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Effect of vasopressin on fertility of male rats

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Abstract

Several drugs, which induce epididymal contractions, have been tested as potential male contraceptives. Vasopressin is one such agent, which has not been tested as a potential male contraceptive acting via epididymis. The aim of this study was to examine the effect of vasopressin on male reproductive competence using rats. Two doses of vasopressin (0.2 I.U. and 0.4 IU./ rat) was injected daily to the cauda epididymides of rats for 7 consecutive days. These rats were individually paired with a receptive female on days 1,3 and 7 of treatment and on day 7-post treatment. The results show that both doses of vasopressin caused the production of oligozoospermic ejaculates. In addition, the higher dose induced marked teratozoospermia (decapitation of sperm heads). However, precoital sexual behavior and fertility of treated rats remained uninhibited. At autopsy, sperm granulomas were evident close to vaso-epididymal junction. The most likely cause for the production of vasopressin-induced oligozoospermia was the presence of sperm granulomas. In addition, ejaculatory dysfunction may play a role in the production of oligozoospermia. The possible cause for teratozoospermia is sperm toxicity.

Key words : Vasopressin, ADH, male contraceptives, oligozoospermia, teratozoospermia, sexual behaviour, fertility

1. Introduction

Still there is no contraceptive pill for men although they are interested in contraception. Hence, there is a strong need for the development of effective, reliable safe and affordable male contraceptives. In the search for a male contraceptive it is generally believed that priority should be given to epididymal approach since such a designed would not interfere with libido, potency or sexual activity (1). In addition, epidiymal contraceptives will have a rapid onset of infertility with the commencement of treatment and fast return of fertility after their withdrawal (1).

Since sperm maturation occurs in the epididymis (2) speeding up of epididymal transit of spermatozoa by inducing epididymal contractions is one approach that is investigated in the development of epididymal contraceptives: for example, sympathomimetics (3,4,5), cholinomimetics (5,6,7). Vasopressin, a neuropeptide, which is found to be present in the male reproductive system (8) is now known to induce contractions in the epididymis of several mammalian species (9,10) and is implicated in sperm transport (10). Vasopressin is also reported to strongly potentiate contractions of the human vas deferens elicited by adrenergic and electrical stimulation (11). What is more, vasopressin appers to facilitate male sexual behaviour (12). Therefore, it is worth examining vasopressin or its analogues as potential male contraceptives acting via epididymis, and this study examines the effects of vasopressin on fertility of male rats.

2. Materials and Methods

Animals

Healthy adult cross-bred albino rats (males weighting 250-300 g and females weighting 200-225 g) of proven fertility from our own colony were used. They were housed under standerdised animal house conditions with free access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and tap water: Due to ethical reasons minimal animal numbers were used.

Intra epididymal administration of drugs

This was done as described previously by Ratnasooriya (13). Briefly, 18 male rats were randomly divided into three equal groups (N=6/group). Their scrotal sacs were cleaned with rectified sprit (95% ethanol). The left cauda epididymis was palpated and held between the left thumb and left index finger of the investigator and 0.1 IU of drug (N=9) or 0.2 IU of drug (N=9) or 0.01 ml of saline (N=9) was slowly injected directly into the cauda using 26 G gauge needle and a plastic syringe through the scrotal sac without using any form of anaesthesia between 15.30-16.00 h. The contralateral cauda epididymis of each animal was then immediately similary injected with the respective dose of drug or saline.

Effects on fertility

Libido, ejaculatory ability and fertility of these rats were assessed 7 days prior to treatment and on day 1,3 and 7 of treatment, and on day 7 post treatment. Each male was paired overnight with a pro-oestrus females (at 16.30-17.00 h). The pre-coital sexual behavior (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 females were taken in the following morning (08.00-08.30 h).

The presence of spermatozoa was considered day 1 of pregnancy. If spermatozoa were present, their numbers were estimated in duplicate using and improvd Neubauer haemocytometer (Fison scientific Ltd., London, UK) and the gross morphology was observed microscopically, (x 100 and x 400). The head defects noted were decapitation, macroceopicaly, microcephaly, pyriform head, bicephaly or rounded heads. Mid piece defects noted were bent necks, thin mid piece, kinked mid piece and irregular mid piece. The tail defects scanned 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 death) were counted to permit analysis of fertility. In addition, the colour, the 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; fertlity 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 implantation] x 100; implantation index = (total number of implants/ number mated) x 100.

Observation on overt signs of toxicity

The rats used in the fertility study were observed 1-2 h following each intraepididymal injection for signs of toxicity (salivation, rhinorrhoea, lacrimation, ptosis, squanted eyes, excessive gnawing and biting movements of jaw, wilting, convulsion, 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 behaviors (such as cleaning of face, self grooming, climbing in the cage, rearings). In addition, aversive behaviours (biting and scratching behaviour, liking at tail, paw and penis, vocalisation) intense grooming behavour were also observed. Food and water intake, consistency of faeces and colour of urine were also noted.

Autopsy

On day 8 post treatment, all the treated and control rats were killed with an overdose of ether. Their abdomianl cavities were opened and the reproductive tracts were grossly examined for presence of sperm granulomas. If sperm granulomas were present their position and the numbers were noted.

3. Results

In the fertility study, all vasopressin treated rats exhibited pre-coital sexual behaviours, which were essentially similar to that of controls. With the lower dose significant ($P<0.05$) impairment in vaginal sperm counts were evident on days 1 (by 78%) and 7 (by 66%) of treatment, and day 7 post treatment (by 79%). On the other hand, with the high dose significant ($P<0.05$) reduction in vaginal sperm counts were seen on days 3 (by 25%) and 7 (by 67%) of treatment, and day 7 post-treatment (by 100%). In addition, significant ($P<0.05$) decapitation of ejaculated sperm were evident with the high dose: days 1,3 and 7 of treatment were respectively , by 36% , 23% and 29%. Further, with the lower dose, all the rats ejaculated at each time point. In contrast, with the higher dose all the treated rats had normospermic ejaculates only on day 1 of treatment whilst on days 3 and 7 of treatment only 3 out of 6 had ejaculated with sperm number exceeding $2.5x10^6$/ml (lowest limit of detection in Neubauer heamocytometer being $2.5x10^6$/ml spermatozoa). On the other hand, on day 7 post treatment all the 6 rats had ejaculated sperm numbers $<2.5x10^6$/ml. In spite the reduction of vaginal sperm numbers none of the fertility parameters investigated were significantly reduced ($P>0.05$) in rats treated with vasopressin. Vasopressin treatment did not induce overt sings of toxicity nor stress nor aversive behaviours.

At autopsy, 5 out of 6 rats treated with lower dose of vasopressin had sperm granulomas close to vasoepididymal junction: 4 of 6 on one side and 2 of 6 on both sides. All the 6 rats treated with the higher dose of vasopressin had sperm granulomas: 3 of 6 on a single side and 3 of 6 on both sides.

4. Discussion

The results show that vasopressin can produce oligozoospermic ejaculates without inhibiting precoital sexual behaviour and provoking undesirable side effects. The onset of this effect was very rapid (within 1 day) suggesting in extratesticular site of action. Spermatogenesis in rats takes approximately 52 days (14). Therefore, considerable time (several days) is required to produce oligozoospermic ejaculates via impairment of spermtogenesis. Mechanical blockage of the epididymis and/vas by sperm granulomas can provoke oligozoospermia (15). This is the most likely mode of action in the present study as both unilateral and bilateral sperm granulomas were evident close to epididdymal vas junction. As the control animals had no sperm granulomas its production is unlikely to be due to the experimental procedure. Further, β-methylene ATP has shown to induce no spermgrnulomas when injected to epididymis in an identical manner (13).

Since production of sperm grnulomas takes time (16) the initial vasopressin induced oligozoospermia cannot be due to grnulomas. Production of oligozoospermic

ejaculates in this study could also be due to ejaculatory dysfunction (17). Several drugs are known to induce ejaculatory dysfunction as a side effect (17). But such an effect is not previously reported with vasopressin or it's analogues (18). However, claims are made that vasopressin could alter affinity characteristics of adrenergic receptors of the vas deferens.(11).

Vasopressin did not provoke overt signs of stress and as such oligozoospermia is unlikely to be due to stress (15). Frequent and excessive matings produce oligozoospermia (15) but such a mode of action is unlikely as the rats were allowed to mate only on specific days. High dose of vasopressin also induced teratozoospermia (decapitated sperm). Decapitation of sprm is usually due to toxicity (14) or prolonged absence of ejaculation (15). Since the rats were allowed to mate within couple of days sperm toxicity is likely to be cause of sperm decapitation.

In spite of oligozoospermia and sperm decapitation (only with the high dose) the fertility of the rats was not inhibited by vasopressin. This is because rats have epididymal sperm stores far in excess that is required for fertilization (14): rats are still fertile after more than 90% depletion of their sprm counts (14). However, if vasoperssin can induce an oligozoospermia in humans in a magnitude that is comparable to that of rats, fertility in humans will be seriously hampered: human fertility is inhibited by small reduction in sperm counts (14) and humans produce only 25% of the spermatozoa per day per gram of testicular tissue compared to mammals (14).

In conclusion, this study demonstrates, for the first time, that vasopressin can provoke oligozoosprmia in rats without compromising sexual behaviour and fertility. It is worthwhile examining orally active vasopressin analogues as potential male contraceptives.

5. References

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Table 1 Effect of intraepididymal administration of vasopressin on some fertility parameters on male rats (mean ± SEM)

Parameter	Dose (IU)	Pre treatment	Treatment Day 1	Day 3	Day7	Post treatment Day7
Vaginal sperm	Control	31.4±6.2	34.6±11.3	29.2±7.5	29.4±4.0	
27.3±3.6						
Counts (10^6/ml)	0.1	27.3±4.0	5.9±1.7**	32.6±8.7	9.4±3.1**	5.6±1.9**
	0.2	25.9±3.7	37.0±2.7	19.5±10.0	8.5±4.6**	0.0±0.0**
Index of libido%	Control	100	100	100	100	100
	0.1	100	100	100	100	100
	0.2	100	100	100	100	100
Quantal pregnancy%	Control	100	100	100	100	100
	0.1	100	100	100	100	100
	0.2	100	100	83.3	100	100
Fertility index%	Control	100	100	100	100	100
	0.1	100	100	100	100	100
	0.2	100	100	100	100	100
Number of implants	Control	8.4	10.2	8.8	7.0	8.8
	0.1	9.1	9.1	9.8	9.1	11.0
	0.2	9.1	11.3	6.3	8.2	8.0
Implantation index%	Control	840	1020	880	700	880
	0.1	916	916	983	916	1100
	0.2	916	1133	633	800	800
Pre implantation	Control	26.5±1.8	16.0±1.7	15.5±2.0	25.1±1.6	13.8±2.2
loss %	0.1	20.1±2.6	26.0±2.0	12.2±2.0	11.6±1.4	10.3±2.0
	0.2	16.7±1.4	13.1±1.4	32.3±6.8	18.6±2.0	13.7±2.0
Post implantation	Control	0.0±0.0	3.5±1.0	2.2±1.0	0.0±0.0	0.0±0.0
loss %	0.1	0.0±0.0	0.0±0.0	1.3±0.5	0.4±0.1	2.5±0.6
	0.2	0.0±0.0	1.1±0.5	3.3±1.4	1.6±0.7	0.0±0.0

As compared to control (Mann - Whitney, U-test and G-test