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EFFECTS OF ATROPINE ON FERTILITY OF MALE RATS

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Abstract

Insertion of silastic rods containing 25% and 50% atropine adjacent to the epididymis of rats produced a significant suppression in fertility which was temporary with the lower dose and permanent with the higher dose. Both doses also impaired the motility of epididymal spermatozoa and nerve-mediated contractile response of isolated vasa deferentia. In addition, the higher dose caused a significant reduction in the mating frequency. It is concluded that the antifertility effect resulted primarily from oligospermia due to dysfunctioning of seminal emission and/or ejaculation.

Introduction

Atropine is a therapeutically used potent cholinoceptor antagonist drug[3,4]. In addition to its specific antagonistic action atropine also has a non-specific effect of stabilizing neuronal membranes — local anesthetic effect[4]. In the field of male reproductive biology, atropine has been shown to : immobilize human ejaculated spermatozoa[9] and rat epididymal spermatozoa[11] *in vitro;* arrest secretory function in the bulbo — urethral and urethral glands and to reduce ejaculated semen volume in the boar.[5] In addition, atropine might impair erectile function of the penis, since normal penile tumescence is mediated primarily through stimulation of the parasympathetic nervous system[6]. These observations raise the possibility that atropine might also reduce fertility of male. The present study was undertaken to test this possibility in rats using a chronic local method of administration of the drug to the epididymis, as this method provides an easy access of the drug to mature spermatozoa.

Materials and Methods

Drug.—Atropine Sulphate (Sigma Chemical Co.) was used.

Animals.—Sexually mature adult male (225-250g) and female (175-200g) Sprague — Dawly rats were used. They were housed in wire meshed cages at a temperature of 28-30°C with a natural photoperiod (about 12h light/24h). All rats had unrestricted access to food (rat pellets, Moosajees Ltd. and green leaves) and water.

Construction of sustained-release drug delivery system.—Silastic rods (3.5 mm diam. and 10-12 mm length) containing 25% and 50% atropine sulphate were constructed by thoroughly mixing known weights of atropine

sulphate and polysiloxane polymer (Silastic 382 Medical Grade Elastomer, Dow Corning Ltd., Midland, U.S.A.) in a pestle and a mortar. The catalyst (Stennous octate) was then added and the resulting viscous homogeneous mixture was forced into polythene tubing and allowed to polymerize. Control rods consisting entirely of silastic were also made. This method of construction of rods is described in detail elsewhere.[13]

Insertion of rods adjacent to epididymis.—A single rod was inserted adjacent to each epididymis under mild ether ansesthesia using aseptic precautions as described by Ratnasooriya *et al*[13]. The day of surgery was designated as day O. The number of animals used in each treatment group were; 25% atropine rods 6, 50% atropine rods 11, and control rods 14.

Assessment of fertility.—Libido, ejaculatory competence and fertility of the operated rats were tested on days 3 and 7 and then approximately at weekly intervals by pairing each male overnight with a pro-oestrous female which had at least three consecutive 4 — day vaginal cycles. The sexual behaviour pattern of these paired rats was noted 2-3h. later. The presence of spermatozoa in the vaginal smears of paired females on the following morning (7-8h) was used as evidence of successful mating. In the absence of spermatozoa, daily vaginal smearing was undertaken to determine the occurance of pregnancy or pseudopregnancy. At 9-12 days *post-coitum*, the females were laparotomized and the number of foetuses present was recorded. For every group of treated males, a fertility index was computed at each time interval following insertion of rods. The fertility index = total number of foetuses ÷ number of successful matings at that time interval.

Vaginal sperm counts.—A 50% atropine rod (6 rats), 25% atropine rod (6 rats) or drug free rod (6 rats) was placed adjacent to each epididymis. Each of these rats was paired with a pro-oestrous female on day 3 and 7 respectively. On the following morning (7-8h), the vaginas of the paired females were flushed five times with 5ml saline (9g NaCl/1000ml distilled water) and the number of sperm present was counted using a haemocytometer.

Motility of epididymal sperm.—Six rats were fitted either with a 50% atropine rod or a 25% atropine rod adjacent to one epididymis and a drug-free rod adjacent to the contralateral epididymis. On day 7, the motility of the spermatozoa from the left and right epididymises was scored using a subjective scale from 0 (immotile) to 5 (greatest motility ever observed) as described elsewhere.[7]

Histology of testes.—Six rats were fitted with 50% atropine rods adjacent to each epididymis and another 3 with drug-free rods. On day 7, these rats were anaesthatized with ether, parts of their testes were removed, fixed in Bouins fluid for 48 h., embedded in paraffin wax, sectioned at 7 m, stained with Harris haemotoxylin and eosin and examined microscopically at x 100 and x 400 magnifications.

Accessory organ weights.—Seminal vesicles together with coagulatory glands, epididymises, vasa deferentia and testes were isolated and weighed from 6 rats fitted with a 50% atropine rod or a drug free rod adjacent to each epididymis on day 7 as described elsewhere[14].

Nerve-induced mechanical response of isolated vasa deferentia.—In 8 rats vasa deferentia were quickly removed under ether anaesthesia and carefully cleaned from loose connective tissue and fat. The vasa were mounted at 1.0g resting tension in a 50ml organ bath maintained at 37 ± 1°C in a Physiological salt solution of the following composition (mM/l) : Na^+, 141; K^+, 5.9; Ca^{++}, 2.6; Mg^{++}, 1.2; Cl^-, 104.8; $H_2PO^-_4$, 2.2; HCO^-_3, 24.9; SO_4^{--}, 1.2; and glucose 11; and was gassed continuously with 95% O_2; 5% CO_2. Contractions were induced by transmural stimulation through platinum ring electrodes using a SRI stimulator for 5 sec. at a frequency of 5 Hz with impulses of 0.5 m sec. duration and 90 V and recorded isometrically on a Bioscience pen recorder.

These stimulatory parameters elicit an initial rapid contraction and a slower more sustained secondary response in vasa deferentia[2] and presumed to be at least partly adrenergic[2]. Atropine sulphate was then added cumulatively increasing the concentration every 15 min and the response to electrical stimulation was recorded at each dose studied (10, 20, 50 and 100 μg/ml). In another set of vasa deferentia, responses to electrical stimulation were recorded in the presence of a single 25% (n = 6) or 50% (n = 8) atropine rod placed within the organ bath. Contractile response in the presence of drug or drug containing rods were expressed as a percentage reduction of their respective pre-drug controls.

Autopsy.—At the completion of the experiment rats with 50% atropine rods were killed and their reproductive systems were examined grossly for any abnormalities.

Release rate from 25% and 50% atropine rods.—A single weighed 50% atropine rod was placed adjacent to each epididymis of 6 rats. On day 8, these rods were removed, blotted free of body fluids and dried at 60°C until a constant weight was recorded.

Results

During the study, the general appearance and the water and food intake of the treated rats appeared normal. However, 9 out of 11 rats fitted with 50% atropine rods developed inflammation and necrosis of the tunica vaginalis and scrotal sacs around the operation site. This inflammatory response was not evident with all the rats with drug-free rods and 5 out of 6 rats with 25% atropine rods. These lesions were treated with Polybactrin R antibiotic spray.

Assessment of fertility.—The results of the fertility experiments are summarized in Tables I and II. The fertility of rats fitted with drug-free rods was normal (6-10 foetuses). In contrast, every drug treated male became subfertile (1-4 foetuses) on several occasions and completely sterile at least on one occasion. This effect on fertility was significant (Student's t-test, $P<0.05$ taken as significant) during the first two matings with 25% atropine rods and at all matings with 50% atropine rods (Table I).

Mating performance, as judged by percentage of successful matings was normal with control and 25% atropine rods. However, this ability was significantly reduced (Fisher Exact test, $P<0.05$ taken as significant) with 50% atropine rods (Table II). In contrast to the mating performance, precopulatory courtship behaviour did not appear to be altered by atropine (25% or 50% rods). Treated animals showed normal sniffing and grooming behaviours of female genital areas, palpation of females sides with forepaws and actual attempts of mounting and intromission.

Vaginal sperm counts.—The vaginal sperm count of females paired with males fitted with drug-free rods was 5.06 ± 0.73 million (Mean $\pm$ S.E. m. $n = 6$). In contrast, the vaginal sperm content of females paired with atropine treated males (25% or 50% rods) was 0.1 million or less than 0.1 million ($n = 6$, for each group) as the minimum numbers that could be counted is 0.1 million.

Motility of epididymal sperm.—The motilities of epididymal spermatozoa on the control side in the two series were 4.75 ± 0.25 and 4.60 ± 0.32 respectively and those on the treated sides were 1.25 ± 0.47 ($n = 6$) with 50% atropine rods and 2.3 ± 0.32 ($n = 6$) with 25% atropine rods. These differences were statistically significant (Student's t-test). Furthermore, neither drug treatment caused any obvious morphological abnormalities in epididymal spermatozoa as judged by light microscopy.

Histology of testes.—The histology of the testes of 6 rats fitted with 50% atropine rods appeared similar to that of 3 rats with drug-free rods, with no obvious interference in the spermatogenic process. In addition, germinal epithelium showed no signs of desquamation.

Accessory organ weights.—The average wet weights of testes, epididymissa, vasa deferentia and seminal vesicular-coagulatory gland complex of rats fitted with drug-free rods were 943 ± 135g, 446 ± 67g, 83 ± 07g and 560 ± 66g respectively. In rats with 50% atropine rods the corresponding values were 1248 ± 46g, 533 ± 65g, 88 ± 09g and 440 ± 51g. There was no significant alteration in the wet weights of any of these organs (Student's t-test).

Nerve-induced mechanical response of isolated vasa deferentia.—The nerve stimulation of vasa deferentia evoked a biphasic contraction consisting of an initial rapid phase (amplitude, 3.54 ± 0.3g, $n=8$) and secondary sustained contraction (amplitude 1.63 ± 0.2g, $n=8$) as described by other workers[9,0] As

Table—I Summarized data showing the effect of atropine on fertility. Fertility has been sxpressed in terms of fertility index + s.e.m. Values within parenthesis = no. of successful matings : no. of pairings.

Treatment group		Fertility Index						
		Time after insertion of rods (in days)						
Control rods	...	8.6 ± .26 (14:14)	8.8 ± .29 (14:14)	8.7 ± .30 (13:14)	7.8 ± .55 (10:11)	8.6 ± .33 (6:6)	7.7 ± .45 (11:11)	8.0 ± .53 (7:8)
25% atropine rods		1.6 ± .68**** (5:6)	3.3 ± 1.8*** (4:6)	5.0 ± 2.4 (6:6)	6.6 ± .49 (6:6)	6.5 ± .67 (6:6)		
50% atropine rods	...	2.8 ± .99**** (7:11)	1.75 ± .60**** (6:11)	1.3 ± 1.3**** (6:11)	3.1 ± 1.2* (6:10)	3.2 ± .93**** (9:11)	2.2 ± .97*** (11:11)	3.1 ± 1.2** (11:11)

**** $p < .001$ ** $p < .02$

*** $p < .002$ * $p < .05$

Table II

Effect of mating performance in male rats of atropine—containing silastic rods

Treatment	*Total number of Pairings*	*Number of successful matings*	*Percentage of successful matings*
Control rods ...	78	75	96.1
25% atropine rods	30	27	90.0
50% atropine rods	76	52	73.6

Table III

Effect of atropine on nerve mediated contractile response of isolated vasa deferentia. Responses are expressed as a percentage reduction of pre-drug control Mean ± S.E. (n = 6 — 8)

Treatment	*Response (% reduction)*	
	Initial rapid contraction	*Secondary sustained contraction*
Atropine (g/ml)		
10	12.2±2.8	21.8± 3.9
20	32.2±4.5	46.6± 6.4
50	55.9±3.5	65.5± 5.0
100	79.9±3.6	80.9± 3.4
Atropine rods		
25%	39.8±6.5	43.2± 6.8
50%	47.8±9.8	53.3±10.5

shown in Table III both components of the contractile response were inhibited considerably by atropine and by silastic rods containing atropine. Repeated washing (4-5) min) subsequent to the addition of 100 μg/ml of atropine restored the initial contraction by $65.7 \pm 9.0\%$ and the secondary contraction by $78.8 \pm 8.4\%$ respectively, indicating that the abolishment of the response is reversible.

Autopsy.—In the 9 rats with the scrotal lesions, bilateral spermatic granulomas of considerable size were evident in the vasa deferentia at the epididymal and close to the vasa/cauda epididymal junction. In the other 2 rats, the cauda epididymises were seen to be distended but no granulomas were evident. In all 11 rats, the rods were seen almost at the site of implantation, encapsulated in dense connective tissue covering.

Atropine release rate.—The average release rate of atropine from 50% atropine rods was 60.3 ± 2.8mg after 7 days. If release kinetics were zero order, this would be equivalent to daily release of 8.4 ± 0.35mg.

Discussion

The results of the current study show that the local application of atropine, a cholinoceptor antagonist[3,4] to the epididymis of rats induced a significant reduction in fertility which was temporary with the lower dose (25% rods) and permanent with the higher dose (50% rods) tested.

The drug treatment also caused an impairment of the motility of epididymal spermatozoa and dysfunctioning in the process of seminal emission (the release of seminal fluid into the prostatic urethra). The latter was evident due to the presence of markedly low numbers of spermatozoa in the vaginas of the females paired with treated males. Either of the above two effects by itself should be sufficient to account for sterility that was evident since, both normal motility and sperm density are crucial for preserving fertility[1]. This impairment in epididymal spermatozoan motility that was observed is in agreement with *in vitro* studies reported earlier with human ejaculated spermatozoa[9] and rat epididymal spermatozoa[11]. As cholinoceptrors do not seem to play a role in the regulation of spermatozoan motility, atleast in the rat[12], it is unlikely that this effect on motility was due to cholinoceptor blocking activity of the drug. However, it is possible that this effect could have resulted from a local anaesthetic effect[4] and/or due to a non-specific toxic effect of the drug. Local anaesthetics have been shown to impair motility of human ejaculated spermatozoa[9].

On the other hand, several mechanisms seem to be responsibile for the failure of emission, depending on the time period of the study. At the initial phase, this could have resulted from an inhibition of the orgasmic contraction of the vas deferens and epididymis as indicated by marked suppression of the nerve-mediated contraction of the isolated vasa deferentia both in the presence of the drug and drug containing rods. This is likely to have been caused by

the local anaesthetic effect of the drug since these contractions were restored rapidly (<5 min) with repeated washings. Indeed, atropine has been shown to possess local anaesthetic properties[4]. In addition, as the cholinergic innervation of the vas deferens and epididymis appears to be secretomotor rather than motor[15] any blockade of cholinoceptors would not be expected to alter contraction of these organs sufficiently to account for oligospermia. At the later stages, the failure of emmission is likely to be triggered by a mechanical block in the vas deferens or epididymis produced by local inflammatory reactions and subsequent necrosis of tissues overlying, due to high local release of the drug. Nevertheless, the lethal dose of atropine in the rat is reported to be 750mg/kg, body weight[4]. Further, the presence of spermatic granulomat in the tract indicates the existence of a mechanical block within it[7,13]. This permanent mechanical obstruction would also account for the inability to restore fertility in the 50% treatment group.

Atropine treatment did not alter sexual desire (libido) nor androgen out-put from the testes as indicated by undiminished courting responses and due to its inability to reduce significantly the wet weights of male sexual accessory organs. Male sexual drive (libido) and structural and functional integrity of male accessory sex organs are androgen-dependant[8]. In contrast, a single vaginal instillation of 2mg of atropine just before coitus has been shown to suppress sexual drive (libido) in female rat[11]. However, the 50% atropine rods caused a significant reduction in the mating frequency. It is possible that this could have resulted from a reduction in penile tumescence and sensitivity in view of anticholinergic[3,4] and local anaesthetic[4] properties of the drug. There have been occasional clinical reports of impotence with anticholinergic agents such as propantheline[6]. Further local anaesthetics have been shown to reduce penile sensitivity and impair copulatory performance in rats[10]. However, the possibility that physical trauma of scrotal lesions impeding the mating performance at least during early part of the study cannot be absolutely ruled out.

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