesterone initially inhibited the stimulatory action of oestradiol-17β. However, on Day 10 (the length of a normal ovine luteal phase), oestradiol again caused an oxytocin-stimulated PGF-2α release, but this time the effect was 50–100 times greater. In the pregnant ewe the oxytocin receptor concentration is significantly lower on Day 16 (see McCracken *et al.*, 1981), suggesting that it is the appearance of oxytocin receptors in the late luteal phase which normally leads to luteolysis and that their formation can be blocked by pregnancy.

The hypothesis that the luteolytic action of oxytocin is mediated via the release of PG is supported by evidence from a number of other workers. Oxytocin injections have been shown to cause a significant increase in the circulating levels of PGF-2α or its metabolite PGFM (13,14-dihydro-15-keto PGF-2α) in pregnant and post-partum ewes (Mitchell, Flint & Turnbull, 1975), non-pregnant goats (Cooke & Homeida, 1982) and heifers (Newcomb, Booth & Rowson, 1977; Milvae & Hansel, 1980). The luteolytic action of oxytocin in the goat can be prevented by the administration of meclofenamic acid (Cooke & Knifton, 1981). About two-thirds of the increases in jugular PGFM in the ewe were accompanied by surges of oxytocin (Flint & Sheldrick, 1983). A similar relationship between oxytocin and PGFM release has been demonstrated indirectly by Fairclough *et al.* (1980) and Fairclough, Moore, McGowan, Smith & Watkins (1983) who measured the levels of ovine neurophysin I/II during luteal regression and found coincident surges of neurophysin and PGFM. The release of this neurophysin is indicative of oxytocin secretion (Moore & Watkins, 1983). However, at the start of luteolysis, neurophysin surges occurred without any marked rise in PGFM levels whereas at the end of luteolysis the reverse situation was found (Fairclough *et al.*, 1983). It has also been shown that oxytocin-induced luteolysis in the cow can be prevented by concurrent administration of hCG (Simmons & Hansel, 1964) or LH (Donaldson &

Hansel, 1966). This situation parallels that in the ewe in which both gonadotrophins will block the luteolytic action of PGF-2α (Henderson & McNatty, 1977; Bolt, 1979).

The important role of steroids in this response has also been confirmed. Sharma & Fitzpatrick (1974) showed that oxytocin injection alone did not cause the release of PGF-2α in the anoestrous ewe, but pretreatment with oestradiol-17β caused potentiation, leading to a 6-fold greater release of PGF-2α than was caused by oestradiol treatment alone. In the heifer the amount of PGF released in response to oxytocin varied with the day of the cycle, being maximal on Day 3 and decreasing thereafter as the progesterone level rose (Newcomb *et al.*, 1977).

*Laboratory animals.* The luteolytic action of oxytocin has not been extensively investigated in laboratory species, although two studies in the rat and guinea-pig have failed to show an effect on cycle length (see above). However, evidence from the rat (Campos, Liggins & Seamark, 1980) and rabbit (Small, Gavagan & Roberts, 1978) showed that endometrial tissue from ovariectomized animals released increased amounts of PGF in response to oxytocin. In the rabbit this effect was potentiated by pretreatment with progesterone, a situation similar to that found in the ewe. Two similar studies in the guinea-pig provide conflicting results. Leaver & Seawright (1982) used slices of endometrial tissue collected at Days 7 and 15 of the cycle and measured the output of PGF-2α *in vitro*. They reported that oxytocin given alone increased PGF-2α release on Day 15 but not Day 7, whereas if oestradiol was given concurrently a response was seen on both days. However, Poyser & Brydon (1983), using superfused guinea-pig uteri also on Days 7 and 15, did not stimulate prostaglandin release with oxytocin. The doses of oxytocin used in the two studies were similar (100 and 50 μU/ml respectively), and as they were both carried out in the same laboratory, the reason for the discrepancy is unclear.

### *Effect of prostaglandins on oxytocin release*

The data presented above show that oxytocin can cause the release of uterine prostaglandins. There is also evidence that the reverse situation occurs. Schams & Karg (1982) tested the luteolytic actions of four different PGF-2α analogues on cattle during the mid-luteal phase of the oestrous cycle. In all cases jugular oxytocin concentrations increased, reaching peak values 15–30 min after treatment and returning to baseline within about 2 h. Flint & Sheldrick (1982) administered cloprostenol (an analogue of PGF-2α) to sheep in the luteal phase and observed a rapid increase in the plasma oxytocin concentration which peaked about 10 min after treatment. As this response was much greater in the ovarian than the jugular vein and there was no measurable arterial–venous difference across the head, they concluded that the oxytocin released was of ovarian origin. On the basis of these observations Flint & Sheldrick (1982, 1983) suggested that during luteolysis ovarian oxytocin and endometrial PGF-2α engage in a positive feedback loop leading to a more rapid decline of the corpus luteum. A rise in plasma oxytocin levels has also been detected after prostaglandin administration in the male (man: PGE-2 and PGF-2α: Gillespie, Brummer & Chard, 1972; rabbit: PGF-2α: Desai & Raghavan, 1982), but the origin of this oxytocin is unknown.

### *Oxytocin and the establishment of pregnancy*

In the ewe luteal oxytocin concentrations are slightly lower on Day 14 of pregnancy than on Day 14 of the cycle (373 ± 256 ng/g compared with 730 ± 266 ng/g, $n = 6$ per group) and the levels continue to fall to $< 5$ ng/g by Day 50 of pregnancy (Sheldrick & Flint, 1983a). Luteal oxytocin concentrations are also considerably lower in pregnant than non-pregnant cows (Wathes *et al.*, 1983a) although the time at which this difference is first apparent has not been defined. In the ewe the drop in luteal oxytocin is accompanied by a decrease in the circulation concentrations of oxytocin (Sheldrick & Flint, 1981, 1983a; Webb *et al.*, 1981) and its associated neurophysin (Moore *et al.*, 1982). Similarly, Homeida & Cooke (1983) have shown that circulating oxytocin levels

decrease from Day 12 onwards in pregnant and non-pregnant goats. In the non-pregnant goat pulses of oxytocin release were superimposed on the overall pattern of decline, but these pulses were absent in pregnant animals.

These data show that luteal and circulating oxytocin levels fall at the same time whether or not the animals are pregnant. McCracken *et al.* (1981) found that the endometrial oxytocin receptor concentration was significantly lower on Day 16 in pregnant compared with non-pregnant ewes and this may explain why the release of oxytocin in pregnant ewes does not induce luteolysis. The decline of oxytocin in the ovary does not appear to be controlled by the presence of a conceptus because luteal levels fall after the cycle has been prolonged by hysterectomy (Sheldrick & Flint, 1983b) and this decline occurs at about the same time in hysterectomized and pregnant ewes (Sheldrick & Flint, 1983c).

*Conclusions*

There is now considerable evidence that oxytocin is involved in the control of luteolysis in the farm species studied. Nevertheless, the available data do not indicate that an increase in endometrial oxytocin receptors is the triggering event for luteal regression. Firstly, exogenous oxytocin has been shown to cause luteolysis in only two species, the cow and the goat, and in the cow this occurs only on Days 3–6 of the cycle. Several workers have shown that during the first few days of its development the corpus luteum of the cow will not respond to a normally luteolytic dose of PGF-2α (see Rowson, Tervit & Brand, 1972; MacMillan, 1978). Therefore, if oxytocin acts by stimulating PG release, it cannot cause luteolysis early in the cycle. The lack of effect later in the cycle can probably be explained by insufficient endometrial receptors for oxytocin although this has only been demonstrated in the ewe (Roberts *et al.*, 1976). Therefore, in the cow and goat there seems to be a short period between Days 4 and 6 during which the endometrium can respond to oxytocin and the corpus luteum to PGF-2α. The ewe is much less susceptible to oxytocin-induced luteolysis. This could be because PGF-2α is only luteolytic after Day 4 (Haresign, 1978) by which time the concentration of endometrial oxytocin receptors may be too low.

The crucial question which has yet to be answered is whether the endometrial oxytocin receptor concentration increases again before or after the progesterone level starts to fall. The only experimental evidence comes from Roberts *et al.* (1976) who found a small increase in receptor number on Day 15 of the ovine cycle, but they did not measure circulating steroid levels at this time. As exogenous oxytocin does not appear able to reduce the length of the cycle when given in the late luteal phase, it seems more likely that the receptor concentration goes up after plasma progesterone has already started to drop, i.e. after luteolysis has begun. At this time oxytocin and PGF-2α could engage in a positive feedback loop as suggested by Flint & Sheldrick (1983). If this is so, an analogy can be drawn with the role of oxytocin in parturition when it is probably not released in increasing amounts until labour has already been initiated. However, this point also remains controversial (see Fuchs, 1978; Dawood, Ylikorkala, Trivedi & Fuchs, 1979; Glatz *et al.*, 1981; Sellers *et al.*, 1981). Viewed in this way the lack of endometrial oxytocin receptors during the luteal phase may be a protective mechanism to prevent luteolysis during the period when the corpus luteum is secreting maximal amounts of oxytocin. As the presence or absence of receptors appears to be the major factor determining whether ovarian oxytocin can have a luteolytic action, further work is required to quantify changes in receptor population at different stages of the cycle and pregnancy in the ewe and additional species.

Luteal oxytocin therefore appears to play a part in the control of luteolysis in the cow, goat and ewe, although it may not initiate this event. At the present time there is no evidence to show an involvement of oxytocin in luteolysis in other species. However, as outlined above for the sheep, the fact that luteolysis cannot be induced with exogenous oxytocin does not necessarily preclude endogenous oxytocin from a role in the completion of this process.

## Relationship with gonadotrophin secretion

*Involvement of the pineal gland*

Kitay & Altschule (1954) reviewed a series of references dating back to 1898 indicating that pineal extract can have pressor or depressor activities and can increase uterine contractility in a variety of mammalian species, but they thought that many of these findings were probably non-specific effects attributable to impure extracts. However, Milcu, Pavel & Neacsu (1963) purified a pineal extract with both pressor and oxytocic properties and suggested that the active principle responsible for these effects was arginine vasotocin (AVT). This idea was supported by Cheesman & Fariss (1970) who compared the biological activities of pineal extract and synthetic arginine vasotocin and by Cheesman (1970) who used mass spectroscopy to determine the amino acid sequence of the extract.

It was also known that the pineal gland had anti-gonadal actions and that it contained melatonin and a number of other indoles (for review, see Reiter, 1980). During the past 2 decades there has been considerable controversy about whether the active antigonadal agent in the pineal is AVT, melatonin, as yet unidentified peptides, or a combination. Another suggestion is that melatonin is a specific releasing factor for AVT (Pavel, 1973; Pavel & Goldstein, 1979). Whilst much of this argument is not directly relevant to the present review, and it is by no means certain that the pineal gland does contain arginine vasotocin, it has led to a considerable amount of work on the actions of AVT and related peptides on the reproductive system, and in particular on gonadotrophin secretion.

*Exogenous gonadotrophins*

Pavel & Petrescu (1966) first reported that synthetic AVT exerted antigonadotrophic effects. They showed that exogenous AVT (0·1 i.u./day s.c.) could block the increase in size of the ovaries and uteri of immature mice induced by treatment with PMSG. A similar effect was noted by Moszkowska & Ebels (1968) who used medium from cultured rat anterior hypophyses to provide gonadotrophic stimulation. Hipkin (1970) found that large doses of AVP (15 i.u.) or oxytocin (6 i.u.) alone would block an hCG-stimulated increase in uterine weight in mice but the two hormones given together produced a similar response at a much lower dose (0·3 i.u.). PMSG-stimulated ovulation was also blocked by exogenous AVT in the rat (11 μg: Smith, Orts & Benson, 1972) but not the mouse (up to 50 μg; Cheesman & Forsham, 1974). All these effects should probably be considered as pharmacological.

*Endogenous gonadotrophins*

*Arginine vasotocin.* The antigonadal action of AVT described above could have been acting at the level of the brain, the pituitary or the gonads. As a low dose of only 0·5 μU AVT inhibited compensatory ovarian hypertrophy when injected into the third cerebral ventricle but not when administered intravenously, Pavel, Petrescu & Vicoleanu (1973) favoured the brain as the target site.

In the female rat it was shown that large doses of AVT (20 μg/h i.v. for 5 h) on the day of pro-oestrus prevented ovulation (Cheesman, Schlegel, Sagasay & Forsham, 1983) and blocked or delayed the LH surge when given intravenously (0·1 μg: Cheesman, Osland & Forsham, 1977a), intra-atrially (> 0·5 μg: Salisbury, Krieg & Seibel, 1980) or into the third ventricle (100 pg: Osland, Cheesman & Forsham, 1977). However, in AVT-treated immature rats stimulated with PMSG the LH surge proceeded as normal but ovulation was still prevented (Johnson, Vaughan, Reiter, Blask & Rudeen, 1978). The effects of AVT on tonic, as opposed to phasic, gonadotrophin release have also been investigated. Cheesman *et al.* (1977a) found that AVT did not reduce circulating LH levels in female rats during dioestrus and after ovariectomy. Johnson *et al.* (1978) even reported higher FSH and LH titres in PMSG-treated immature rats after AVT injections, but as they did not

appear to have allowed for any possible cross-reactivity of PMSG in their radioimmunoassays these data may be unreliable.

Treatment of immature male mice and hamsters with daily intraperitoneal injections of 1 μg AVT caused a significant decrease in the size of the ventral prostate and male accessory organs compared with those in control animals (Vaughan, Reiter, McKinney & Vaughan, 1974a; Vaughan, Vaughan & Klein, 1974b). Vaughan, Blask, Johnson & Reiter (1979) found that lower doses of AVT (2 μg s.c. every 2 h) inhibited tonic LH secretion in castrated male rats whereas higher doses (5 μg s.c. every 3 h) inhibited both LH and FSH. Similarly, Pavel, Luca, Calb & Goldstein (1979) noted a significant reduction in LH but not FSH levels in urethane-anaesthetized male rats 15–60 min after an injection of only $10^{-4}$ pg AVT into the third ventricle, but not after injection into the pituitary.

Based upon these and other data, Pavel *et al.* (1979) suggested that AVT acts by preventing the release of LH-RH from the hypothalamus rather than by interfering with LH release at the pituitary level. AVT did not prevent the release of LH induced by PGE-2 or LH-RH in the whole rat (Osland *et al.*, 1977; Pavel *et al.*, 1979) and physiological levels of AVT did not affect LH or FSH release into the medium by male rat anterior pituitary cells in monolayer culture (Demoulin, Hudson, Franchimont & Legros, 1977). Pavel *et al.* (1979) also showed that AVT increases the hypothalamic 5-hydroxytryptamine levels in the male rat and that *p*-chlorophenylalanine (an inhibitor of 5-hydroxytryptamine synthesis) blocked the inhibitory action of AVT on LH release. They therefore suggested that AVT acted at a site above that of the LH-RH neurone to inhibit LH-RH release by interfering with 5-hydroxytryptamine neurotransmission. However, this idea is not supported by data for the dog: Yamashita, Mieno & Yamashita (1979, 1980) and Mieno, Yamashita, Koba, Iimori & Yamashita (1980) measured 17 oxo-steroid levels in the spermatic venous blood of anaesthetized dogs as an estimate of circulating LH levels and found that an arterial injection of AVT inhibited the testicular response to LH-RH treatment. It would therefore appear either that there is a species difference in the response of the rat and the dog, or that in the dog AVT was acting directly on the testis.

The conclusion that can be drawn at present is that AVT can reduce tonic gonadotrophin secretion in the male and delay the LH surge in the female. Tonic LH release in the female requires further investigation. Whether or not these positive effects can be considered 'physiological' depends on further work to confirm the presence of AVT in the pineal gland or some other site in the mammal.

*Arginine vasopressin.* Early work provided indirect evidence that vasopressin stimulated gonadotrophin release. Martini, Mira, Pecile & Saito (1959) used a mouse uterus bioassay to measure the total gonadotrophin content of female rabbit urine samples before and after treatment with lypressin or lysine vasopressin. Both preparations increased the gonadotrophin levels in the urine. Several studies have shown that vasopressin at doses of 0·2–5 units *in vivo* is active in the rat ovarian ascorbic acid depletion test which was used as a bioassay for LH (Parlow, 1958; McCann & Taleisnik, 1960; Giuliani, Martini, Pecile & Fochi, 1961; Ramirez & McCann, 1963). McCann & Taleisnik (1960) found that LH and vasopressin were equally active when administered to rats that had been hypophysectomized 1 h earlier, suggesting that the action of arginine vasopressin (AVP) was not mediated via LH release. In contrast, Giuliani *et al.* (1961) showed that vasopressin was ineffective if given 24 h after hypophysectomy, but as they did not also test LH at this time it is possible (as suggested by McCann & Taleisnik, 1960) that the ovary is by then incapable of responding normally. Further direct evidence for an effect on LH secretion was provided by Ramirez & McCann (1963). They injected vasopressin intravenously into ovariectomized rats and used blood collected from these animals 10 min later to test for ascorbic acid depletion in another group of rats. A positive response was obtained with doses of vasopressin greater than 200 mU.

More recently several studies have examined the effect of exogenous AVP on LH release as measured by radioimmunoassay. In the female rat large doses given intra-atrially or intravenously blocked the LH surge (Salisbury *et al.*, 1980) and inhibited ovulation (Cheesman *et al.*, 1983).

Cheesman *et al.* (1983) thought that their original failure to demonstrate an effect of AVP on the LH surge (Cheesman *et al.*, 1977a) was due to impurities in their standard preparation. In contrast Koyama & Hagino (1983) have shown that subcutaneous injections of 10 units vasopressin given to female baboons in the early luteal phase produced a significant elevation in LH 30 min later. However, several studies on the effect of AVP on tonic LH secretion in the male failed to show any effect (rat: Pavel *et al.*, 1979; Vaughan *et al.*, 1979; man: Franchimont & Legros, 1968). A similar lack of effect on FSH secretion was also noted (Franchimont & Legros, 1968; Pavel *et al.*, 1979). AVP caused a slight stimulation of prolactin release in the female rat *in vivo* (Salisbury *et al.*, 1980) but resulted in a slight decrease in prolactin release from cultured pituitary cells from male rats (Lumpkin, Samson & McCann, 1983).

It therefore appears that vasopressin does not have a significant influence on FSH or prolactin release. It can alter LH secretion in the female but an effect has not been shown in the male.

*Oxytocin.* Large doses of oxytocin given less than 20 min before mating will block ovulation in the rabbit (Brinkley & Nalbandov, 1963) and will prevent an hCG-stimulated increase in uterine weight in mice (Hipkin, 1970). The block to ovulation could therefore be acting at the ovary rather than on the pituitary or brain.

The majority of studies have not found any effect of oxytocin on LH or FSH secretion. This has been demonstrated for male and female rats (McCann & Taleisnik, 1960; Giuliani *et al.*, 1961); Cheesman *et al.*, 1977a; Pavel *et al.*, 1979; Vaughan *et al.*, 1979; Salisbury *et al.*, 1980; Lumpkin *et al.*, 1983), cows (Donaldson *et al.*, 1965; Harms *et al.*, 1969; Wilks & Hansel, 1971a, b) and men (Ditlove & Faiman, 1970; Dawood, Ylikorkala, Trivedi & Gupta, 1980). Oxytocin-induced luteolysis still occurs in cows with hypophysial stalk transections, suggesting that this effect is not mediated via the hypothalamus (Oxenreider, 1968). In mice and hamsters oxytocin treatment did not affect the size of the ventral prostate and male accessory organs (Vaughan *et al.*, 1974a, b).

However, a few studies have shown a positive effect. Martini *et al.* (1959) found that oxytocin treatment increased the gonadotrophin levels in urine samples of female rabbits. Franchimont & Legros (1968) reported a rise in circulating FSH levels 10 min after an injection of 2 units oxytocin i.v., into human males whereas Vaughan *et al.* (1979) found that 1 unit oxytocin s.c. decreased FSH levels in castrated male rats. Armstrong & Hansel (1958) found that daily treatment of immature male rabbits with 10 i.u. oxytocin a day over a period of 11 weeks resulted in a significant decrease in testis weight, seminiferous tubule diameter and prostate epithelial height, whereas the growth of the Leydig cells was stimulated. They postulated that this effect was caused by an alteration in gonadotrophin secretion but provided no direct evidence for this.

There is also some controversy over the effect of oxytocin on prolactin secretion. The early work on this subject was reviewed by Martini (1966). Evidence that oxytocin could stimulate prolactin release was provided indirectly by experiments showing that oxytocin could retard the involution of the mammary gland, initiate lactation in oestrogen-primed rabbits and induce pseudopregnancy in uterine-traumatized rats. However many of these effects either lacked specificity or were not confirmed by other workers. In-vitro experiments in which prolactin release from rat pituitary glands was measured by a pigeon crop bioassay likewise failed to show an effect of vasopressin or oxytocin (Nicoll & Meites 1962); Talwalker, Ratner & Meites, 1963). Gala & Reece (1965) did report an increase in prolactin release *in vitro* after treatment with 100 mU oxytocin/ml but thought that this was due to contamination of the preparation with epinephrine.

Injection of oxytocin into women had no effect on circulating prolactin levels (del Pozo, Kleinstein, Brun del Re, Derrer & Martin-Perez, 1980). In the cow, Schams (1972) found that large doses of oxytocin (160 i.u.) did cause an increase in prolactin whereas in the lactating rat Kühn, Krulich & McCann (1973) found that large doses (10 i.u.) blocked the suckling-induced rise in prolactin. However, both papers pointed out that these were likely to be pharmacological effects. Two experiments on the female rat provide contradictory results. Cheesman *et al.* (1977b) found that oxytocin had no effect on the natural prolactin surge in pro-oestrous rats, whereas Salisbury *et al.* (1980) induced prolactin release by steroid treatment of ovariectomized rats and found that

oxytocin infusion (1 μg intra-atrially) augmented the surge. The work by Lumpkin *et al.* (1983) provides considerable support for the view that oxytocin does affect prolactin secretion. They found that oxytocin ($10^{-8}$ and $10^{-7}$ M) stimulated prolactin release by dispersed rat anterior pituitary cells and doses of $10^{-6}$ and $10^{-5}$ M stimulated release from hemipituitary glands. The intravenous injection of 1 or 10 μg oxytocin into conscious male rats elevated plasma prolactin significantly 5 min later. However, when given into the third ventricle oxytocin (0·1 and 1 μg) significantly lowered plasma prolactin concentrations.

Therefore, the data on FSH and LH favour the view that the release of these gonadotrophins is not affected by oxytocin. In the case of prolactin further work is required before a conclusion can be reached.

*Conclusions*

Although the data are not yet conclusive, there is evidence to suggest that all three peptides can influence gonadotrophin secretion providing that the dose is sufficient. Vasotocin and vasopressin both have effects on LH levels, whereas oxytocin may influence prolactin. Further work is required to determine whether arginine vasotocin is actually present in mammals before it can be concluded that its action is of physiological relevance. In addition the interpretation of much of this information is complicated by a lack of knowledge about the site of action. Pavel *et al.* (1979) found that doses of AVT as low as $10^{-4}$ pg into the third ventricle depressed LH levels whereas doses up to $10^{-1}$ pg were ineffective when given into the pituitary, indicating that a central site of action was the most likely.

The majority of the experiments referred to above involving oxytocin and vasopressin used large concentrations injected either subcutaneously or intravenously. In his behavioural work on rats, de Wied (1976) noted that 722 mg vasopressin was needed to obtain an effect when the hormone was administered subcutaneously, but 25 pg could elicit a response when given intraventricularly. Two studies using injections of radioactively-labelled vasopressin administered intravenously into dogs and rats showed that only very small amounts of radioactivity (<1·5%) reached the brain while relatively large amounts accumulated in both the anterior and posterior pituitaries (Ang & Jenkins, 1982; Janáky, László, Sirokmán & Morgat, 1982). This suggests that there is a blood–brain barrier for vasopressin. Therefore if the action of either vasopressin or oxytocin is on the pituitary or gonad it is likely that the hormone concentrations reached in that gland in many of the experiments referred to would have been above the normal range. However, if the action is on the brain, a large dose may be required to produce a small rise in hormone concentration at the relevant site. It should also be remembered that, in the case of vasopressin, a large dose given systemically is likely to cause a short-term increase in blood pressure and this could also influence hormone secretion patterns.

The levels of oxytocin and vasopressin found in the systemic circulation in normal circumstances are almost certainly not sufficient to affect gonadotrophin secretion, and a feedback system from the gonads operated by this means is therefore unlikely. However, there is now considerable evidence from immunocytochemical techniques that nerve fibres from the hypothalamus, containing vasopressin and to a lesser extent oxytocin, terminate on the hypophysial portal vessels (Silverman, 1976; Zimmerman & Antunes, 1976; Buijs, de Vries, van Leeuwen & Swaab, 1983; Sofroniew, 1983). The levels of vasopressin in the portal blood vessels of monkeys are 300 times higher than those in the systemic circulation (Zimmerman *et al.*, 1973). It therefore appears that vasopressin and possibly oxytocin have direct access to the anterior pituitary. In addition, vasopressinergic- and oxytocinergic-neurones have been found in many other regions of the brain, in particular the limbic system and brain stem, and both peptides have been measured in cerebrospinal fluid (Silverman, 1976; Zimmerman & Antunes, 1976; Buijs *et al.*, 1983; Robinson, 1983; Sofroniew, 1983). This raises the possibility that they could influence gonadotrophin release at higher centres in the brain and this subject merits further investigation.

## Direct actions on the gonads

### *Male*

In a number of the studies reported in the previous section it is unclear whether the exogenously administered peptide was acting at the level of the brain, the pituitary, or the gonad. Three studies have shown that treatment with oxytocin or arginine vasotocin can alter the development of the male tract. In immature mice and hamsters 3 daily injections of AVT (but not oxytocin or vasopressin) will inhibit the growth of the ventral prostate amd male accessory organs (Vaughan *et al.*, 1974a, b). In the rabbit long-term (11 weeks) oxytocin treatment stimulated the development of the testis, leading to increased testis weight, seminiferous tubule diameter and a probable increase in the number and size of the Leydig cells (Armstrong & Hansel, 1958). The height of the epithelial cells of the prostate was also increased. In the ram treatment with oxytocin for 6 weeks caused a decrease in the number of spermatozoa per ejaculate and an increased percentage of abnormal spermatozoa (Knight & Lindsay, 1970). It is impossible to say whether any of these actions were directly on the testis. The work of Yamashita *et al.* (1979) provided slightly more evidence for a gonadal site of action as they found that an hCG-stimulated rise in total 17-oxo-steroid secretion into the spermatic veins of dogs could be blocked by the simultaneous administration of AVT, whereas if the AVT was given 3 h earlier it had no effect.

Adashi & Hsueh (1981a) confirmed a possible gonadal site of action by demonstrating that AVT and other peptides could inhibit hCG-stimulated testosterone secretion by cultured rat testicular cells in a dose-related manner. AVP, AVT and lysine vasopressin were the most potent peptides tested with median effective doses of around $10^{-10}$ M. Oxytocin, mesotocin (Ile$^8$-oxytocin) and valitocin (Val$^8$-oxytocin) were less potent (median effective doses $\sim 10^{-8}$ M) and various related neuropeptides had little effect. The specificity of this response was further confirmed by the demonstration that pressor-selective but not oxytocin-selective or antidiuretic-selective agonists of neurohypophysial hormones also inhibited hCG-stimulated testosterone secretion in a dose-related manner *in vitro*. Pressor, but not oxytocic, antagonists could block the inhibition normally caused by AVP (Adashi & Hsueh, 1981b). AVT did not appear to act by preventing hCG binding to its receptors but at a site in the cell distal to cAMP formation (Adashi & Hsueh, 1982). On the basis of measurements of the concentrations of steroid precursors for testosterone in the culture medium Adashi & Hsueh (1982) suggested that at least part of the inhibitory action of AVT on testosterone production was due to selective suppression of the enzymes 17α-hydroxylase and 17–20 desmolase.

### *Female*

In the female a number of studies have shown an effect of neurohypophysial peptides and AVT on the ovary although again it is unclear whether these actions were direct. Thus AVT given to rats at the appropriate time will block ovulation (Cheesman & Forsham, 1974; Johnson *et al.*, 1978; Cheesman *et al.*, 1983) and Johnson *et al.* (1978) showed that this block occurred despite an apparently normal LH surge. Large doses of oxytocin given less than 20 min before mating blocked ovulation in the rabbit (Brinkley & Nalbandov, 1963). AVT, AVP and oxytocin can prevent the increase in the size of the ovaries and uteri of mice normally evoked by exogenous gonadotrophin stimulation (Pavel & Petrescu, 1966; Moszkowska & Ebels, 1968; Hipkin, 1970). It has also been shown that vasopressin is active in the ovarian ascorbic acid depletion test developed as a bioassay for LH (Parlow, 1958; McCann & Taleisnik, 1960; Giuliani *et al.*, 1961; Ramirez & McCann, 1963). Whilst Giuliani *et al.* (1961) showed that this effect was abolished in rats which had been hypophysectomized 24 h previously, suggesting that the effect was mediated via changing LH secretion, McCann & Taleisnik (1960) found that in hypophysectomized rats the ovary no longer responded normally to LH, invalidating this result. These authors showed that, if the test was carried out immediately (1 h) after hypophysectomy, LH and vasopressin were equally effective. In addition vasopressin caused ascorbic acid depletion when administered directly to the ovary via retrograde injection into the ovarian vein.

There have been two in-vitro studies on the action of oxytocin on progesterone production by the corpus luteum. Mares & Casida (1963) reported a preliminary trial in which oxytocin did not affect progesterone production by slices of bovine luteal tissue *in vitro*. However, Tan, Tweedale & Biggs (1982a, b) showed that oxytocin concentrations in the range 4–40 mU/ml stimulated progesterone production by isolated bovine and human luteal cells whereas concentrations of oxytocin > 400 mU/ml inhibited the cells' capacity to respond to hCG by increasing progesterone output. As no other related peptides were tested in this system and as high doses were required the specificity of this response requires confirmation.

In-vivo studies in the ewe have shown that progesterone secretion can remain at the same level when oxytocin concentrations are changing markedly. Sheldrick & Flint (1983c) found that peripheral progesterone levels were maintained following hysterectomy or the establishment of pregnancy, but in both cases luteal oxytocin concentrations dropped about 2 weeks after ovulation. Similarly, Schams *et al.* (1983) found that progesterone levels remained the same in ewes in which the circulating oxytocin level had decreased after immunization procedures.

### *Conclusions*

There is good evidence that vasopressin, in concentrations similar to those actually present in the testis of the rat and man, can inhibit testosterone production in a dose-related and specific manner. Although oxytocin is present in slightly higher concentrations in the testis it is also less potent than vasopressin in causing this effect. In the cow and sheep luteal concentrations of oxytocin are much higher, but the in-vitro evidence for an effect of oxytocin on progesterone production is at present inadequate. Vasopressin is known to cause ovarian ascorbic acid depletion, but it is not clear whether this is a direct effect on the ovary or mediated via an alteration in LH secretion. Further work is therefore required to determine whether ovarian oxytocin and vasopressin affect other systems within the ovary.

## Final Conclusions

It is now known that the ovaries of the sheep and cow contain high concentrations (μg/g) of a peptide which is almost certainly oxytocin. Much lower (pg/g) concentrations of another peptide which is probably vasopressin have also been found in cows and women. Measurements of arterial–venous differences in oxytocin concentrations across the ovary in the sheep show that the corpus luteum can secrete oxytocin (Flint & Sheldrick, 1982, 1983) and the idea that the hormone is synthesized locally is supported by the observation that the bovine ovary contains neurophysin (Wathes *et al.*, 1983a). Oxytocin is also present in human ovaries and preliminary evidence from our laboratory suggests that it can be isolated from the ovaries of rats and pigs. However, in these species, from 3 different orders of mammal, the concentrations appear to be considerably lower. The very high levels in the cow and sheep may therefore represent a ruminant specialization.

In ruminants exogenous oxytocin can cause luteolysis by stimulating the release of PGF-2α from the uterine endometrium (McCracken, 1980). It is possible that the formation of endometrial oxytocin receptors is the triggering event in luteolysis, although this observation requires confirmation. The importance of endogenous oxytocin in luteolysis is indicated by the prolongation of oestrous cycles in ewes immunized against oxytocin (Sheldrick *et al.*, 1980; Schams *et al.*, 1983). Nevertheless, the highest ovarian and peripheral oxytocin concentrations are found during the first half of the luteal phase, when they cannot have a luteolytic action because of the lack of uterine receptors. This leads to the question of whether all the oxytocin produced in the early luteal phase in cows and sheep is 'wasted' or has another function.

Several possible sites of action have been examined. Luteal oxytocin is unlikely to affect motility of the tract as the oviducts and myometria of cattle and sheep are unresponsive to

oxytocin stimulation during the luteal phase (Ruckebush & Bayard, 1975; Roberts & McCracken, 1976). Oxytocin may influence prolactin secretion (Lumpkin *et al.*, 1983) but not all workers are in agreement, and the dose required exceeds that found in the peripheral circulation. The most likely alternative is that oxytocin has an action within the ovary itself. In-vitro experiments in the cow (Tan *et al.*, 1982a) indicate that it can alter basal and LH-stimulated progesterone production. No experiments so far carried out *in vivo* have examined the influence of oxytocin on progesterone release in detail, but such evidence as is available does not indicate a direct positive or negative correlation. The lifespan of the corpus luteum can be prolonged by pregnancy, hysterectomy or immunization against oxytocin, and in all three cases peripheral progesterone levels remain similar to those in the mid-luteal phase at a time when the oxytocin concentration is dropping by a factor of about 200 (Schams *et al.*, 1983; Sheldrick & Flint, 1983c).

I should like to speculate that oxytocin does have an additional action which is important in the early luteal phase. One possible idea is based on the observation of Adashi & Hsueh (1981a) that oxytocin, vasopressin and arginine vasotocin can all inhibit LH-stimulated testosterone production. In the case of arginine vasotocin, Adashi & Hsueh (1982) demonstrated that this inhibition was probably caused by the selective suppression of the enzymes 17α-hydroxylase and 17–20 desmolase which are involved in the conversion of progesterone to androstenedione. In these circumstances there was a 20-fold increase in progesterone accumulation in the medium of cultured rat testicular cells. In pre-ovulatory ovine and bovine follicles thecal cells are involved in the production of androstenedione (Moor, 1977; Fortune & Hansel, 1979) and this is converted to oestradiol-17β by aromatization within the granulosa cells. After the LH surge follicular progesterone production increases (Moor, Hay & Seamark, 1975; Dieleman, Kruip, Fontijne, de Jong & van der Weyden, 1983). This rise is generally attributed to the functional luteinization of the granulosa cells. However, Fortune & Hansel (1979) also demonstrated that after the LH surge the cells of the theca interna showed a large increase in progesterone production. In the corpora lutea of cows and sheep it is known that both large and small luteal cells are involved in progesterone biosynthesis (Ursely & Leymarie, 1979; Rodgers, O'Shea & Findlay, 1983) and it is thought that the small luteal cells are derived from the theca interna. It is therefore possible that oxytocin (or vasopressin) produced by the developing corpus luteum could suppress the conversion of thecal progesterone into androgens, thus causing an increase in overall progesterone production. Oxytocin and vasopressin are both present in luteal tissue soon after ovulation in the cow (D. C. Wathes, unpublished observations) but it is not known whether their appearance occurs sufficiently soon after the LH surge to have this effect. By the same line of reasoning high oxytocin levels during the luteal phase of the cow and sheep could restrict follicular oestrogen production, thus limiting follicular development potential at this time. In women the corpus luteum continues to produce both androstenedione and oestradiol-17β (Swanston, McNatty & Baird, 1977) which could be correlated with the much lower oxytocin level. All these ideas are at present purely speculative and the possibility that oxytocin can influence progesterone metabolism requires confirmation before they can be accepted.

As stated above ovarian oxytocin levels in man are considerably lower than in the cow and sheep but the hormone is nevertheless present. Endometrial PGF-2α is not involved in the control of luteolysis in women. Oxytocin could stimulate PGF-2α production within the ovary but this possibility has not been examined. It is again improbable that oxytocin affects the motility of the tract as the non-gravid uterus is very unresponsive to oxytocin stimulation (Coutinho & Lopes, 1968). The concentrations of oxytocin which affect progesterone production by human luteal cells *in vitro* (Tan *et al.*, 1982b) are slightly higher than those which have been found within the ovary. Therefore the function of human ovarian oxytocin is also unknown and lack of information prevents speculation on other species.

Vasopressin has been found in bovine and human ovaries at a concentration about two orders of magnitude greater than that in the peripheral circulation, indicating that it is either taken up or manufactured at this site (Wathes *et al.*, 1982, 1983a, b). It is possible that luteal vasopressin may

stimulate uterine activity at the beginning of menstruation in women but the low levels of AVP in the ovary and circulation at this time make this relatively unlikely. Vasopressin can inhibit testosterone biosynthesis as discussed above. It has many other actions within the body, for example on blood pressure, fluid retention and memory, but concentrations of vasopressin within the ovary are probably too low to elevate peripheral blood levels sufficiently to affect distant receptors.

In the male oxytocin and vasopressin at concentrations similar to those measured in rat and human testes (Nicholson *et al.*, 1983) can inhibit testosterone production *in vitro* (Adashi & Hsueh, 1981a, b). However, the long term study by Armstrong & Hansel (1958) on the rabbit indicated that daily oxytocin injections stimulated testicular growth and indirect evidence suggests that testosterone production in these animals was enhanced rather than inhibited. The effect of oxytocin and vasopressin on testosterone production *in vivo* needs to be investigated. Oxytocin and vasopressin can also influence the motility of the epididymis and vas deferens and oxytocin has been shown to increase the frequency of peristaltic contractions in the seminiferous tubules (Niemi & Kormano, 1965). Both hormones could therefore be involved in controlling the movement of spermatozoa from the testis to the lower parts of the tract, but further data are required to substantiate this idea.

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