Effect of local application of sympathomimetic drugs to the epididymis on fertility in rats*

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Summary. Insertion of Silastic rods containing the directly acting sympathomimetic drug, methoxamine, adjacent to the epididymis of rats caused a temporary reduction in fertility with no loss of ability to mate. This effect lasted up to 3 weeks. At the time of the maximal antifertility action (3–7 days after insertion), the number of spermatozoa in the ejaculate fell to almost zero, and there was a reduction in the total number of spermatozoa in the epididymis resulting from a significant drop in the number present in the cauda. Methoxamine also caused immotility and decapitation of the remaining epididymal spermatozoa. The indirectly acting sympathomimetics, tyramine and norephedrine, did not affect fertility. It is postulated that methoxamine acts to induce infertility principally by bringing about a reduction of sperm numbers in the ejaculate. This could have been produced either by a failure of the vas and cauda to contract normally at copulation or because the sperm store in the cauda had fallen below a critical threshold level.

Introduction

Testicular spermatozoa are infertile (Amann & Griel, 1974; Cooper & Orgebin-Crist, 1975) and the cumulative weight of evidence indicates that the epididymis is the region within which the spermatozoa acquire their fertilizing potential (Orgebin-Crist, 1967; Cummins, 1976; Cooper & Orgebin-Crist, 1977). The possibility of interrupting epididymal sperm maturation suggests an attractive method of male contraception. Since spermatogenesis would be unaltered, such a technique would be prompt in action and equally rapidly reversible. Furthermore, it is unlikely that libido would be affected. One possible way to achieve such a goal would be to decrease sperm transit time by using sympathomimetic drugs to induce rhythmic contractions in the epididymis (Hib, 1976; Ratnasooriya, 1978).

This paper reports the effect of fertility of male rats when sympathomimetic drugs were applied locally to the epididymis.

Materials and Methods

Adult Sprague–Dawley or Wistar rats (males weighing 300–350 g and females weighing 200–250 g) were used. The animals were housed under constant environmental conditions with free access to food (Diet 41B: Millers) and water.

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Fertility tests

Rods containing an appropriate weight of the powdered sympathomimetic drugs together with a known volume of polysiloxane polymer (Silastic 382 Medical Grade Elastomer: Dow Corning Corp., Midland, Michigan, U.S.A.) were prepared exactly as described for collars (Ratnasooriya, Gilmore & Wadsworth, 1979). The rods were cut into lengths of 15–20 mm with a diameter of 3.5 mm. Each weighed approximately 200 mg. Control rods consisting entirely of Silastic were also constructed.

One or two rods were inserted through the tunica vaginalis adjacent to each epididymis of 21 rats lightly anaesthetized with ether. The incision in the tunica vaginalis was sutured and that in the scrotal sac closed with 12 mm Michel wound clips. The drugs used were methoxamine hydrochloride, tyramine hydrochloride (Wellcome) and norephedrine hydrochloride (Sigma) and the numbers of animals treated in each group are listed in Table 1. The fertility of the treated and control males was tested from Day 3 after the operation by separately pairing each male overnight with a pro-oestrous female.

Sperm distribution in the male tract and ejaculate sperm count

To investigate the effects of methoxamine hydrochloride on sperm distribution a rod containing 50% of the drug by weight was placed adjacent to the right epididymis in 6 rats. Within the left tunica vaginalis a control rod was inserted. On Day 3 after the operation the animals were anaesthetized with ether and the excurrent duct from each side was removed and divided into caput, corpus and cauda epididymidis and vas deferens. Each region was homogenized and the number of spermatozoa in it was counted using an improved Neubauer haemocytometer.

Three male rats were each separately paired overnight with one mature pro-oestrous female. Between 09:00 and 10:00 h the following morning, the females were anaesthetized with ether and the uteri and vaginae were removed. The total number of spermatozoa in the female reproductive tract was counted using an improved Neubauer haemocytometer. Counts were made of repeated ejaculations of each individual male, with not less than 3 days rest between consecutive matings. At least two control counts were made for each rat, before a rod containing 50% methoxamine hydrochloride was inserted adjacent to each epididymis as described above. Further counts were carried out on Days 3 and 7 after the operation.

Histology and motility of the epididymal spermatozoa

Following the last mating the 3 male rats used to estimate sperm counts in the female tract were killed and parts of the testis and excurrent duct (cauda, corpus, caput epididymidis and the vas deferens) were removed. The tissue was fixed in Bouin's fluid for subsequent histological examination. Sections were cut at 7 μ m and stained with haematoxylin and eosin before microscopic examination at $\times 100$ and $\times 400$ magnification.

Spermatozoa were extruded into saline (9 g NaCl/l) from a portion of the cauda epididymidis immediately before its fixation. The motility of the spermatozoa was scored on a subjective scale of 0–5, 0 being absence of motility and 5 the greatest observed motility.

Sperm counts in the urinary bladder

A single rod containing 50% methoxamine hydrochloride was placed adjacent to each epididymis in 4 rats. On Day 4 after operation, the animals were paired individually with a pro-oestrous female at 17:00–18:00 h. The paired animals were observed until mating occurred. Immediately after copulation the males were killed by an overdose of ether. The urinary bladder was exposed and the entire contents were withdrawn using a 1 ml syringe. A drop of the urine

was transferred to a microscope slide and examined for any spermatozoa. A vaginal smear was also taken from the female to check for the presence of spermatozoa.

Release rate from 50% methoxamine hydrochloride rods

This was calculated by subtracting the final from the initial weight of the rods, after they had been dried at 60–70°C until a constant weight had been reached (about 3 days). Release of the methoxamine was such that 3 days after insertion the rods contained 60.4–82.8% ($N = 3$) of their original content. After 8 days only 1.2–5.0% ($N = 4$) of the methoxamine remained.

Statistics

The significance of differences between the treated and the control groups was assessed using Mann–Whitney or Wilcoxon rank-sum non-parametric tests (Seigel, 1956) taking $P < 0.05$ as significant.

Results

Fertility tests

As shown in Table 1 all 3 control males were fertile and the females with which they were mated had normal numbers of embryos.

Of the 7 rats into which one 50% methoxamine rod was inserted next to each epididymis, 1 died 2 days after the operation and is excluded from Table 1. None of the others was fertile at the first mating, but all 6 subsequently showed a progressive return to fertility. However, up to Day 28, there was a significant reduction in the average number of embryos recorded, compared to those produced by the control group (results combined for Days, 3, 7, 14 and 21: $P < 0.05$). No abnormalities were detected amongst the litters born from the last two matings.

With the exception of a single mating on Day 3, none of the 3 males into which a single rod containing 25% methoxamine was inserted was appreciably affected by the treatment. However, the average number of embryos resulting from the matings was less than in the controls. The insertion of rods containing tyramine or norephedrine next to each epididymis had no significant effect on fertility and no gross abnormalities were encountered in the litters derived from tyramine-treated males.

Histological observations and motility of epididymal spermatozoa

In slides made of tissues taken from the 3 rats killed 8 days after insertion of one 50% methoxamine rod adjacent to each epididymis, no interference with the spermatogenic process in the testes was apparent. All the stages from spermatogonia to spermatozoa were observed.

The epithelium of the epididymides was normal except in one male in which that of the corpus epididymidis showed disintegration. This was presumably due to some local action of the rod since the cauda and the caput were normal. Numerous spermatozoa were seen in the epididymal lumen and there was an accumulation of cell debris in the proximal caudae. No change was seen in the muscle layers of the vasa deferentia and either no or only very minor damage was caused to the vas epithelium of the treated animals.

The motility score of the cauda spermatozoa, extracted into saline, from these 3 animals was 0 in contrast to control animals which had a sperm motility score of 5. In addition, large numbers of spermatozoa in the treated animals were decapitate.

Table 1. The effect (shown by implantation number) of local application of sympathomimetic drugs to the epididymis on the fertility of male rats tested individually with a different female at various times after insertion of the rods

Animal no.	Number of implantations												
	Days after operation												
	3	4	7	11	14	21	23	28	31	34	44	51	65
<i>Control rods</i>													
56		14	15	14			14		10			14	
57		10	13	14			18		14			17	
90		14	11	8			16		10			12	
<i>50% methoxamine</i>													
1	0		4		9	11		7		10*			12*
2	0		16		10	11		8		16*			14*
3	0		4		0	5		7		12*			10*
4	0		0		0	0		11		14*			14*
5	0		0		6	6		12		10*			—
6	0		0		0	4		10		11*			—
<i>25% methoxamine</i>													
7	0		12		9	12							
8	10		15		5	11							
9	12		7		6	10							
<i>50% tyramine</i>													
58		13	14	0			11		12				17
59		0	11	0			15		11				16
<i>2 × 50% tyramine</i>													
70	15		10		16	0					9*		
74	16		14		15	7					3*		
75	11		14		15	16					15*		
<i>2 × 50% norephedrine</i>													
66	14		12		0	12							
67	8		10		6	3							
68	14		16		8	0†							

* Females allowed to litter.

† Adhesions in ovary.

Sperm distribution in the male tract and ejaculate sperm count

In the 6 rats investigated 3 days after the insertion of a control rod on one side and a 50% methoxamine-containing rod on the contralateral side the most consistent effects seen were (1) a reduction in sperm numbers in the cauda and the whole epididymis, and (2) the decapitation of a varied proportion of the spermatozoa in the cauda epididymidis and vas deferens (Table 2). A granuloma was observed in the vas deferens of the treated side in one rat. Before removal of the tracts for assessment of sperm content, it was observed that the vas deferens in all 6 animals was contracting rhythmically, but on the treated side only. The ejaculated sperm numbers in the 3 rats investigated ranged from 1×10^6 to 105×10^6 on 2 occasions before the insertion of 50% methoxamine rods. By 3–7 days after treatment the sperm count of the ejaculate was reduced to very low numbers ($<5 \times 10^4$) or to zero, and the spermatozoa were mostly decapitate.

Sperm counts in the urinary bladder

No spermatozoa were seen in the urine in any of the 4 treated males. However, a few spermatozoa were seen in the vaginal smears from 2 of the mated females. Copulatory plugs devoid of spermatozoa were obtained from the other two females.

Table 2. Effect of methoxamine on the sperm distribution in the genital tract of male rats

	Total sperm no. ($\times 10^6$)			
	Control side (left)	Side with a 50% methoxamine rod (right)	% decrease	% spermatozoa decapitated (no. of rats)
Caput epididymidis	132 $\pm$ 6	88 $\pm$ 19	33 $\pm$ 15	25 (1)
Corpus epididymidis	21 $\pm$ 4	19 $\pm$ 4	4 $\pm$ 23	—
Cauda epididymidis	175 $\pm$ 9	93 $\pm$ 13*	46 $\pm$ 9*	95 (6)
Whole epididymis	328 $\pm$ 9	200 $\pm$ 18*	39 $\pm$ 5*	—
Vas deferens	35 $\pm$ 20	48 $\pm$ 22	53 $\pm$ 22	92 (6)

* Significantly different from control at $P < 0.05$ (Wilcoxon test).

Discussion

The main result of this study showed that local administration to the epididymis of a directly acting sympathomimetic drug, methoxamine, caused a dramatic reduction in fertility in male rats, but not in their ability to mate. The gradual return to fertility over a 4-week period was presumably due to exhaustion of the drug which had nearly all been released after 8 days. The single death observed probably resulted from the marked cardiovascular stimulation caused by methoxamine. The toxic effects of this drug were most pronounced within the first 2–3 days after administration whereas the antifertility effect lasted at least 3 weeks. Although it is not possible to rule out an effect of methoxamine on spermiogenesis, such an action could not account for the extremely rapid antifertility effect that was observed.

The indirectly acting sympathomimetics, tyramine (Ambache, Dunk, Verney & Zar, 1972; Wadsworth, 1973) and norephedrine (Ratnasooriya & Wadsworth, 1979), whose effect is brought about by the release of endogenous noradrenaline from adrenergic neurones, failed to produce any antifertility affect when administered in a similar manner. This would imply that methoxamine induces sterility by its effect upon the non-innervated section of the tract, i.e. caput and proximal corpus epididymidis, rather than upon the cauda and vas deferens which receive a dense adrenergic innervation (El-Badawi & Schenk, 1962; Norberg, Risley & Ungerstedt, 1967) and would also be susceptible to the drugs norephedrine and tyramine.

Although the total number of spermatozoa recovered from the female genital tract always showed considerable individual variation (in agreement with the results of Matthew & Alder, 1977), the number of ejaculated spermatozoa fell dramatically by 3–7 days after the application of methoxamine-containing rods. While this must be the main cause of the observed infertility, the loss of motility and extensive decapitation which also occurred would by themselves be sufficient to produce sterility.

Histological observations demonstrate that the severely oligospermic ejaculates in methoxamine-treated animals cannot be attributed either to obstruction of the epididymis or vas or to spermatogenic arrest. We can also rule out retrograde ejaculation into the bladder since no spermatozoa were detected in the urine after copulation. This leaves two possible explanations for the results obtained. (1) The residual number of spermatozoa in the cauda epididymidis may have been too low for any to enter the ejaculate. The sperm distribution studies showed that the sperm store in the cauda was reduced by about half, but there were still considerable numbers present (about 90×10^6). In the hamster 40–45% of the spermatozoa in the cauda epididymidis remain after repeated ejaculations (Chang & Sheaffer, 1957), and there may be a similar substantial 'reserve pool' in rats which remains unavailable. (2) The smooth muscle of the cauda epididymidis and vas deferens may have failed to contract at copulation. In theory it seems possible that this could occur as a result of stimulation of presynaptic α -receptors (Farnebo & Malmfors, 1971) or because of desensitization (Wadsworth, 1974). We have no evidence that could allow a choice to be made between possibilities 1 and 2.

The reduction in sperm numbers in the cauda epididymidis was not due to redistribution of spermatozoa within the excurrent ducts, since the total number in the whole epididymis was also reduced. It must therefore have been due to enhanced expulsion into the vas deferens or to a reduction in the rate of release from the seminiferous tubules. Since there was no histological evidence of reduced sperm output from the testis, it is likely that the output of spermatozoa into the vas was increased.

Sympathomimetic drugs have been shown to enhance rhythmic contractions in the cauda epididymidis (Da Silva, Gimeno & Gimeno, 1975; Hib, 1976) and probably also cause contractions in the caput and corpus since these also contain smooth muscle. If such contractions were being induced by methoxamine rods, then this might cause expulsion of sufficient spermatozoa to account for our results. Consistent with this explanation is the observation that tyramine and norephedrine did not affect fertility, since these indirectly acting sympathomimetics would not be expected to cause contraction in the corpus and caput. If increased numbers of spermatozoa were being transferred into the vas, they must subsequently have been lost from there because no accumulation within the vas was found. Such a loss could be accounted for by accelerated transport of spermatozoa through the epididymis and vas, due to rhythmic contractions throughout, eventually resulting in either spontaneous ejaculation (Orbach, 1961; Kihlström, 1966; Ågmo, 1976) or spermatorrhoea (Fernández-Collazo, Videla & Pereyra, 1971; Vreeburg, Van Andel, Kort & Westbrook, 1974). Indeed, rhythmic contractions of the vas deferens were observed in our experiments 3 days after the insertion of 50% methoxamine rods.

The decapitation and loss of motility of the caudal spermatozoa could have resulted from: (1) a direct toxic effect on the spermatozoa themselves; (2) the environment becoming hostile, perhaps by an alteration in the blood supply because methoxamine is a potent vasoconstrictor (Hoffbrand & Forsyth, 1973); or (3) enhanced transport, leaving insufficient time for the spermatozoa to become fully mature. Of these three possibilities (2) seems the most likely since decapitation was always first observed in the cauda epididymidis.

Our intention was to apply drugs only to the epididymis, but diffusion of drug to the testis, vas and their blood and nerve supplies may have occurred and we have therefore considered all of these tissues as possible sites of action. The effects observed were similar to those produced by sympathomimetic drugs applied directly to the vas deferens in the form of Silastic collars (Ratnasooriya *et al.*, 1979). However, while the possibility exists that the collars might themselves have obstructed the passage of spermatozoa along the vas, there was no evidence that the rods had this effect. Furthermore, as decapitated spermatozoa were produced only by animals which had received rods this method of application appears to supply the greatest concentration of drug to the epididymis.

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