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REVERSIBLE SUPPRESSION OF FERTILITY IN MALE RATS BY SALBUTAMOL, A SELECTIVE β—ADRENOCEPTOR AGONIST.

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Summary

The aim of this study was to investigate the potential of β—adrenergic agonists as male contraceptives acting via epididymis. This was tested in rats with salbutamol which was applied directly to the epididymis using silastic rods containing 50% drug. Two doses were used (1x50% drug/epididymis and 2x50% drug/epididymis). The higher dose had no significant effect on fertility. Lower dose, on the other hand, reduced fertility temporarily (on days 3 and 7 following the insertion of rods) without concomittant loss of libido, potency, motility of sperm, ejaculated sperm count or post-implantation losses. However, the antifertility effect was accompanied with a significant loss in pre-implantation losses. It is concluded that salbutamol induced antifertility action resulted from an inhibition on the fertilising competence of sperm due to changes in the secretory profile of the epididymis.

Key Words : Salbutamol, epididymis, male fertilty, fertilizing potential, ejaculation, contraception.

1. Introduction

The Epididymis is an attractive target for attack in the development of new, safe and reversible contraceptive agents for men (Cooper, 1992). In this context, several α - adrenoceptor agonists [methoxamine (Ratnasooriya, *et al.*, 1980), tyramine (Ratnasooriya, *et al.*, 1980), norephedrine (Ratnasooriya, *et al.*, 1980) and clonidine (Ratnasooriya and Wadsworth, 1987a)], α - adrenoceptor antagonists [guanethidine (Ratnasooriya and Manatunga, 1981), phenoxybenzamine (Ratnasooriya and Wadsworth, 1987b), prazosin (Ratnasooriya and Wadsworth, 1984 ; Ratnasooriya and Wadsworth, 1990), terazosin (Ratnasooriya, *et al.*, 1992) and tamsulosin (Ratnasooriya and Wadsworth, 1994)] and β - adrenoceptor antagonists [atenolol (Ratnasooriya, 1991)] have been tested in rats as potential male contraceptives. Except for tyramine, norephedrine (Ratnasooriya, *et. al.*, 1980), guanethidine (Ratnasooriya and Manatunga, 1981) and clonidine (Ratnasooriya and Wadsworth, 1987a) the other drugs tested (Ratnasooriya, *et. al.*, 1980 ; Ratnasoorriya and Wadsworth, 1987b ; Ratnasooriya and Wadsworth, 1984 ; Ratnasooriya

and Wadsworth, 1990 ; Ratnasooriya, *et al.*, 1992 ; Ratnasooriya, *et al.*, 1994 ; Ratnasooriya, 1991) have yielded considerable promise for evaluating adrenoceptor drugs further as potential male contraceptives. However, as yet, the potential of β - adrenoceptor agonists as male contraceptives acting via the epididymis has not been investigated. Exploration of this possibility forms the basis of this study.

Salbutamol was a drug under investigation. It is a β - adrenoceptor agonist drug commonly used clinically as a smooth muscle relaxant especially in the treatment of asthma (Goth, 1978). The pharmocological action of salbutamol is known to be mediated by elevating intracellular concentrations of adenosine $3^1 : 5^1$ — cyclic monophosphate (cAMP) (Goth, 1978). The testing model employed was rats and the mode of administration of salbutamol was topical to the epididymis using a silastic drug delivery system (Ratnasooriya, *et. al.*, 1980).

2. Material and methods :

Cross bred healthy adult albino rats of proven fertility from our own colony were used (males weighing 200 - 250g and females 200 - 225g). They were housed under standardised animal house conditions (temperature ; 28-31^{0}C ; photoperiod : approximately 12h light and 12h dark daily ; relative humidity ; 50-55%). Food [pelleted chow (Oils and Fats Co. Ltd., Seeduwa, Sri Lanka) and vegetables] and tap water were available without restriction throughout the period of study.

Silastic (Silastic 382, Medical grade Elastomer, Dow Corning Ltd., USA) rods (2mm. in diameter and 8mm. long) containing 0% and 50% salbutamol were made as described in detail previously (Ratnasooriya, *et al.*, 1980 ; Ratnasooriya, *et al.*, 1981).

Either a single (0% salbutamol rod, N=6 ; 50% salbutamol rod, n=6) or two (0% sabutamol rod n=6 ; and 50% salbutamol rod n=8) rods were implanted adjacent to each epididymis under mild ether (BDH Chemicals Ltd., Poole, UK) anaesthesia using aseptic precautions through a mid incision made in the scrotal sac and lateral incisions in the tunica vaginalis on each side (Ratnasooriya, *et al*, 1980). The day of insertion of rods was designated as day 0. The rectal temperature of these rats were monitored 1h before insertion of rods and 1, 2, 3, 4, 5, or 16th following surgery. Furthermore, all treated animals were examined 2-3h post surgery and twice daily(9.00-10.00h and 15.00 - 16.00h) thereafter for mortality, general physical conditions and behaviour.

Libido (sexual drive), ejaculatory ability and fertility were evaluated at regular intervals (7 days prior to insertion of rods and on day 3, 7 or 14 after insertion) by placing each male (between 17.00 - 18.00h) overnight with a proestrous female having had a regular 4 day oestrous cycle on at least four complete vaginal cycles before the study was started. The co-housed pair was observed for 1-2h with respect to their sexual behaviour patterns.

Successful mating was confirmed by the presence of sperm in the vaginal smear on the following morning (7.00 - 8.00h). If sperm were present, (considered as onset of pregnancy) their numbers in the vagina were estimated (in duplicate) using an improved Neubauer type haemocytometer (Fisons Scientific Equipment, Loughborough, UK) after flushing the vagina with 0.05mL of isotonic saline (0.9% NaCl, W/V). This was used as a vaginal sperm count index (10^6 mL^{-1}). Concomittantly, the sperm recovered from the vagina were examined microscopically (at x 400) under phase contrast optics for any gross morphological defects. In the absence of sperm, the females were checked daily by vaginal saline lavage for the occurence of pregnancy or pseudopregnancy.

At day 12, post-coitum the mated females were laporotomised under ether anaesthesia, and the number of fetuses (both viable and dead) was counted to permit analysis of fertility. The number and the gross appearance of the corpora lutes in each ovary was also noted during laporotomy.

The following reproductive indices were then computed : 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 = [(total number of corpora lutea — total number of implantations)/total number of corpora lutea] × 100; and post-implantation loss = [(total number of implantations — total number of viable implantations)/total number of implantations)] × 100.

On day 15, the treated and control rats were killed with an overdose of ether. The gross morphology of the testes, epididymis, vas deferens, seminal vesicle and ventral prostrate were noted with respect to their size and appearance.

Twelve rats were fitted with 1 x 0% salbutamol rod adjacent to the left epididymis and 1 x 50% salbutamol rod adjacent to the right epididymis as described previously under methodology. On day 3 (n=6) and on day 7 (n=6) these rats were anaesthetised with ether, and their genital tracts and testes were exposed through pelvic and scrotal incisions. The gross morphology of these organs were recorded especially with respect to their size and appearance. The sperm from a portion of each cauda epididymis were

extracted into isotonic saline and immediately examined at x 100 magnification for assessment of their motility using a subjective scale from 0 (immotile) to 5 (maximum perceived motility). Scoring was done in whole number units only.

Epididymides were then removed from the rats and the caudae portion of each was separated. These were dissected free of fat, weighed and placed in petri dishes containing 1mL of isotonic saline. The cauda epididymides were then minced with a razor blade and homogenised in a ground glass, homogeniser (BDH Ltd., Dagenham, Essex, UK). After suitable dilution, sperm numbers in each cauda epididymis were determined (in duplicate) under phase contrast optics using an improved Neubauer type haemocytometer. Results are expressed as 10^6 sperm g^{-1} tissue.

In a separate set of experiments 3 rats were fitted with 0% salbutamol rods adjacent to each epididymis and another 3 rats with 50% salbutamol rods. Their drinking frequency, feeding frequency and the time spent on a feeding bout were monitored for a 90 min period on days 1 and 2 using an event counter (Columbus Instruments, Ohio, USA).

A weighed 1 x 50% salbutamol rod was inserted adjacent to each epididymis of 6 rats. On day 7, these rods were removed under ether anaesthesia, blotted free of body fluids and dried at 55°C until a constant weight was reached. The amount of salbutamol released was estimated by substracting the final weight from the initial weight.

The results are expressed as means $\pm$ SEM. Where appropriate data were statistically evaluated using Mann-Whitney U-test, G-test and Student's t-test. Correlation coefficients were determined by the method of least squares. The 5% level of significance was considered statistically significant.

3. Results

No deaths were recorded during the study. Salbutamol treatment did not alter the rectal temperature (as compared to pretreatment) significantly $p > 0.05$; Student's t-test) (data not shown). Furthermore, the drug treatment did not elicit any other overt clinical signs of toxicity (both physical and behavioural).

In the feeding experiments, there were no significant differences ($p > 0.05$; Mann-Whitney U-test) in the frequency of drinking [days 1 and 2 (treatment vs control) : 2.8 ± 2.8 vs. 6.3 ± 5.3 min^{-1} and 5.4 ± 0.8 vs. $5.0 \pm 0.5 min^{-1}$ respectively], frequency of feeding [days 1 and 2 : 0.3 ± 0.1 vs. 1.7 ± 0.7 min^{-1} and 0.6 ± 0.1 min^{-1} respectively] and in the time spent on a single feeding bout [days 1 and 2 ; 0.2 ± 0.1 vs. 1.6 ± 0.9 min^{-1} and 0.9 ± 0.1 vs. 3.6 ± 0.5 min^{-1}]. In addition, at the termination of the study, none of the rats exhibited symptoms of wasting syndrome (thin body, thin skin, rough hair coats or lethargic activity).

In the mating studies, the precoital sexual behaviour (chasing, nosing, anogenital sniffiing, attempts at mounts and intromission) of salbutamol treated rats remained essentially similar to that of control rats. Moreover, as shown in Table 1, the index of libido was not significantly ($p > 0.05$; G-test) inhibited : with the lower dose (1 x 50% salbutamol rods) all the 24 pairings and with the higher dose (2 x 50% salbutamol rods) 20 out of 21 pairings being successful.

With the higher dose of salbutamol the ejaculated sperm number was not significantly ($p > 0.05$; Mann-Whitney U-test) altered (Table 1). However, the lower dose, at day 7 caused a marked reduction (by 46%) in the vaginal sperm count although this reduction did not reach statistical significance ($p > 0.05$; Mann-Whitney U-test). Salbutamol treatment also failed to render any ejaculate completely azoospermic. Importantly, a significant linear correlation was found between the vaginal sperm count and the number of uterine implants on day 7 with the lower dose of salbutamol ($r=0.82$; $p< 0.05$) but not on day 3 ($r = 0.03$; $p>0.05$). The external morphology of the ejaculated sperm always appeared normal.

Salbutamol treatments had no significant effect ($p>0.05$; Mann-Whitney U-test) either on the quantal pregnancy or on fertility index. On the other hand, the lower dose of salbutamol significantly ($p <0.05$; Mann-Whitney U-test) impaired the number of uterine implants (Table 1) both on day 3 (by 35%) and on day 7 (by 30%). Albeit, none of the matings with the lower dose was completely sterile. However, with the higher dose 3 out of 20 successful matings (15%) were completely sterile.

With the reduction in uterine implants there was an enhancement in pre-implantation losses (at day 3 by 344% and at day 7 by 294%), which reached statistical significance ($p<0.05$, Mann-Whitney U-test) on day 7. In addition, with the lower dose there was a significant linear correlation between the number of uterine implants and the pre-implantation loss (at day 3 : $r=0.99$, $p<0.05$ and at day 7 ; $r=0.95$, $p<0.05$). In contrast, salbutamol treatment had no significant effect on post implantation loss ($p<0.05$, Mann-Whitney U-test).

On autopsy, the testes, epididymides, vasa deferentia, seminal vesicles and ventral prostrate of treated rats were found to be essentially similar to those of control with respect to their sizes and gross appearance.

Following insertion of 1 x 50% salbutamol rod adjacent to the left epididymis the motility of the cauda epididymis sperm recovered from the treated side (day 3, 4.0 ± 0.6 at day 7, 4.7 ± 0.3) was not significantly ($p >0.05$; Mann Whitney U-test) altered from that of the control side (day 3, 4.8 ± 1.7 and

day 7, 4.7 $\pm$ 0.3). On the other hand, there was a significant ($p < 0.05$, Mann-Whitney U-test) reduction in sperm numbers (by 44%) in the cauda epididymis on day 3, in of the treated side (283.21 $\pm$ 27.87 x 10^6 g^{-1}) in comparision with the control side (504.43 $\pm$ 28.18 x 10^6 g^{-1}). However, on day 7, there was no significant ($p < 0.05$; Mann-Whitney U-test) difference in the sperm numbers in the cauda epididymis between the treated side (503.49 $\pm$ 97.99x10^6) and control side (441.93 $\pm$ 167.31 x 10^6). On both these days the external appearance of sperm in the cauda epididymis of the treated side was essentially similar to those obtained from the control side.

The average release rate of salbutamol from the rods was 2.8 $\pm$ 0.16 mg day^{-1}.

4. Discussion

The results show that, salbutamol when applied locally to the epididymis can suppress fertility (in terms of number of uterine implants and not as quantal pregnancy or fertility index). The topical mode of administration of the drug and the rapid onset (within 3 days) of the antifertility effect suggest that the main target site of action is the epididymis. The short duration of the antifertility effect appears to be due to exhaution of drugs in the silastic formulation : as evident from the release rate of the drug.

A notable feature of the antifertility effect of salbutamol was that it was not dose-related. Generally, a lack of a dose-response relationship indicates a non receptor mediated action. However, it is unlikely to be the case here : because, (a.) a fairly high density of β-adrenoceptors is found in the epididymis (Wong, *et al.*, 1992), (b.) salbutalmol is a well recognized β-adrenergic agonist (Goth, 1978) and (c.) the antifertility effect was seen at a reasonably low dose level. Thus, the inability of the higher dose of salbutamol to inhibit fertility may have resulted from tolerance. Indeed, development of tolerance is frequently reported with β-adrenoceptor agonists like salbutamol (Dowing, and Hollingworth, 1992).

Salbutamol caused no suppression in food intake. More over, it was not reprotoxic as there were no sperm granulomas and/or lesions in the vas deferens and/or epididymis. Thus, the antifertility effect is not secondary to general toxicity or reprotoxicity. Salbutamol did not suppress libido (both in terms of pre-coital sexual behaviour and index of libido) and size of sexual accessory organs. Therefore, antifertility action is unlikely to have arisen from an inhibition of sexual drive resulting from an androgen deficiency. It is noteworthy that p - adrenoceptor antagonist drug, atenalol, also had no effect on libido when applied to epididymis of rats in an identical manner (Ratnasooriya, 1991). Depression in erectile function of the penis, which impairs mating

performance, is also unlikely to be a causative factor in salbutamol induced antifertility action : as β-adrenergic stimulation is known to enhance the production of penile tumescence and rigidity (Wein, *et al.*, 1983).

Salbutamol induced an enhancement in pre-implantation losses indicating disruptions in sperm function. This appears to be the main, if not the sole, mechanism of the antifertility action of salbutamol. However, this elevation in the pre-implantation loss has not resulted from a reduction in the ejaculated sperm numbers, inhibition in sperm motility or from defects in the external morphology of sperm. Therefore, the salbutamol induced pre-implantation loss is likely to have caused by an impairment in the fertilizing competence of sperm : adrenergic agents are known to inhibit fertilizing potential of human sperm (Chan and Tang 1984). An epididymal dysfunction, such as changes in its secretions, can easily inhibit fertilizing potential of sperm. In rat, salbutamol is shown to alter epididymal secretory profile (see ref. 16 for details) Such a change in epididymal secretion could impair the functional competence of sperm in this study. Salbutamol also reduced the number of cauda epididymal sperm on day 3 (by 44%). But, this effect is unlikely to have contributed to the antifertility effect ; a reduction in caudal sperm count in excess of 90% is necessary to inhibit fertility in the rat (Aafjes, *et al.*, 1980).

Summerizing, this study for the first time, shows the promise of β-adrenoceptor agonists as potential male contraceptives.

Table 1. Effect of 50% salbutamol rods on some reproductive parameters of rats (means ± SEM)

Parameter	Treatment	n	Pre treatment	Days of Treatment		
				3	7	14
Index of Libido	1 x control rod	6	100%	100%	100%	100%
	2 x control rod	6	100%	100%	100%	100%
	1 x 50% rod	6	100%	100%	100%	100%
	2 x 50% rod	6	100%	87.5%	100%	100%
Vaginal sperm count index (10^6 mL^{-1})	1 x control rod	6	18.6 ± 2.80	18.26 ± 4.83	12.88 ± 1.49	14.34 ± 2.47
	2 x control rod	6	13.91 ± 2.36	11.75 ± 1.45	11.87 ± 0.96	12.79 ± 1.85
	1 x 50% rod	6	13.04 ± 3.19	12.37 ± 2.96	7.00 ± 0.51	10.50 ± 1.63
	2 x 50% rod	6	14.71 ± 2.26	18.82 ± 3.70	14.62 ± 1.42	13.45 ± 1.47
Quantal pregnancy	1 x control rod	6	100%	100%	100%	100%
	2 x control rod	6	100%	100%	100%	100%
	1 x 50% rod	6	100%	100%	100%	100%
	2 x 50% rod	6	100%	87.5%	100%	100%
Number of implants	1 x control rod	6	7.33 ± 0.72	7.33 ± 0.55	7.00 ± 0.71	8.50 ± 0.24
	2 x control rod	6	8,83 ± 1.17	9.33 ± 0.55	8.50 ± 0.96	9.80 ± 0.96
	1 x 50% rod	6	10.50 ± 0.62	6.83 ± 1.05*	7.33 ± 0.88*	9.33 ± 0.33
	2 x 50% rod	6	8.62 ± 0.50	7.71 ± 1.39	7.75 ± 1.50	7.00 ± 1.63

Table 1. Effect of 50% salbutamol rods on some reproductive of rates (means ± SEM)

Parameters	Treatment	n		Days of Treatment 3	7	14
Fertility index	1 x control rod	6	100%	100%	100%	100%
	2 x control rod	6	100%	100%	100%	100%
	1 x 50% rod	6	100%	100%	100%	100%
	2 x 50% rod	6	100%	75%	87.5%	83.3%
Pre-implantation Loss (%)	1 x control rod	6	29.57 ± 4.74	27.25 ± 6.04	31.09 ± 7.53	18.95 ± 2.58
	2 x control rod	6	28.53 ± 9.47	25.17 ± 6.38	27·29 ± 7.72	21.66 ± 7.22
	1 x 50% rod	6	7.75 ± 3.03	34.43 ± 11.48	30.50 ± 7.59*	8.18 ± 3.07
	2 % 50% rod	6	21.64 ± 3.80	30.04 ± 12.42	18.63 ± 12.30	39.39 ± 13.40
Post implantation loss (%)	1 x control rod	6	0.00	0.00	4.44 ± 4.44	0.00
	2 x control rod	6	3.33 ± 3.34	3.70 ± 2.35	0.00	1.38 ± 1.39
	1 x 50% rod	6	0.00	10.87 ± 3.98	4.23 ± 2.71	1.85 ± 1.85
	2 x 50% rod	6	7.66 ± 4.28	3.37 ± 2.22	2.50 ± 1.65	3.33 ± 3.34

* As compared with the pre-treatment value $p < 0.05$

5. References

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