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Localization of oxytocin receptors in the human and macaque monkey male reproductive tracts: evidence for a physiological role of oxytocin in the male

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The peptide oxytocin is present in tissues of the male reproductive tract from a variety of mammalian species. In the human, specific mRNA for oxytocin and the peptide itself have been identified in the testis, epididymis and prostate. The peptide has been shown to modulate both steroidogenesis and contractility in the male reproductive tract and may be involved in the pathogenesis of benign prostatic hyperplasia. We have performed Western blots and immunohistochemistry using a specific antibody to the human oxytocin receptor (OTR) to investigate the distribution and localization of the receptor in the human and macaque monkey (*Macaca fasicularis*). An immunoreactive band of ~55 kDa was detected in human and monkey uterine, testicular and prostatic tissues and in preparations of monkey caput and cauda epididymis. A second, less intense, band of 60 kDa was also seen in testicular and uterine tissue samples. No specific bands were detected in monkey muscle or in any tissue following incubation with mouse immunoglobulin (Ig)M. In the human and monkey testis staining for the OTR was present in the interstitial tissue and in Sertoli cells. Localization of the OTRs varied throughout the epididymis being expressed by epithelial cells proximally but confined to cells at the base of the epididymal ducts and to the surrounding smooth muscle layers distally. In the prostate OTR were localized to the stromal tissue surrounding the ducts. These findings correlate with sites of local production of the peptide and the observed biological actions of oxytocin, and thus support the evidence that oxytocin may play a physiological role in the male reproductive tract.

Key words: epididymis/oxytocin/oxytocin receptor/prostate/testis

Introduction

The hormone oxytocin has well-recognized functions in the control of milk ejection and parturition in the female. The peptide has more recently been implicated in the regulation of steroidogenesis and contractility in the male reproductive tract and there is growing evidence that these effects may be mediated by locally produced, rather than hypothalamic oxytocin. Oxytocin is produced by the rat and human testis (Nicholson and Hardy, 1992; Ivell *et al.*, 1997) and both oxytocin mRNA and peptide have been identified in the human epididymis and prostate (Nicholson *et al.*, 1985; Ivell *et al.*, 1997) suggesting local synthesis.

Oxytocin can modulate contractile activity throughout the male reproductive tract. In the testis the peptide increases contractility of the seminiferous tubules (Niemi and Kormano, 1965; Worley *et al.*, 1985) in a stage-dependent fashion (Harris and Nicholson, 1998a). This contractile activity is thought to be important in the movement of sperm out of the testis and may also play a part in the process of spermiation, when spermatozoa are shed from the seminiferous epithelium (Frayne *et al.*, 1996). Oxytocin also increases contractility of the epididymis, vas deferens and prostate (Hib, 1974; Knight, 1974; Bodanszky *et al.*, 1992) and may be involved in ejaculation (Melin, 1970).

Oxytocin has peptide specific effects on testosterone production in the rodent testis (Frayne and Nicholson, 1995) and can modulate steroid production both *in vitro* and *in vivo*. Perhaps more importantly oxytocin can stimulate the conversion of testosterone to the biologically active dihydrotestosterone (DHT) in the testis, epididymis and prostate by increasing activity of the enzyme 5 α-reductase (Nicholson and Jenkin, 1994). Both the epididymis and prostate are androgen-dependent organs relying on DHT to stimulate normal growth and function. In the rat, oxytocin has been shown to increase prostatic growth and we have demonstrated that tissue from dogs with hyperplastic prostates has higher concentrations of oxytocin, 5 α-reductase and DHT (Nicholson and Jenkin, 1995). Thus, local paracrine actions of oxytocin may be involved in the pathophysiology of benign prostatic disease.

A prerequisite for the actions of oxytocin in the male reproductive tract is the presence of specific receptors. Oxytocin receptors have been localized by autoradiography to the Leydig cell of the rat testis (Bathgate and Sernia, 1994) and the peptide was localized in the reproductive tissues of the marmoset (Einspanier and Ivell, 1997). While mRNA for the receptor has been identified in human tissue, the cellular localization of the receptor is not known. The aim of this study was to identify and localize oxytocin receptors in the male reproductive tract of the human and macaque monkey (*Macaca fasicularis*) and to determine whether the localization of these receptors correlates with the observed biological actions of oxytocin.

Materials and methods

Tissues

Testicular, epididymal, prostatic, skeletal muscle, kidney and uterine tissues were obtained post-mortem from adult macaque monkeys. Human prostatic, testicular and uterine tissue was obtained peri-operatively during routine transurethral prostatectomy, orchidectomy and hysterectomy following approval by the local United Bristol Hospital Trust (UBHT) Ethical Committee. All tissue was immediately frozen in liquid nitrogen and stored at –80°C until use.

Electrophoresis and Western blotting

For gel electrophoresis, membrane preparations were prepared by homogenizing tissues in phosphate-buffered saline (PBS; 154 mM NaCl, 1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.2) containing 1 mM 4-(2-aminoethyl)benzenesulphonyl fluoride (ICN Biomedicals Ltd, Thame, UK) followed by incubation, on ice, in the same buffer containing 1% Triton X-100 (Sigma Chemical Co, Poole, UK). Homogenates were then centrifuged at 10 000 *g* and supernatants stored at –20°C until required.

To remove oxytocin receptors from membrane preparations, aliquots were incubated for 2 h with oxytocin peptide coupled to CNBr-activated Sepharose-4B (Pharmacia Biotech, St Albans, UK) in 0.05 M Tris–HCl buffer containing 3 mM magnesium sulphate. Absorbed preparations were then centrifuged at 10 000 *g* and the supernatant collected.

Proteins (20 μg/lane) were separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions on 12% polyacrylamide gels and electroblotted onto polyvinylidene difluoride (PVDF; NEN Life Science Products, Brussels, Belgium) membranes. Blots were probed with anti-human OTR monoclonal antibody, 2F8 (raised against the peptide corresponding to the 20th to 40th amino acids of the human receptor sequence) at 2.5 μg/ml for 2 h at room temperature. The antiserum was a kind gift from Dr T.Kimura, Osaka, Japan. Membranes were then washed and incubated with horseradish peroxidase (HRP)-conjugated swine anti-mouse immunoglobulins (1:2000 dilution in PBS; Dako Ltd, High Wycombe, UK) for 1 h. Detection of bound antibody was achieved by enhanced chemiluminescence (Amersham ECL™; Amersham International, Amersham, UK) and exposure of membranes to Hyperfilm (Amersham).

To ensure that absorbed membrane preparations were specifically devoid of oxytocin receptors, Western blots of macaque testicular samples were also incubated with antibodies raised against a different macaque testicular membrane protein, tMDC II (a member of a family of proteins containing a *M*etalloproteinase-like domain, a *D*isintegrin-like domain and which are *C*ysteine rich) (Frayne *et al.*, 1998).

Control blots of all samples were incubated with normal mouse immunoglobulin (Ig)M in place of the primary antibody.

Immunocytochemistry

Frozen tissue sections (12 μm) were cut using a cryostat and air-dried overnight. The sections were then fixed for 10 min in acetone at 4°C and air-dried. Non-specific antibody binding was blocked with 10% normal swine serum in PBS for 1 h. Tissue sections were then incubated overnight at 4°C with anti-human OTR antibodies (2F8), at a concentration of 10 μg/ml. After washing, sections were incubated with fluorescein isothiocyanate (FITC)-labelled swine anti-mouse immunoglobulins for 1 h at room temperature, washed again, mounted in antifade solution (Dako Ltd) and viewed on a Zeiss microscope. Control sections were incubated with pure mouse IgM in place of the primary antibody.

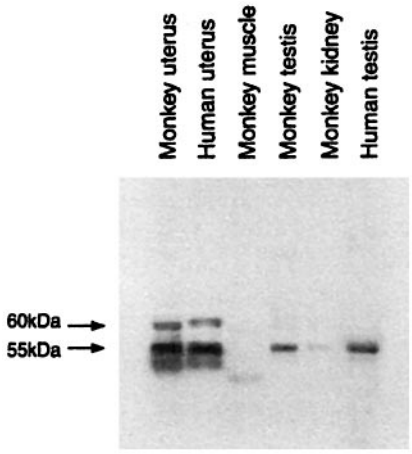

Figure 1. Western blot of membrane preparations (20 μg/ml) from human uterus and testis, and macaque uterus, testis, muscle and kidney probed with the anti-oxytocin receptor monoclonal antibody 2F8 (2.5μg/ml).

Results

Detection of oxytocin receptors in adult macaque and human tissues

Initially it was necessary to establish the specificity of the human oxytocin receptor monoclonal antibody (2F8) in our system, and in particular whether it would cross-react with a macaque oxytocin receptor. Western blots of membrane preparations from human uterus, as a positive control tissue, human testis and macaque uterus, testis, skeletal muscle and kidney were probed with monoclonal antibody 2F8 (Figure 1). An intensely stained band of 55 kDa was detected on Western blots of both human and monkey uterine samples. A second, less intense band of 60 kDa was also detected in uterine tissue from both species.

Similarly, in both monkey and human testicular tissue two bands were detected; a major band at 55 kDa and a much fainter band at 60 kDa.

No specific bands were detected in membrane preparations of macaque skeletal muscle. However, a faint band of ~55 kDa was detected in the membrane preparation from macaque kidney which is in accord with the known expression of such receptors in this tissue (Maeda *et al.*, 1993, Ostrowski *et al.*, 1995).

Having verified cross-reactivity of the antiserum 2F8 with a macaque oxytocin receptor, we next probed Western blots of macaque prostatic, caput and caudal epididymal membrane preparations and human prostatic membrane preparations. A single band of 55 kDa was detected on Western blots of all these samples (Figure 2).

Incubation of monkey uterine and testicular tissue with oxytocin peptide coupled to CNBr-activated sepharose prior to electrophoresis and Western blotting resulted in loss of the 60 kDa band and a marked reduction in the intensity of the 55 kDa band in both tissues (Figure 3). In contrast, when Western blots of both absorbed and total macaque testicular membrane preparations were probed with the macaque tMDC II antisera no difference was seen in the intensity of the bands detected (Figure 3).

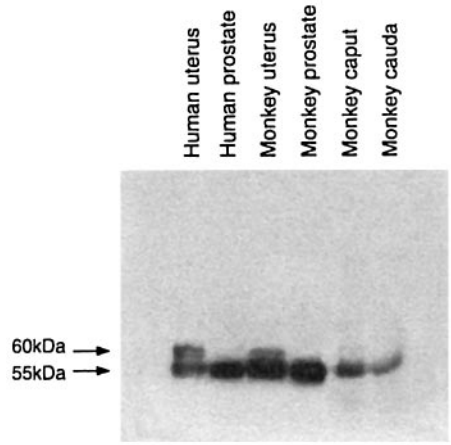

Figure 2. Western blot of membrane preparations (20 μg/ml) from human prostate and macaque prostate, caput and cauda epididymis probed with antibody 2F8 (2.5 μg/ml). Human and macaque uterus were included as positive controls for comparison.

Localization of oxytocin receptors in macaque and human tissues

Having established the specificity of antiserum 2F8 for an oxytocin receptor in both the human and macaque monkey by Western blot analysis, the localization of these receptors in a variety of tissues by indirect immunofluorescence was investigated.

In both the human and macaque testis oxytocin receptors were localized to the interstitial Leydig cells, although few Leydig cells were present in the sections from human testis. Interestingly, in both species receptors were also detected on Sertoli cells, the staining clearly seen forming a contour around the nucleus of the cells (Figure 4A,B).

No staining for oxytocin was detected on sections of macaque skeletal muscle (Figure 4C), whereas on sections of human uterus, oxytocin receptors were localized to most, but not all, myometrial cells (Figure 4D).

Oxytocin receptors were also localized to distinct cell types within the macaque epididymis where the pattern of staining varied with progression from the initial to the terminal segment (Figure 5). In the initial segment receptors were localized to many, but not all, epithelial cells (Figure 5A). In contrast, in the caput epididymis no staining for OTR was detected in the epithelial cells, however in this region receptors were within the layer of smooth muscle surrounding the tubule (Figure 5B). Similarly, in the cauda epididymis oxytocin receptors were located in the smooth muscle layer surrounding the tubule (Figure 5C). The increase in the amount of staining appeared to correlate with the increase in the thickness of this layer in the distal regions of the epididymis. Additionally, in this region of the epididymis oxytocin receptors were also detected on a second layer of cells closely apposed to the base of the epithelium. It is difficult to specifically identify these cells as information in the literature is scant, however it is likely that they represent the layer of peritubular smooth muscle cells which surround the epididymal tubule.

In both human and macaque prostate oxytocin receptors were confined to the stromal tissues surrounding the ducts (Figure 6).

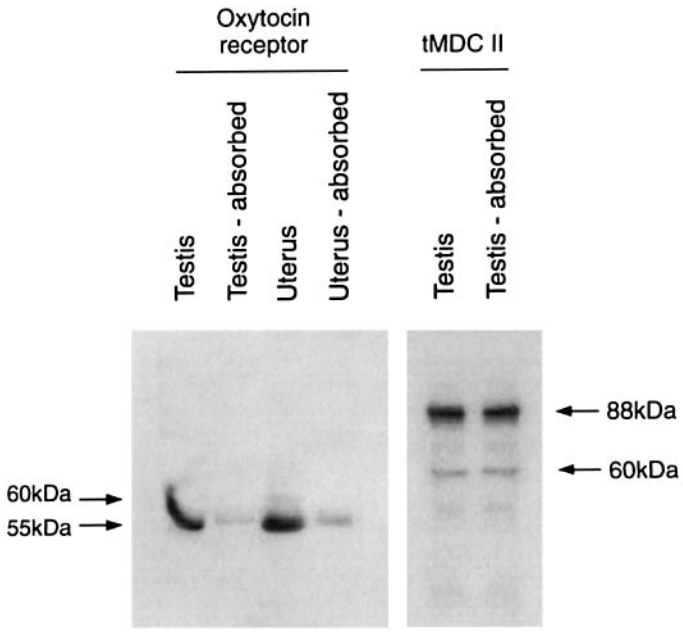

Figure 3. Western blot of membrane extracts from macaque uterus and testis (20 μg/ml) before and after absorption with oxytocin coupled to CNBr-activated sepharose. The blot was probed with antibody 2F8 (2.5 μg/ml). Control shows some testis membrane extract before and after absorption with oxytocin probed with antibody to tMDCII.

Discussion

These data demonstrate the expression and localization of oxytocin receptors in the male reproductive tract of the human and macaque monkey. In all reproductive tract tissues studied an oxytocin receptor with a molecular mass of ~55 kDa was detected, with a second, less abundant receptor of ~60 kDa present in the uterus and testis. The sizes of the receptors are similar to those described in the rabbit uterus and mammary gland (Hinko *et al.*, 1992) and rat myometrium (Strakova and Soloff, 1997) but slightly smaller than that reported in the human myometrium (Kimura *et al.*, 1996). The presence of two bands has been observed by other workers (Hinko *et al.*, 1992) in female reproductive tissues. Since only a single gene transcript has so far been identified for the OTR (Einspanier and Ivell, 1997) it is more likely that the two bands are derived from the same gene. The size of the human OTR as determined by immunoblotting is much larger than the estimated molecular weight of 43 kDA (Kimura *et al.*, 1996) and it has been suggested that the increase in size is due to *N*-terminal glycosylation. Three glycosylation sites are present in the extracellular *N*-terminal domain of the human OTR and recent studies, using site-directed mutagenesis, have demonstrated that full glycosylation is not essential for the receptor to be biologically active (Kimura *et al.*, 1997). Further work is necessary to investigate the nature and biological activity of these two receptors.

Oxytocin is produced locally in the testis (Pickering *et al.*, 1990; Nicholson and Hardy, 1992) and immunoreactive peptide has been localized to the Leydig cells (Guldenaar and Pickering, 1985; Weindl *et al.*, 1986). The peptide has been shown to modulate testosterone secretion in the rodent (Adashi *et al.*,

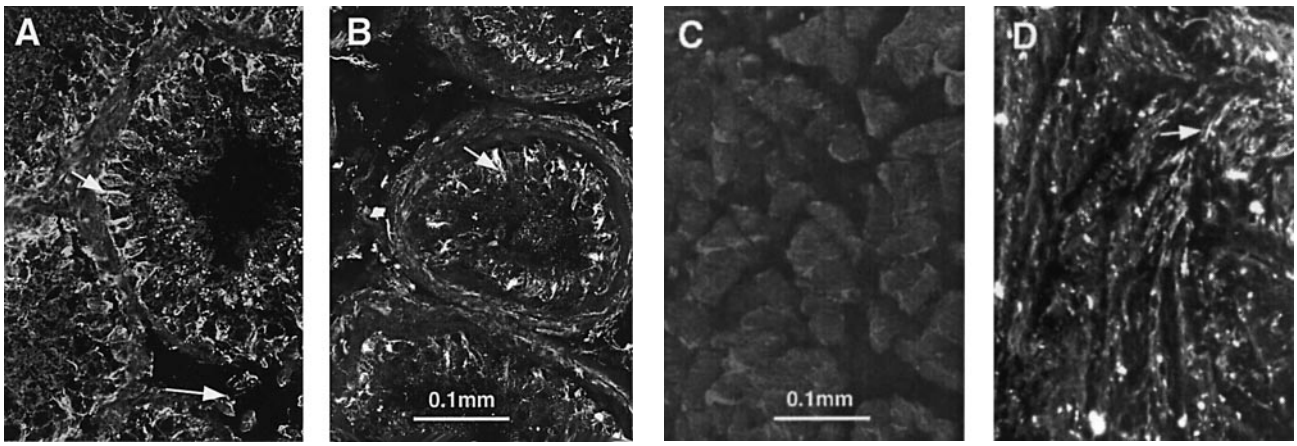

Figure 4. Localization of oxytocin receptors in frozen tissue sections by indirect immunofluorescence using the anti oxytocin receptor antibody 2F8 (10 μg/ml). (**A**) macaque testis; short arrow indicates Sertoli cells, long arrow indicates Leydig cells; (**B**) human testis; arrow indicates Sertoli cells; (**C**) monkey skeletal muscle; (**D**) human uterus; arrow indicates myometrial cells.

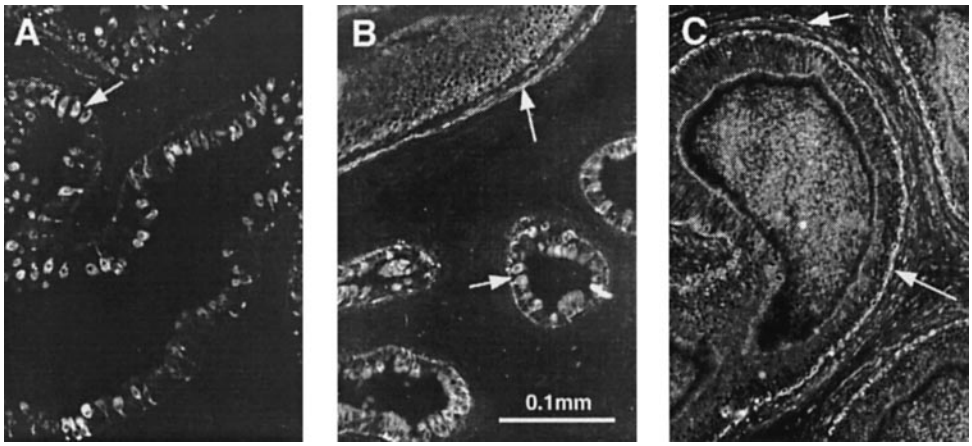

Figure 5. Localization of oxytocin receptors in frozen sections of macaque epididymis by indirect immunofluorescence using antibody 2F8 (10 μg/ml). (**A**) initial segment; arrow indicates positively stained epithelial cells; (**B**) caput region and initial segment; long arrow indicates positively stained layer of smooth muscle surrounding a tubule in the caput region, short arrow indicates stained epithelial cells within the initial segment; (**C**) cauda region; long arrow indicates layer of positively stained peritubular cells, short arrow indicates smooth muscle layers surrounding the epididymal tubule.

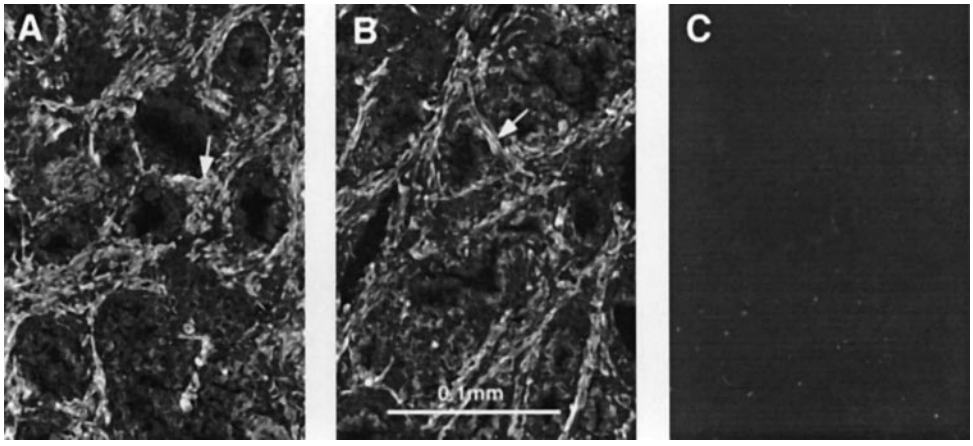

Figure 6. Localization of oxytocin receptors in frozen sections of (**A**) human prostate and (**B**) monkey prostate by indirect immunofluorescence using antibody 2F8; arrows indicate positive staining in the stromal tissue surrounding the prostatic ducts. (**C**) control section of macaque prostate with primary antibody replaced by an equivalent amount of pure mouse immunoglobulin (Ig)M.

1984). This effect can be mimicked by a specific oxytocin agonist suggesting that oxytocin exerts its biological action via a specific OTR (Frayne and Nicholson, 1995). This is supported by autoradiographical evidence showing the presence of OTRs only on the Leydig cells in the adult rat (Bathgate and Sernia, 1994). The localization of OTRs on monkey and

human Leydig cells in our study suggests that oxytocin may play a similar role in the primate and agrees with the findings in the marmoset monkey (Einspanier and Ivell, 1997) and the human (Ivell *et al.*, 1997). However, unlike the rat and marmoset, clear staining for OTRs was also found in the Sertoli cells within the seminiferous tubules confirming a recent report by Ivell *et al.* (1997). While oxytocin has typically been located in Leydig cells, oxytocin gene expression has been identified in the Sertoli cells from bulls (Ungefroren *et al.*, 1994) and transgenic mice expressing the bovine oxytocin transgene (Ang *et al.*, 1994). Weak staining for the peptide has been identified in the Sertoli cells of the marmoset monkey (Einspanier and Ivell, 1997) and there is evidence in the rat and the sheep that the seminiferous epithelium may secrete oxytocin into the lumenal fluid of the seminiferous tubules (Veeramachaneni and Amann, 1990; Nicholson *et al.*, 1994). The function of the oxytocin receptors on the Sertoli cells is not known. Sertoli cells are the main site for 5 α-reductase activity in the adult testis thus, oxytocin may act to increase local dihydrotestosterone concentrations. We have recently shown that oxytocin treatment in the rat can affect spermiation (Frayne *et al.*, 1996) and it is possible that the peptide may act directly to assist in the shedding of sperm from the Sertoli cell.

OTRs were not found on the peritubular myoid cells in this study and have not been identified on myoid cells in the marmoset or rat (Bathgate and Sernia, 1994; Howl *et al.*, 1995; Einspanier and Ivell, 1997). Oxytocin at physiological concentrations has, however, been shown to stimulate seminiferous tubule contractility in the rat (Worley *et al.*, 1984) and human (Nicholson; personal communication). Thus, it would seem that oxytocin either acts via the V_{1a} receptor (Howl *et al.*, 1995) or a novel oxytocin receptor (Harris and Nicholson, 1998b) on the myoid cells. Alternatively oxytocin may act indirectly via the Sertoli or Leydig cells so that binding to these receptors results in the production of an intermediary factor which stimulates contractility.

Oxytocin receptors were detected in all regions of the macaque epididymis but interestingly the localization of the receptors varied along the length of the epididymal duct. The epididymis is dependent on DHT to maintain its growth and functions. Testosterone is converted to DHT via the enzyme 5 α-reductase and high concentrations of the enzyme are present in the epithelial cells of the proximal epididymis (Gloyna and Wilson, 1969). Regulation of 5 α-reductase activity in the proximal epididymis occurs by molecules which enter the epididymis via the testicular lumenal fluid (Brown *et al.*, 1983). Oxytocin is present in the luminal fluid of the testis and epididymis and has also been identified within the epididymal epithelial cells of the sheep and rat (Knickerbocker *et al.*, 1988; Veeramachaneni and Amann, 1990; Harris *et al.*, 1996). The expression of OTRs by epithelial cells of the initial segment supports a role for oxytocin in the modulation of 5 α-reductase in these cells.

As in the testis, oxytocin also affects contractility of the epididymis. Binding sites for the peptide have been described in the pig epididymis (Maggi *et al.*, 1987) and treatment with systemic oxytocin results in increased sperm transport within the epididymis (Voglmayr, 1975). In both the caput and cauda epididymis OTRs were present in the smooth muscle layer(s) surrounding the epididymal duct, and also in the cauda epididymis in a layer of cells, presumably peritubular myoid cells, closely apposed to the epididymal duct. This distribution of receptors would suggest that while in the proximal epididymis oxytocin may modulate 5 α-reductase activity, elsewhere in the epididymis it is more concerned in stimulating contractility and the resultant sperm transport.

Within the prostate oxytocin has been demonstrated to modulate epithelial cell growth (Plecas *et al.*, 1992), stimulate contractility and affect the muscular tone of the gland (Bodanszky *et al.*, 1992). Preliminary data suggest that the effect of oxytocin on cell growth is mediated by DHT, thus oxytocin stimulates 5 α-reductase activity which increases DHT concentrations resulting eventually in prostatic growth (Nicholson, 1996). Oxytocin is present in the human prostate and furthermore concentrations of the peptide and 5 α-reductase activity are increased in tissue taken from men or dogs with benign enlargement of the gland (Nicholson and Jenkin, 1995). mRNA for the OTR has recently been demonstrated in extracts of human prostate and in prostatic carcinoma cell lines (Ivell *et al.*, 1997). The localization of these OTRs in the stromal tissue of the prostate would allow both modulation of DHT concentrations and possible action on smooth muscle cells. Further studies are necessary to investigate whether the distribution and density of OTRs vary in different pathological states of the prostate.

In conclusion, this study provides evidence for oxytocin receptors within the monkey and human male reproductive tract and demonstrates that the localization of these receptors fits with the previously described functions of oxytocin in the male in regulating steroidogenesis and contractility.

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References

Adashi, E.Y., Tucker, E.M. and Hsueh, A.J.W. (1984) Direct regulation of rat steroidogenesis by neurohypophysial hormones. *J. Biol. Chem.*, **259,** 5440–5446.

Ang, H.-L., Ivell, R., Walther, N. *et al.* (1994) Over-expression of oxytocin in the testis of a transgenic mouse model. *J. Endocrinol.*, **140,** 53–62.

Bathgate, R.A.D. and Sernia, C. (1994) Characterisation and localisation of oxytocin receptors in the rat testis. *J. Endocrinol.*, **141,** 343–352.

Bodanszky, M., Sharaf, H., Roy, J.B. and Saids, I. (1992) Contractile activity of vasotocin, oxytocin and vasopressin on mammalian prostate. *Eur. J. Pharmacol.*, **216,** 311–313.

Brown, D.V., Amann, R.P. and Wagley, L.M. (1983) Influence of rete testis fluid on the metabolism of testosterone by cultured principal cells isolated from the proximal or distal caput of the rat caput epididymis. *Biol. Reprod.*, **28,** 1257–1268.

Einspanier, A. and Ivell, R. (1997) Oxytocin and oxytocin receptor expression in reproductive tissues of the male marmoset monkey. *Biol. Reprod.*, **56,** 416–422.

Guldenaar, S.E.F. and Pickering, B.T. (1985) Immunocytochemical evidence for the presence of oxytocin in rat testis. *Cell Tiss. Res.*, **240,** 485–487.

Frayne, J., Jury, J.A., Barker, H.L. *et al.* (1998) Macaque MDC family of proteins; sequence analysis, tissue distribution and processing in the male reproductive tract. *Mol. Hum. Reprod.*, **4,** in press.

Frayne, J and Nicholson, H.D. (1995) Effect of oxytocin on testosterone production by isolated rat Leydig cells is mediated by a specific oxytocin receptor. *Biol. Reprod.*, **52,** 1268–1273.

Frayne, J., Townsend, D. and Nicholson, H.D. (1996) Effects of oxytocin on sperm transport in the pubertal rat. *J. Reprod. Fertil*, **107,** 299–306.

Gloyna, R.E. and Wilson, J.D. (1969) A comparative study of the conversion of testosterone to 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone) by rat prostate and epididymis. *Endocrinology*, **29,** 970–977.

Harris, G.C., Frayne, J. and Nicholson, H.D. (1996) Epididymal oxytocin in the rat: its origin and regulation. *Int. J. Androl.*, **19,** 278–286.

Harris, G.C. and Nicholson, H.D. (1998a) Stage-related differences of rat seminiferous tubule contractility *in vitro* and their response to oxytocin. *J. Endocrinol.*, in press.

Harris, G.C. and Nicholson, H.D. (1998b) Characterisation of the biological effects of neurohypophysial peptides on seminiferous tubules. *J. Endocrinol.*, **156,** 35–42.

Hib, J. (1974) The *in vitro* effects of oxytocin and vasopressin on spontaneous contractility of the mouse cauda epididymis. *Biol. Reprod.*, **11,** 436–439.

Hinko, A., Soloff, M.S. and Potier, M. (1992) Molecular size characterization of oxytocin receptors in rabbit amnion. *Endocrinology*, **130,** 3554–3559.

Howl, J., Rudge, S.A., Lavis, R.A. *et al.* (1995) Rat testicular myoid cells express vasopressin receptors: receptor structure, signal transduction and developmental regulation. *Endocrinology*, **136,** 2206–2213.

Ivell, R., Balvers, M., Rust, W. *et al.* (1997) Oxytocin and male reproductive function. *Adv. Exp. Med. Biol.*, **424,** 253–264.

Kimura, T., Makino, Y., Bathgate, R. *et al.* (1997) The role of *N*-terminal glycosylation in the human oxytocin receptor. *Mol. Hum. Reprod.*, **3,** 957–963.

Kimura, T., Takemura, M., Nomura, S. *et al.* (1996) Expression of oxytocin receptor in human pregnant myometrium. *Endocrinology*, **137,** 780–785.

Knickerbocker, J.J., Sawyer, H.R., Amann, R.P. *et al.* (1988) Evidence for the presence of oxytocin in the ovine epididymis. *Biol. Reprod.*, **39,** 391–397.

Knight, T.W. (1974) A qualitative study of factors affecting the contractions of the epididymis and ductus deferens of the ram. *J. Reprod. Fertil.*, **40,** 19–29.

Maeda, Y., Han, J.S., Gibson, C.C. and Knepper, M.A. (1993) Vasopressin and oxytocin receptors coupled to calcium mobilization in rat inner medullary collecting duct. *Am. J. Physiol.*, **265,** F15–F25.

Maggi, M., Malozowski, S., Kassis, S. *et al.* (1987) Identification and characterization of two classes of receptors for oxytocin and vasopressin in porcine tunica albuginea, epididymis and vas deferens. *Endocrinology*, **120,** 986–994.

Melin, P. (1970) Effects *in vivo* of neurohypophyseal hormones on the contractile activity of accessory sex organs in male rabbits. *J. Reprod. Fertil.*, **22,** 283–292.

Nicholson, H.D. (1996) Oxytocin: a paracrine regulator of prostatic function. *Rev. Reprod.*, **1,** 69–72.

Nicholson, H.D., Greenfield, H.M. and Frayne, J. (1994) The effect of germ cell complement on the presence of oxytocin in the interstitial and seminiferous tubule fluid of the rat testis. *J. Endocrinol.*, **143,** 471–478.

Nicholson, H.D. and Hardy, M.P. (1992) Luteinizing hormone differentially regulates the secretion of testicular oxytocin and testosterone by purified adult rat Leydig cells *in vitro*. *Endocrinology*, **130,** 671–677.

Nicholson, H.D. and Jenkin, L. (1994) 5 α-reductase activity increased by oxytocin in the rat testis. In Bartke, A. (ed.), *Function of Somatic Cells in the Testis*. Serono Symposia, USA, pp. 278–285.

Nicholson, H.D. and Jenkin, L. (1995) Oxytocin and prostatic function. In Ivell, R. and Russell, J. (eds), *Oxytocin*. Plenum Press, New York, USA, pp.529–537.

Nicholson, H.D., Peeling, W.B. and Pickering, B.T. (1985) Oxytocin in the prostate and semen? [Abstr. no. 127] *J. Endocrinol.*, **104** (suppl.).

Niemi, M. and Kormano, M. (1965) Contractility of the seminiferous tubule of the postnatal rat testis and its response to oxytocin. *Ann. Med. Exp. Biol. Fen.*, **43,** 40–42.

Ostrowski, N.L., Young, W.S. and Lolait, S.J. (1995) Estrogen increases renal oxytocin receptor gene expression. *Endocrinology*, **136,** 1801–1804.

Pickering, B.T., Ayad, V.J., Birkett, S.D. *et al.* (1990) Neurohypophyseal peptides in the gonads: are they real and do they have a function? *Reprod. Fertil. Dev.*, **2,** 245–262.

Plecas, B., Popovic, A., Jovovic, D and Hristic, M. (1992) Mitotic activity and cell deletion in ventral prostate epithelium of intact and castrated oxytocin-treated rats. *J. Endocrinol. Invest.*, **15,** 249–253.

Strakova, Z. and Soloff, M.S. (1997) Coupling of oxytocin receptor to G proteins in rat myometrium during labour: G_1 receptor interaction. *Am. J. Physiol.*, **272,** E870–E876.

Ungefroren, H., Davidoff, M and Ivell, R. (1994) Post-transcriptional block in oxytocin gene expression within the seminiferous tubules of the bovine testis. *J. Endocrinol.*, **140,** 63–70.

Veeramachaneni, D.N.R. and Amann, R.P. (1990) Oxytocin in the ovine ductuli efferentes and caput epididymis: immunolocalization and endocytosis from the luminal fluid. *Endocrinology*, **126,** 1156–1164.

Voglmayr, J.K. (1975) Output of spermatozoa and fluid by the testis of the ram and its response to oxytocin. *J. Reprod. Fertil.*, **43,** 119–122.

Weindl, A., Braun, J. and Rust, M. (1986) Immunohistochemical demonstration of oxytocin in the testis. *Acta Endocr.*, **S274,** 105–106.

Worley, R.T.S., Nicholson, H.D. and Pickering, B.T. (1984) Testicular oxytocin: an initiator of seminiferous tubule movement? In Saez, J.J.M., Forest, M., Dazord, A. and Bertrand, J. (eds), *Recent Progress in Cellular Endocrinology of the Testis.* INSERM, Paris, France, pp. 205–212.

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