LH and testosterone cause the development of seminiferous tubule contractile activity and the appearance of testicular oxytocin in hypogonadal mice

MRC Neuronal Peptides Research Group, Department of Anatomy, The Medical School, Bristol BS8 1TD
*Department of Human Anatomy, University of Oxford, Oxford OX1 3QX

RECEIVED 14 January 1986

ABSTRACT

Immunoreactive oxytocin is present in the testis and it has been shown that this hormone increases the contractility of seminiferous tubules. We have investigated the relationship between testicular oxytocin, tubular movements and the effects of LH and testosterone using, as a model, the hypogonadal (hpg/hpg) mouse, which is deficient in hypothalamic LH-releasing hormone (LHRH). Whilst both testicular oxytocin and seminiferous tubule movements, resembling those seen in the rat, can be found in normal adult mice, neither can be found in hypogonadal mice. After 2 weeks of treatment with LH (200 ng to 100 μg daily) low levels of testicular oxytocin and tubular movements were observed. Treatment with large doses of testosterone for 2–12 weeks led to higher concentrations of testicular oxytocin and tubular movements resembling those seen in the normal adult mouse. The results support the evidence that testicular oxytocin modulates seminiferous tubule movements. We suggest that testosterone may play a part in the accumulation of oxytocin in the testis. J. Endocr. (1986) 110, 159–167

INTRODUCTION

Following on from our discovery of an oxytocin-like peptide in the testes of rats and men (Nicholson, Swann, Burford et al. 1984) we have begun to investigate its possible functions. It seemed reasonable to begin by looking at the contractility of the seminiferous tubules since it has been demonstrated that the seminiferous tubules of the rat exhibit contractile activity in vitro (Roosen-Runge, 1951; Niemi & Kormano, 1965), that this activity can be increased by treatment with oxytocin (Niemi & Kormano, 1965) and that concentrations of oxytocin similar to those found in the testis in vivo also increase contractile activity in vitro (Worley, Nicholson & Pickering, 1985). Moreover, in the neonatal rat we have shown that the appearance of testicular oxytocin on day 8 post partum is accompanied by increasing contractile activity of the seminiferous tubules (Worley et al. 1985).

These findings have led us to ask what determines the appearance and increase of oxytocin during the development of the testis and, in particular, the relationships between testicular oxytocin, tubular movement and other pituitary and gonadal hormones. The hypogonadal (hpg/hpg) mouse has provided us with an excellent model in these investigations. Hypogonadal mice are deficient in hypothalamic luteinizing hormone-releasing hormone (LHRH), resulting in a very much reduced secretion of gonadotrophins and failure in the postnatal development of the gonads (Cattanach, Iddon, Charlton et al. 1977). Development of the testes of these animals can, however, be promoted by the administration of LHRH (Charlton, Halpin, Iddon et al. 1983), luteinizing hormone (LH) or testosterone (Ward, 1980; Lyon, Cattanach & Charlton, 1981). We report here our findings on the effects of LH and testosterone on the presence of testicular oxytocin and seminiferous tubule movements in hpg/hpg mice. Preliminary reports of some of these data have been given already (Pickering, Birkett, Charlton et al. 1986).

MATERIALS AND METHODS

Animals

Male hypogonadal and normal mice of the same
strain, reared in the Department of Human Anatomy, Oxford, were used in this study. Groups of ten normal and ten hypogonadal male mice were weighed and then killed by decapitation. The testes were removed immediately and processed either for oxytocin radioimmunoassay or for measurement of seminiferous tubule contractile activity. The seminal vesicles were also removed from each animal and weighed, the weight being used an an indicator of the androgen levels of the animals (Miescher, Wettstein & Tschopp, 1936).

**Testicular oxytocin determinations**

The testes were frozen immediately after removal from the animals and stored at −80°C until assayed for oxytocin. Extracts of the testes from each group of animals were made using the method of Walsh & Niall (1980). The extracts were pooled and radioimmunoassayed for oxytocin using an antiserum (79/3) raised in the Department of Anatomy, Bristol. Oxytocin content was expressed as fmol/g wet weight or fmol/animal (i.e. per pair of testes) (1 fmol = 1 pg).

**Measurement of seminiferous tubule contractile activity**

*Tubule preparation and recording*

Isolated seminiferous tubules were prepared using a technique similar to that previously described for rat seminiferous tubules (Worley et al. 1985). Single testes were taken from animals in each group and placed immediately in culture medium at 30–35°C. The medium was Dulbecco’s modified Eagles Minimum Essential Medium (DMEM; pH 7.25; 295–300 mosmol/kg, buffered with HEPES, 15 mmol/l) without serum or antibiotics. The testicular capsule was cut and the seminiferous tubules were drawn apart, very gently, to allow lengths of individual tubules to be observed, clear of other tissue. The tubules were rinsed by passing them through fresh, warm medium to remove red blood cells and tissue fragments. They were then placed in a culture chamber arranged with No. 5 glass coverslips for its upper and lower surfaces to ensure an optically good light path through the preparation while retaining adequate mechanical rigidity. The thickness of the coverslips was 3 mm. The 0.5 ml chamber was provided with a continuous flow of fresh, pre-oxygenated culture medium at the rate of 4.35 ml/h. The chamber was placed on the stage of an inverted microscope (Nikon Diaphot) in a temperature-controlled cabinet maintained at 35 ± 0.5°C. The tubule preparation was examined and photographed using phase contrast or differential interference contrast microscopy. Videomicrographic recordings were made via a National WV-1850 high-resolution, high-sensitivity television camera to a National NV-8050B time-lapse video recorder. The ratio of real time to tape playback time, that is the apparent speeding-up of recorded events, was 12:1.

**Experimental treatment of isolated seminiferous tubules**

Images of a seminiferous tubule were recorded over the whole of an experimental period which was typically up to 6 h. Initially, the seminiferous tubule was untreated. This established the base level of spontaneous contractile movement. Then oxytocin was added to the perfusion medium at a concentration of 1–5 nmol/l, corresponding to the range of oxytocin concentration measured in normal adult mouse testes (0.6–3.5 pmol/g wet weight). The oxytocin-containing medium was perfused for 30 min and then replaced by oxytocin-free medium again. Free, added oxytocin was cleared from the chamber within 30 min. Perfusion of oxytocin-free medium was continued until the end of the experiment. The video tape recording contained a record of the movements of one section of the surface of a tubule over the experimental period. The movements of the tubule were measured as a series of distances (from the left-hand boundary of the video field to the edge of the tubule image) at a constant rate governed by the video framing frequency (25 measurements per second, i.e. one for each frame). This provided, for example, 45 000 measurements for a 6-h experiment (30 min of tape time). The measured values were stored in a BBC microcomputer, building up a frame by frame record of the movements of the tubule. Data were automatically transferred to disk storage at regular intervals.

**Analysis of movement data**

Because of the complexity of the contractile movements we adopted a method of spectral analysis based on the premise that a complex motion can be approximated mathematically as a linear combination of a set of sinusoidal functions of different frequencies (Yuen & Fraser, 1979). The distribution of these functions is the power spectrum of the motion; the relative contribution of each frequency to the total motion is described by the magnitude of the power component of that frequency. The power components are the root mean squares of all the displacements in each sampling period, so the larger the power component the greater the contribution of that range of frequency of contraction to the total contractile activity.

Each experiment was divided into equal length time-periods, typically 30 min of real time (150 s of tape time; 3750 samples). The data from the time-periods were analysed into power spectra, using the fast Fourier transform (FFT) algorithm (Yuen & Fraser,
1979) on a ROM fitted to the BBC computer. Figure 1 shows frequency calibration spectra plotted in the same way as spectra from tubule contraction experiments. The spectral data from whole experiments were represented using a pseudo three-dimensional graphics technique. The x and y axes are the frequency and power, while the third axis is used as a time scale on which sequential spectra are plotted. Alterations in tubule contractile behaviour were detected as shifts in the peak frequencies, as alterations in power over any frequency band, or as a combination of these. An increase or decrease in the contractile activity of a tubule is reproduced as an increase or decrease in the power component of the spectrum, which results from both the proportion of tubule contractions of a particular frequency in a time period and the amplitude of those contractions. The power component is not in any way to be equated with the amplitude of the individual contractions. All Figures are drawn to the same scale.

**LH treatment**

Groups of ten adult male hypogonadal mice were injected subcutaneously, twice daily for 2 weeks, with LH (NIH oLH-22, National Hormone and Pituitary Program, NIH, Baltimore, MD, U.S.A) in 0-2 ml 0-5% (w/v) bovine serum albumin (BSA) or with 0-2 ml BSA solution alone. The doses of LH given to each group were 100 ng, 1, 10 and 50 µg, twice daily. Animals were killed on day 15 after the first injection and testicular oxytocin content and seminiferous tubule activity were determined as above. Fresh hormone was made up at the beginning of each week and divided into 0-2 ml aliquots and kept frozen at –20 °C. Enough hormone for each individual injection was removed from the freezer 30 min before use.

**Testosterone treatment**

Testosterone implants were made from medical grade tubing (cat. no. 602-305, Dow-Corning, Reading, Berks: 1·98 mm internal diameter, 3·18 mm outside diameter) by sealing one end with Dow-Corning 382 medical grade elastomer and then packing a length of 1 cm with testosterone (T-1500, Sigma Chemical Co. Ltd, Poole, Dorset) before sealing the other end with the elastomer. The lengths of tubing were implanted subcutaneously into the middle of the backs of adult male hypogonadal mice. Groups of ten treated animals were killed 2, 6 and 12 weeks later and each animal was checked to ensure that the implant was still in situ. Testicular oxytocin content and tubule activity were measured as above.

**Statistics**

For comparison of contractile activity of pre- and post-oxytocin time-periods the spectra were divided into wide, equal-width, high- and low-frequency bands, each comprising half of the frequency range. Comparisons were made: between spectra for time-periods before oxytocin treatment, to test stability of the contractile behaviour of the tubules; between spectra for time-periods after oxytocin treatment, to test recovery from oxytocin stimulation; between pre-oxytocin spectra and spectra during and after oxytocin treatment, to test alterations in contractile behaviour coinciding with hormone treatment of the tubules. In each case, the high-frequency band and the low-frequency bands were compared separately. Thus for each experiment the pre-oxytocin periods were the internal controls for the periods during and after oxytocin treatment. The significance of differences between the means of contractile activities (power components) of high- or low-frequency bands of pairs of spectra were assessed using multiple two-group *t*-tests. Testicular immunoreactive oxytocin values are given as the mean and range of three estimations of pools of nine pairs of testes. Organ weights are given as means ± S.E.M.
TABLE 1. Testicular oxytocin and organ weights in the hypogonadal mouse: effect of LH and testosterone

<table>
<thead>
<tr>
<th></th>
<th>Immunoreactive oxytocin in testis*</th>
<th>Organ weights (mg/pair)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fmol/g (range)</td>
<td>fmol/animal (range)</td>
</tr>
<tr>
<td>Normal mouse</td>
<td>945 (800–1120)</td>
<td>197 (150–221)</td>
</tr>
<tr>
<td>Hypogonadal mouse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>&lt; 50</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>LH-treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vehicle alone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 µg/day</td>
<td>452 (410–487)</td>
<td>6.2 (5.8–6.6)</td>
</tr>
<tr>
<td>2 µg/day</td>
<td>254 (235–268)</td>
<td>6.4 (5.9–6.8)</td>
</tr>
<tr>
<td>20 µg/day</td>
<td>343 (330–356)</td>
<td>13.2 (12.7–13.7)</td>
</tr>
<tr>
<td>100 µg/day</td>
<td>240 (221–265)</td>
<td>7.4 (7.2–7.8)</td>
</tr>
<tr>
<td>Testosterone implanted</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>342 (330–353)</td>
<td>8.3 (8.0–9.0)</td>
</tr>
<tr>
<td>6 weeks</td>
<td>4197 (4150–4225)</td>
<td>134 (132–136)</td>
</tr>
<tr>
<td>12 weeks</td>
<td>1600 (1550–1690)</td>
<td>167 (160–174)</td>
</tr>
</tbody>
</table>

*Mean and range of three estimations of pools of nine pairs of testes.
†Mean ± S.E.M. (n = 9).

RESULTS

Testicular oxytocin content of adult normal and hypogonadal mice

Oxytocin immunoreactivity was present in the testes of normal adult mice (Table 1). No oxytocin immunoreactivity could be detected in the testes of adult hypogonadal mice.

Seminiferous tubule contractile activity in normal and hypogonadal mice

The seminiferous tubules of normal adult mice exhibited two types of spontaneous contractile activity in vitro. These movements resemble the type A and type B movements which have been described in the rat (Worley et al. 1985): type A contractions being small, frequent dimpling movements each involving only a small area of the tubule wall but, collectively, covering the whole surface myoid layer of the tubule with contractile events, while type B movements are less frequent, larger movements involving a whole section of the tubule and associated with bulk movements of the luminal contents along the tubule, corresponding to those described by Niemi & Korman (1965).

Figures 2 and 3 show contractile activity of seminiferous tubules from normal mice. Figure 3 shows that introduction of oxytocin into the perfusion medium increased the contractile activity of the tubule in both the high- and low-frequency bands. The seminiferous tubules of adult hypogonadal mice differ from those of normal mice, being smaller (70–100 µm diameter) than normal mouse tubules (300–350 µm diameter), poorly developed, and with no visible lumen. Time lapse videomicrography revealed no contractile activity (Fig. 4).

Effects of LH treatment

Testicular oxytocin

No oxytocin immunoreactivity could be detected in the testes of hypogonadal mice treated with subcutaneous

FIGURE 2. Normal adult mouse: seminiferous tubule contractile activity in vitro. Power spectra of 30-min periods. No oxytocin treatment. There were no significant differences in contractile activity between the spectra for any of the time-periods.
FIGURE 3. Normal adult mouse: seminiferous tubule contractile activity in vitro. Power spectra of 30-min periods. Medium containing oxytocin (1 μg/l) was perifused during the fourth 30-min period (arrow). Contractile activity contributing to both the high-frequency and low-frequency halves of the spectra was greater in spectra 4 and 5 (post-oxytocin) than in spectra 1–3 (pre-oxytocin) (0·03 > P > 0·0001).

FIGURE 4. Untreated hypogonadal mouse: seminiferous tubule contractile activity in vitro. Power spectra of 30-min periods. Medium containing oxytocin (1 μg/l) was perifused during the fifth 30-min period (arrow). There were no significant differences in contractile activity between the spectra for any of the time-periods.
movements were all BSA Movements or and, hypogonadal and from weight could the (Table untreated seminal (001 spectra Activity with figure Contractile (1 spectra tubule pg/1) was of weight in adults, mice in normal mouse, as was the seminal vesicle weight. The testes, however, remained smaller than in the normal adult mouse, so that the oxytocin content when calculated per gram of tissue was considerably higher than that seen in the normal adult. After 12 weeks of treatment the size of the testes had increased so that although there was little change in the amount of oxytocin per animal, the oxytocin content per gram of testicular tissue had fallen.

Effects of testosterone treatment

Testicular oxytocin

Oxytocin immunoreactivity could be detected in all the animals treated with testosterone (Table 1). After 6 weeks of treatment the oxytocin content per animal was approaching that seen in the normal adult mouse, as was the seminal vesicle weight. The testes, however, remained smaller than in the normal adult mouse, so that the oxytocin content when calculated per gram of tissue was considerably higher than that seen in the normal adult. After 12 weeks of treatment the size of the testes had increased so that although there was little change in the amount of oxytocin per animal, the oxytocin content per gram of testicular tissue had fallen.

Seminiferous tubule contractile activity

After 2 weeks of testosterone treatment the seminiferous tubules were larger than those seen in the untreated hypogonadal mouse, but still remained smaller than in the normal adult mouse. Some development of the tubule had occurred but the lumen from mice receiving the higher doses of LH moved more than those from animals receiving 100 ng twice daily, but the activity was less than in normal adult mice (Figs 5 and 6).

BSA injections. In these animals the testicular and seminal vesicle weights did not differ from those in untreated adult hypogonadal mice. Testicular oxytocin could be detected in all the animals treated with LH (Table 1) but no obvious trend was seen in relation to the dose. There was an LH-stimulated increase in the weight of both seminal vesicles and testes, but the increases were small and the organs did not approach the weights seen in normal mice.

Seminiferous tubule contractile activity

No contractile activity could be detected in tubules from hypogonadal mice treated with BSA injections, and their tubules resembled those of untreated adult hypogonadal mice. The tubules from LH-treated hypogonadal mice were also small and poorly developed and, with even the highest dose of LH, no clear lumen or luminal contents were seen in the perfused tubules. Movements of the tubules were, however, evident in all of the LH-treated animals. These movements were small and discontinuous and resembled type B movements of rat tubules more than type A. Tubules

J. Endocr. (1986) 110, 159–167
FIGURE 7. Testosterone-treated hypogonadal mouse: seminiferous tubule contractile activity in vitro. The mouse was treated for 2 weeks with an implant of testosterone. Power spectra of 30-min periods. Medium containing oxytocin (1 μg/l) was perfused during the fourth 30-min period (arrow). Contractile activity contributing to the high-frequency halves of the spectra was greater in spectra 4–6 (post-oxytocin) than in spectra 1–3 (pre-oxytocin) (0.006 > P > 0.002). Activity contributing to the low-frequency halves of the spectra was greater in spectra 4 and 5 than in spectra 1–3 (0.02 > P > 0.0001).

was still indistinct. By 6 weeks of treatment the tubules, although smaller, were morphologically indistinguishable, when observed in the perfusion chamber, from those of normal adult mice, with both lumen and contents present. Both type A and type B movements could be seen after 2 weeks of testosterone treatment. After 6 and 12 weeks of testosterone treatment the contractile activity of the tubules was similar to that of normal adult mice (Figs 7 and 8).

DISCUSSION
An oxytocin-like peptide is present in the normal mouse testis in concentrations similar to those seen in the rat, man (Nicholson et al. 1984), cat, dog, sheep and rabbit (Nicholson, Worley, Guldenaar & Pickering, 1985) suggesting that testicular oxytocin may be common to all mammals. As in the rat (Nicholson et al. 1984), the oxytocin immunoreactivity extracted from mouse testes diluted in parallel with authentic oxytocin and coeluted with it on high performance liquid chromatography, and its testicular concentration was slightly lower in the mouse than in the rat (Nicholson et al. 1984). Two types of contractile activity are exhibited by normal adult mouse seminiferous tubules in vitro, resembling the movements seen in the rat (Worley et al. 1985), cat and dog (Nicholson et al. 1985). Furthermore, in all these species the activity of the tubules can be increased by the administration of small amounts (1 nmol/l) of oxytocin to the bathing medium in vitro.

Testicular oxytocin is undetectable in untreated adult hypogonadal mice and their seminiferous tubules do not contract. The testes of these animals are poorly developed, with no spermatogenesis (Cattanach et al. 1977). There are two possible reasons for the lack of immunoreactive oxytocin and tubular activity. First, they may be stimulated directly by gonadotrophins or by androgens, so their absence may be due purely to the deficiency of these gonadal hormones. Secondly, the appearance of testicular oxytocin and the onset of tubular movements may be secondary to the effects of LH and androgens on the development of the testis, in particular the tubular and peritubular tissues, and the initiation of spermatogenesis. It has been suggested that tubular movements are involved in the transport of sperm out of the testis (Hargrove, MacIndoe & Ellis, 1977). It would be possible that the advent of spermatogenesis triggers the accumulation of oxytocin
in the testis and the initiation of contractile activity. Certainly in the rat, where spermatogenesis begins around day 4 post partum (Clermont & Huckins, 1961), testicular oxytocin and increased tubular movements closely follow, starting around day 7 to 8 (Worley et al. 1985) and by day 33 when the first sperm are shed into the lumen, tubular contractile activity is similar to that seen in adults.

Treatment of hypogonadal mice with LH resulted in low levels of oxytocin immunoreactivity and the presence of small tubular movements. The absence of a dramatic increase in testicular oxytocin content per animal with increasing doses of LH may indicate that the gonadotrophin does not have a direct effect on testicular oxytocin but that it may act indirectly, for example via testosterone. Hypogonadal mice treated for 2 weeks with testosterone have tubules which are better developed than those seen after LH treatment and which exhibit both of the types of movement seen in the normal adult mouse. After 6 weeks of testosterone treatment, testicular oxytocin content per animal, tubule morphology and contractile activity are indistinguishable from those of the normal adult mouse, apart from tubule diameter which is smaller. The evidence that testosterone treatment, alone, can bring about tubular movement associated with the presence of testicular oxytocin supports the suggestion that the similar changes seen with LH are due to its effects on androgen production.

Seminiferous tubule movements were seen only in mice in which testicular oxytocin was present and, in general, contractile activity was greater the higher the levels of testicular oxytocin. Co-ordinated tubular movements of typical A and B type, however, were seen only in normal and in testosterone-treated mice, even though after 2 weeks of testosterone treatment oxytocin levels were not much higher than those seen after LH treatment. This raises the question of whether the tubular movements are modulated by the local androgen content rather than, or as well as, by testicular oxytocin. Hib & Poncio (1977) reported that androgens are necessary for contractile activity in the mouse epididymis and our data suggest that this may be true for the developing seminiferous tubule also. The results presented here are compatible with the idea that testosterone alone might be responsible for the modulation of tubular movement. Preliminary results from experiments in which Leydig cells were selectively destroyed by treatment of rats with ethane-dimethanesulphonate showed, however, that tubular movement which was substantially decreased could not be restored by treatment with testosterone (Pickering et al. 1986).

The developing testis presents a different problem. While in the normal adult all components of the testis are well developed, in the prepubertal and the hypogonadal mouse testis this is far from true. Testosterone has been shown to be necessary to promote the growth and development of the peritubular (Paranko, Pelliniemi, Dym et al. 1981) and tubular structures (Hovatta, 1972). Thus the action of testosterone on tubular movements may be to promote the growth of peritubular structures such as myoid cells so that movements become physiologically possible. Our data support the hypothesis that testicular oxytocin has a role in modulating seminiferous tubule contractile activity and possibly, therefore, sperm transport. Moreover, they suggest that testosterone may be involved in the accumulation of an oxytocin-like peptide in the mouse testis.

The oxytocin immunoreactivity of the rat testis has been localized immunocytochemically to the Leydig cells (Guldenaar & Pickering, 1985). While we cannot be sure that the peptide is indeed synthesized in the testis, as it is in the corpus luteum (Swann, O'Shaughnessy, Birkett et al. 1984; Ivell & Richter, 1984), our preliminary evidence is pointing that way (H. D. Nicholson, R. T. S. Worley and B. T. Pickering, unpublished observations). Our present results suggest that oxytocin production may be dependent on androgen levels. This does not necessarily contradict the evidence that oxytocin inhibits testosterone production by dispersed cells of the testis in vitro (Adashi & Hsueh, 1981), since these hormones may be components of the same feedback loop. Thus, in both male and female gonads, evidence is accumulating that an oxytocin-like peptide and a steroid are synthesized in the same cell and that their production and functions may be interrelated.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of J. A. Leendertz (electronics and Fourier analysis), J. Black and A. Jones (expert technical assistance), D. Rogers and S. Kelly (graph plotting and advice on statistics). This work was supported by the MRC.

REFERENCES


