Effect of oxytocin on fertility of male rats

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Abstract

It is now recognised that oxytocin (OT) modulates several aspects of male reproduction. However, its overall impact on male fertility remains unknown. Thus, the aim of this study was to examine the effect of OT on male fertility. This was investigated in rats using three doses [0.1 International Units (IU) of OT once a day (n = 9), twice a day and thrice a day, given intraperitoneally for 7 consecutive days] and a non-competitive serial mating trial (on days 1,3 and 7 of treatment and day 7 post-treatment). The OT caused marked oligozoospermia without provoking toxic side effects (in terms of teratozoospermia, overt signs of toxicity, renotoxicity, hepatotoxicity or haemotoxicity) or compromising libido, mating performance and fertility (in terms of quantal pregnancy, number of uterine implants and indices of implantation and fertility). The oligozoospermia was mediated by retrograde ejaculation and by impairment of ejaculatory contraction of vas (as judged by inhibition of electrically elicited contraction of isolated vas) and probably epididymis. It is possible that OT analogues may be developed as post-testicular male contraceptives.

Key Words: oxytocin, male fertility, oligozoospermia, retrograde ejaculation, ejaculatory contraction

Introduction

Oxytocin (OT), a pituitary neuropeptide, is also synthesised in the testes (1), and modulates several aspects of male reproduction such as steroidogenesis (1,2), contractility of seminiferous tubules (2), epididymis (1,3,4,5), vas deferens (2,5), motility of spermatozoa (6), penile erection (7,8), sexual interest and/or copulatory performance (7,8), sexual arousal (9), orgasm (9), sexual satiety (10), and spontaneous ejaculation (11). With these modulatory activities a possibility exists that OT may impair male fertility without disturbing sexual function. So far, the effects of OT on male fertility have not been investigated. If OT can inhibit male fertility rapidly with no unacceptable toxic effects, and restore fertility when withdrawn, it may be possible to develop an effective post-testicular male contraceptive based on oxytocin analogues. Currently, there is a need for development of new male contraceptive which do not interfere with libido, potency and sexual activity(12).

The aim of this study was to investigate the effects OT on fertility of male rats when given intraperitoneally .

Material and Methods

Animals

Healthy adult cross-bred albino rats (males weighing 250-300g and females weighing

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200-225g) of proven fertility from our own colony were used. They were housed under standardised animal house conditions and had free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and tap water. Separate groups of animals were used in different sets of experiments undertaken.

Drugs

Oxytocin (Neon Laboratories Ltd., Mumbai, India) and diethylether (Fluka Chemica, Buchs, Switzerland) were used.

Effects on fertility

Fifty four male rats were randomly assigned into six equal groups (n=9 / group) and treated intraperitoneally in the following manner for 7 consecutive days: group1, 0.5 IU of OT once a day; group 2, 0.5 IU of OT twice a day; group 3, 0.5 IU of OT thrice a day; and group 4, 0.1ml of isotonic saline (0.9% w/v NaCl) once a day; group 5, 0.1 ml saline twice a day; and group 6, 0.1 ml saline thrice a day. Libido, ejaculatory ability and fertility of these rats were assessed 7 days prior to treatment and on days 1, 3, and 7 of treatment, and on day 7 post-treatment. Each male was paired overnight with a pro-oestrous female (at 16.30-17.00h). The pre-coital sexual behaviour (chasing, nosing, anogenital sniffing, genital grooming, attempted clasping, mounting and intromission) of the paired rats was observed 1-2 h later. Vaginal smears of the female were taken in the following morning (08.00-08.30h). The presence of spermatozoa was considered day 1 of pregnancy. If spermatozoa were present, their numbers were estimated in duplicate using an improved Neubauer haemocytometer (Fison Scientific, London, UK) and the gross morphology was observed microscopically (x 100 and x 400). The head defects noted were decapitation, macrocepahaly, microcephaly, pyriform head, bicephaly and rounded heads. Mid piece defects noted were bent necks, thin mid piece, kinked mid piece and irregular mid piece. The tail defects scanned for were double, coiled, hairpin and irregular width.

At day 14 post-coitum, the mated females were subjected to laparotomy under ether anaesthesia and the number of conceptus (both viable and dead) were counted to permit analysis of fertility. In addition, the colour, number and gross morphology of the corpora lutea in each ovary were recorded.

The following reproductive parameters were then calculated: index of libido = (number mated / number paired) x 100; quantal pregnancy = (number pregnant/ number mated) x 100; fertility index = (number pregnant/ number paired) x 100; pre-implantation loss = (number of corpora lutea-number of implants) / number of corpora lutea x 100; post-implantation loss = (total number of implants-number of viable implants)/ total number of implantations x 100; implantation index = (total number of implants/number mated) x 100.

Observations on overt signs of toxicity

The rats used in the fertility study were observed 1 to 2 h following each intraperitoneal injection for signs of toxicity (salivation, rhinorrhoea, lacrimation, ptosis, squinted eyes, excessive gnawing and biting movements of jaw, wilting, convulsions, stupor, tremors, rapid rotational movement of head, neck and / or entire body around the spinal axis, yellowing of fur, pallor of lips, loss of hair), abnormal postural changes,

stress (erection of fur, exopthalmia) and non sexual behaviours (such as cleaning of face, self grooming, climbing in the cage, rearings). In addition, adverse behaviours (biting and scratching behaviour, licking the tail, paw and penis, intense grooming behaviour or vocalisation) were also observed. Body weights were determined on days 1 and 30 of treatment. Food and water intake, consistency of faeces and colour of urine were also noted.

Effect on sperm number in urine

This was done as previously described by Ratnasooriya et al (13). Briefly, 18 male rats were randomly divided into two equal groups (n=9/group) The first group was intra peritoneally administered with 0.5 IU of OT thrice a day for 7 consecutive days and the other group was similarly treated with 0.1ml saline. These rats were individually placed in wire-meshed cages which were placed on wooden stands attached with polythene funnels and glass bottles, to collect urine samples continuously 7 days of treatment. Urine collected over 24 h was removed, centrifuged (at approximately 1600 x g for 5 min) and the precipitate was resuspended in normal saline. Then the sperm numbers were estimated (in duplicate) using an improved Neubauer type haemocytometer.

Effect of sperm number in urinary bladder

Twelve male rats were randomly divided into two equal groups (n=6/ group). One group was treated intraperitoneally with 0.5 IU of OT three times a day as described previously and the other with 0.1ml saline. Shortly after treatment each of these rats were placed individually with a pro-oestrus female in a plexi glass cages and their pre-coital sexual behaviour was observed until ejaculation occurred. Immediately afterwards the male was removed, anaesthetised with ether, pelvic cavity opened and the urinary bladder was exposed. The fluid content of the urinary bladder was removed using a 27 gauge needle, fixed to a 0.1 ml plastic syringe and its sperm numbers were estimated (in duplicate) after suitable dilution with normal saline using an improved Neubauer type haemocytometer as described elsewhere (13). The gross morphology of the spermatozoa was also noted.

Effects on the sperm count and gross morphology of sperm in the epididymis and vas deferens

Twelve male rats were randomly assigned into two equal groups (n = 6/ group). One group was treated intraperitoneally with 0.5 IU of OT thrice a day for 3 consecutive days. The other group was similarly treated with saline. Approximately 6 h later, these rats were anaesthetised with ether and their epididymides and vasa deferentia were carefully exposed and removed, and was placed in petri-dishes containing isotonic saline. The right vasa deferentia, cauda epididymis and caput plus corpus epididymis were separated, weighed (Chyo Balance Cooperation, Tokyo, Japan) homoginised separately with a known volume of isotonic saline in a ground glass homogeniser. The number of spermatozoa present in each of these organs was determined in duplicate and were expressed as 10^6/mg tissue as described elsewhere (14). Spermatozoa were extracted from each of these segments on the left half to isotonic saline and their gross morphology was carefully examined(14).

Effect on contractility of isolated cauda epididymal tubules

Nine rats were anaesthetised with ether and their epididymides removed through a 4-5 cm midline lower abdominal incision. These were immediately placed in a 25 ml glass beaker containing fresh oxygenated (95% O_2 and 5% CO_2) physiological salt solution of the following composition (mmol/L): Na^+ 143; K^+ 5.8; Ca^{++} 2.6; Mg^{++} 1.2; Cl^- 128; $H_2PO_4^-$ 1.2; HCO^-_3 25; SO^{2-}_4 1.2; and glucose 11.1.

A small portion of the cauda epididymides (approximate length 25mm) was uncoiled. These portions of the cauda epididymides were suspended in a 50 ml organ bath at 37±1°C under a resting tension of 0.25g.

Following an equilibration period of 15-30 min four different concentrations of OT (0.002, 0.004, 0.006 or 0.008 IU/ml) were added cumulatively to the organ bath at 5 min intervals. Contractile responses were recorded isometrically with an isometric sensor (Star Medicals, Tokyo, Japan) and displayed on a pen recorder (Rikadenki Kogyo Co. Ltd., Tokyo, Japan).

Effects on electrically induced contractions of isolated vasa deferentia

Six rats were anaesthetised with ether and their vasa deferentia were removed. The isolated vasa deferentia (approximate length: 40mm) were suspended in a 50 ml organ bath containing physiological salt solution used in the above section under 1.0g tension and were allowed to equilibrate for 30 to 45 min. Contractile responses were elicited by transmural stimulation via a platinum ring electrode placed towards epididymal end (13), using a SRI stimulator (SRI Scientific and Research Instrument Ltd., London, UK) for 5 sec at a frequency of 5HZ with impulses of 0.5 msec and 90V in the presence of OT (0.01, 0.02, or 0.03 IU/ml).

Effects on liver and kidney function

Twelve male rats were randomly divided into two equal groups (n=6). One group was treated intraperitoneally with 0.5 I.U of OT thrice a day for 7 consecutive days. The other group was similarly treated with 0.1 ml of isotonic saline. On day1 post-treatment, these rats were mildly anaesthetised with ether and 3ml blood collected from the tail under aseptic conditions.

The blood was allowed to clot (25-30 min) at room temperature (28-30°C) and subjected to 15 min centrifugation at 3200 x g. Serum was seperated and serum glutamic-oxaloacetate transaminase (SGOT), serum glutamic-pyruvate transaminase (SGPT), creatinine and urea levels were estimated using Randox assay kits(Randox Laboratories Ltd,.Antrium, UK) and a spectrophotometer (JASCO V500, JASCO Corporation, Tokyo, Japan). All readings were taken within 10 min following incubation.

Effects on haematology

Using some of the blood collected in the above experiment, red blood cell (RBC) counts, white blood cell (WBC) counts and haemoglobin content were estimated .

Statistical analyses

Data are given as mean ±SEM. Statistical comparisons were made using non parametric ANOVA (Kruskal-Wallis test) followed by Nemenyi multiple comparison test, G-test and Mann-Whitney U-test as appropriate. P value < 0.05 was accepted as indicating significance.

Results

Effects on fertility

Since there was no significant difference ($P > 0.05$, ANOVA) in the results of the mating experiment between the control groups these data are pooled and shown in Table 1. In the mating experiments, the precoital sexual behaviour of OT-treated rats appeared as vigorous as the control rats, if not facilitated. Thick milky exudates were frequently seen in the penises of the OT-treated rats during the period. Furthermore, index of libido remained uninhibited (Table 1)

OT caused marked and significant impairment of vaginal sperm count. ($P<0.05$; ANOVA) The lower dose induced profound and significant ($P<0.05$: ANOVA) oligozoospermic ejaculates on day 7(by 81%), mid dose by day 3 (by 79%) and high dose by day 1 itself (by 80%). Interestingly, all rats showed ejaculations during the study. In addition, there was no decapitated sperm or sperms with other head, mid piece or tail defects evident in the ejaculates of OT-treated rats as with the control. Cessation of treatment improved sperms counts in the ejaculates markedly by day 7: lower dose (by 43%), mid dose (by 75%) and high dose (by 41%). On the other hand, none of the fertility parameters determined/ computed was significantly altered by OT treatment ($P>0.05$). The corpora luteae of females mated with OT-treated males and control males were red in colour and appeared similar.

Adverse effects

OT treatment was well tolerated. None of the treated rats died or developed overt signs of toxicity, stress or aversive behaviour. The food intake and general health remained essentially normal throughout the study period. The body weights of OT- treated rats were not significantly impaired (data not shown).

Sperm in urine

In the urine collection study, no sperm were collected in the urine of both OT- treated rats (high dose) and control rats.

Sperm in bladder

In the experiment carried out to investigate sperm in the urinary bladder, during the initial precoital sexual behaviour period, spontaneous penile erections were evident in OT-treated rats. The sexual behaviour of the treated rats was almost similar to that observed in the fertility study. In all the OT-treated rats, sperm in considerable numbers were found in the urinary bladder immediately after ejaculation. The mean sperm count was 300 ± 63.0 million/ml. In contrast, no sperm were detected in urinary bladder of the control rats.

Sperm count in epididymis and vas deferens

Table 2 summarises the sperm count in the epididymis and vas deferens following OT treatment. A considerable reduction in sperm numbers in the cauda epididymis (by 18%), caput and corpus epididymis (by 8%) and vas deferens (by 28%) were evident compared to the controls. This reduction was not statistically significant ($P> 0.05$). Further, the gross morphology of the cauda epididymal sperm of the OT-treated rats were essentially similar to that of controls and no sperm granulomas were evident in any region of the vas deferens or epididymis.

Table 1: Effect of intraperitoneal administration of oxytocin on some fertility parameters of male rats. (mean ± SEM)

Parameter	Dose	Pre-treatment	Treatment			Post treatment
			Day 1	Day 3	Day 7	Day 7
Vaginal sperm counts 10^6/ml	Saline	31.4±6.2	31.4±6.9	29.2±7.5	29.4±4.1	27.3±3.6
	0.5 I.U daily	0.5 I.U 24.3+4.8	19.6±8.7	15.7±4.1	5.5±2.1*	9.5±2.4
	twice daily	0.5 IU 33.1±3.7	17.9±3.4	6.0±1.1*	9.8±4.3*	39.4±3.0
	thrice daily	25.5±4.5	6.3±3.0*	9.3±3.4*	7.9±4.3*	13.4±2.0
Index of libido %	Saline	100	100	100	100	100
	0.5 I.U daily	100	100	100	100	100
	0.5 I.U twice daily	100	100	100	100	100
	0.5 I.U thrice daily	100	100	100	100	100
Quantal pregnancy %	aline	100	100	100	100	100
	0.5 I.U daily	100	100	100	100	100
	0.5 I.U twice daily	100	100	100	100	100
	0.5 I.U thrice daily	100	100	100	100	100
Fertility index %	Saline	100	100	100	100	100
	0.5 I.U daily	100	100	100	100	100
	0.5 I.U twice daily	100	100	100	100	100
	0.5 I.U thrice daily	100	100	100	100	100
Number of implants	Saline	8.4±1.0	10.2±0.6	8.8±0.6	7.0±0.7	8.8±0.4
	0.5 I.U daily	8.6±0.5	7.0±1.8	7.4±1.3	8.8±1.2	8.8±1.0
	0.5 I.U twice daily	10.2±1.0	8.3±0.8	10.5±1.0	8.0±1.7	7.6±1.2
	0.5 I.U thrice daily	7.3±1.1	8.5±1.0	9.6±0.6	7.5±1.0	8.6±0.6
Pre-implantation loss %	Saline	24.5±3.6	13.1±4.9	15.5±4.0	23.8±3.3	12.5±3.9
	0.5 I.U daily	23.1±6.7	17.0±5.8	27.3±11.1	34.4±7.2	16.7±4.0
	0.5 I.U twice daily	16.5±3.0	21.7±2.9	14.0±5.1	20.0±5.5	28.2±9.0
	0.5 I.U thrice daily	25.9±3.9	15.9±4.5	16.5±3.6	28.5±4.1	21.3±4.5
Post-implantation loss %	Saline	0.0±0.0	3.8±2.3	2.5±2.5	0.0±0.0	0.0±0.0
	0.5 I.U daily	0.0±0.0	0.0±0.0	6.5±4.4	8.2±3.0	4.5±1.5
	0.5 I.U twice daily	0.0±0.0	4.2±4.2	0.0±0.0	8.2±3.1	42.4±24.0
	0.5 I.U thrice daily	0.0±0.0	4.3±2.8	7.1±3.6	0.0±0.0	1.8±1.8
Implantation index %	Saline	840	980	860	700	880
	0.5 I.U daily	820	700	680	840	840
	0.5 I.U twice daily	1016	800	1050	716	633
	0.5 I.U thrice daily	700	816	900	750	850

As compared to control (Mann-Whittney, U-test and G-test) *P<0.05

Table 2: Effect of oxitocin on sperm distribution in male reproductive tract

	Caput and corpus epididymis	Cauda epididymis	Vas deferens
Control	0.455 ±0.02	1.398 ±0.08	0.148 ±0.03
Oxytocin	0.418 0.05	1.181 ±0.07	0.109 ±0.02

(sperm counts 10^6/ mg of body weight) (mean ± SEM)

Table3: Effect of oxytocin on contractility of isolated cauda epididymal tubules.

Treatment	Frequency of contraction(min^{-1})	Amplititude of contraction (g)
Oxytocin		
0.002 IU/ml	1.58±0.30	0.035±0.003
0.004 IU/ml	2.23±0.30	0.052±0.007
0.006 IU/ml	2.63±0.37	0.061±0.008
0.008 IU/ml	3.03±0.60	0.075±0.014

(mean ±SEM) g = grams

Table 4: Effect of oxytocin on electrically induced contraction of isolated vas deferens

Treatment	% inhibition of initial twitch	% inhibition of secondary twitch
Oxytocin (IU/ml)		
0.01	22.0 ± 5.0	35.0 ± 6.2
0.02	38.0 ± 6.0	48.5 ± 6.0
0.03	35.0 ± 9.0	56.5 ± 6.5

(mean ± SEM)

Contractility of isolated cauda epididymal tubules

In the organ bath study with isolated cauda epididymal tubules, no spontaneous contractions were evident prior to OT addition. OT addition induced rhythmic contractions whose frequency (r^2=0.9: P<0.05) and amplitude (r^2=0.9, P<0.05) increased with the concentration of OT. (see Table 3).

Electrical stimulation of isolated vasa deferentia

Electrical nerve stimulation of isolated vasa deferentia evoked an initial twitch (amplitude: 1.9 ± 0.2 g) and a secondary sustained contraction (amplitude: 1.5 ± 0.2 g). As depicted in Table 4, OT significantly (P <0.05) inhibited both components of the contractile response in a dose dependent manner (initial twitch, r^2=0.9, P<0.001 and secondary sustained contraction, r^2 = 0.9, P<0.001).

Liver and kidney function tests

OT treatment did not significantly alter the SGOT (control vs treatment: 39.5 ± 2.2 vs 43.6 ± 4.5 Units /L), SGPT (control vs treatment:21.4 ± 1.0 vs 18.4 ± 2.1 Units / L), and serum creatinine (control vs treatment: 0.77 ± 0.1vs 0.9 ± 0.1mg/dl) or serum urea (control vs treatment: 33.8 ± 2.5 vs 37.2 ± 7.2 mg/dl) levels.

Haematology

OT treatment did not significantly change the red blood cell count (control vs. treatment: 6.5 ± 0.2x10^6 vs.5.6 ± 0.2 x10^6 cells/mm^3), white blood cell count (control vs. treatment:6.0 ± 8.8x10^6 vs.4.5 ± 9.0x10^6) and haemoglobin content (control vs. treatment:19.4 ± 1.0 vs.20.5 ± 0.5 g/dl).

Discussion

In a serial mating study intraperitoneal administration of OT reduced the sperm numbers in the ejaculates (measured as vaginal sperm counts) markedly. Importantly, all the rats showed ejaculation during the study. This OT-induced oligozoospermia was dose and time-dependent, and normozoospermia resumed soon after cessation of treatment. Furthermore, the OT treatment was well tolerated and did not show toxic effects (in terms of overt signs of toxicity, stress, hepatotoxicity, renotoxicity or haemotoxicity). Interestingly, OT is known to exerts potent antistress effects (7). Some workers have shown an increase in sperm numbers in ejaculates with OT (3,5), and others have not shown such improvement (11,15). These discrepancies may be due to species differences and to differences in doses, route and time of administration of OT relative to semen collection.

The production of oligozoospermic ejaculates with OT was extremely quick suggesting post testicular sites of action: as spermatogenesis takes approximately 52 days in rats (16), considerable time is needed to decrease sperm counts via effects on spermatogenesis. Mechanical blockage of reproductive excurrent ducts can provoke oligozoospermia (17), but this mode of action is unlikely as there was no sperm granulomas evident in OT-treated rats. Enhanced epididymal sperm resorption can elicit oligozoospermia (18), but is unlikely to be operative as it is a slow process. Frequent and excessive sexual intercourse can produce oligozoospermia (17), but seems unlikely in view of the fact that these rats were allowed only to mate on specific

predetermined days during OT treatment. OT-induced oligozoospermia was not due to stress (as judged by lack of any overt signs of stress). Spermatorrhoea can impair sperm numbers in the ejaculates but this mechanism is also unlikely as there was no sperm in the urine of OT-treated rats. This finding was similar to that of controls.

In the rats, OT induced spontaneous ejaculations having appreciable numbers of sperm (11). Although sperms counts were not done thick milky exudates were frequency evident at the tips of penises of OT-treated rats. These would obviously contribute for the production of oligozoospermic ejaculates.

As reported by others (3,5), OT in this study caused rhythmic contractions in isolated rat cauda epididymal tubules. Such rhythmic contraction can reduce the cauda epididymal sperm reserves as evident in this study. Sperm toxicants are also reported to impair epididymal sperm counts (16) and as such could play an auxiliary role in this regard.

However, profound toxic effects of OT on sperm seems unlikely as there were no decapitated or morphologically abnormal sperm in the ejaculates and because pre-implantation losses were not increased. Unaltered preimplantation loss suggests that functional competence of the ejaculated sperm remains undisturbed. Since the majority of sperm in the ejaculates of rats come from the cauda epididymis (14), the OT induced reduction of sperm numbers in cauda epididymis could contribute to the production of oligospermic ejaculates in this study.

It is known that retrograde ejaculation results in oligozoospermia and/or azoospermia (19). Sperm retrieval from the urinary bladder showed that OT treatment caused marked retrograde ejaculation and this is one of the major mechanisms through which oligozoospermia was achieved in this study. However, it remains to be seen whether this retrograde ejaculation is due to myogenic or neurogenic causes.

The other main mechanism which is responsible for the production of oligozoospermic ejaculates with OT is impairment of ejaculatory contraction of the vas deferens and cauda epididymis. The experimental evidence in support of this mechanism comes from the fact that OT impaired, dose-dependently, both phases of the biphasic contraction of the isolated rat vas deferens, produced by field stimulation: the ATP mediated initial rapid phase and the noradrenaline mediated secondary slow phase (20). This is a novel and an interesting finding. In spite of the marked oligozoospermia OT failed to suppress fertility (in terms of uterine implants, quantal pregnancy, fertility and implantation indices), of rats in this study. However, if the harem mating system was employed to evaluate effects on fertility (21), it is likely that fertility would have impaired as rats have epididymal sperm stores far in excess required for fertilization : rats are still fertile after more than 90% depletion of sperm counts (16). In contrast, human fertility is impaired by small reductions in sperm counts (16,17). Humans produce only 25% of the spermatozoa per day per gram of testicular tissue compared to other mammals (16), and several studies have revealed that human sperm count is dropping drastically(17). Thus, if OT can induce an oligozoospermia in humans in a magnitude comparable to that of rats, fertility in humans

would be seriously inhibited. Although qualitative measurements were not made the overall sexual competence of OT-treated rats was essentially similar, if not superior, to that of controls (as evident from pre coital sexual behaviour and index of libido). This is to be expected as OT is shown to facilitate pair bond formation (7), and promote sexual behaviour of male rats(10).

In conclusion, this study demonstrates, for the first time, that intraperitoneal administration of OT to male rats induces severe oligozoospermia without compromising fertility. In addition, this effect was reversible and was without toxic effects. If the results are applicable to humans it may be possible to develop a post-testicular male contraceptive based on OT analogues.

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