

animals were kept in controlled temperature ($25 \pm 2^\circ\text{C}$) under 14:10 h light-dark schedule (Lights on at 6:00 a.m.). Food and water were provided *ad libitum*. The rats were screened for sexual behavior and those having a sex drive score (SDS) above four were chosen for the study (2, 5). Under sodium pentobarbital anesthesia (40 mg/kg body wt, I.P.), 26 gauge bilateral guide cannulae with indwelling styli were implanted in the brain, one mm above the LS, at the co-ordinates 7.8 mm anterior, 0.75 mm lateral, and 2.75 mm above the interaural zero, as per DeGroot atlas (6). The whole assembly was firmly fixed to the skull with four implanted anchoring screws and dental cement. Seven days after the implantation of the cannulae, the rats were tested for their copulatory activity on three occasions, at an interval of three days between the tests. Only those rats, which showed consistency in behavior, were used for experimentation. Sex behavior scoring was performed under dim illumination in a wooden box ($45 \times 30 \times 30$ cm) with a sliding glass front, during the dark phase of the light-dark cycle (6:00–11:00 p.m.). Bilaterally ovariectomized females of the same strain, primed with 25 μg of estradiol benzoate and 1 mg of progesterone, were used as receptive partners. The male rat was introduced into the test arena 5 min prior to the introduction of the female, and the recording was initiated at the entry of the female into the box. A computer program was used to record the latencies of pursuit, mount, intromission and ejaculation, frequencies of pursuit, mount, intromission, intervals of post-ejaculation and mean inter-intromission and SDS in these rats. This software quantifies the sexual behavior on an IBM-compatible PC (2, 5).

2 μg of β_1 -antagonist atenolol (4,2-Hydroxy-3-propoxyl-phenylacetamide) and 1 μg of β_2 -antagonist butoxamine hydrochloride obtained from Sigma Chemicals Co (St Louis, USA), were injected in the LS in two different groups of rats. The mixture of these two drugs (2 μg of atenolol and 1 μg of butoxamine) and vehicle (i.e. 0.9% NaCl) were injected in the LS in two other groups of rats. The selection of drug dose was based on some previous reports (7, 9). The drugs and vehicle were infused bilaterally in small volumes (0.2 μl) by a slow injector at the rate of 0.1 $\mu\text{l}/\text{min}$. The injector cannula was left in place for one min following injection. The cannula was then replaced with the stylus. The injection was given only once in any one brain site in each of the animals. The sex scoring started after 10 min of administration of the drug. At the end of the experiment, the brain sites and the spread of injection were verified histologically by injecting 0.2 μl of 2% ferric chloride, through the implanted guide cannulae, and then perfusing the brain with 10% formalin saline containing 3% potassium ferrocyanide. The experiments were performed in accordance with the guidelines laid down by the Animal Ethics Committee of the All India Institute of Medical Sciences, New Delhi, India.

The scores of all the parameters of sex behavior were analysed using Friedman test to find out the variation among the three control readings. Studies were done only on those rats where there was no significant variation among the three control scores. The mean of these three values (control) of each rat was taken for comparison with the post-injection values.

Preinjection parameters of different groups were analysed using Kruskal-Wallis test. The effects of drugs were analysed by comparing preinjection scores of different components of sexual behavior, with post-injection scores, using Wilcoxon matched-pairs signed-ranks test.

RESULTS

The effects of drugs, including vehicle, were tested on the animals, which showed consistent sex behavior. The injection of saline in the LS did not produce any significant effect on any of the parameters of the sexual behavior (Fig. 1, Tables I, II). The injection of either atenolol or butoxamine at the LS produced stimulation of sexual behavior, as is evident from the significant decrease in ejaculation latency and mean inter-intromission interval, and increase in intromission frequency and SDS.

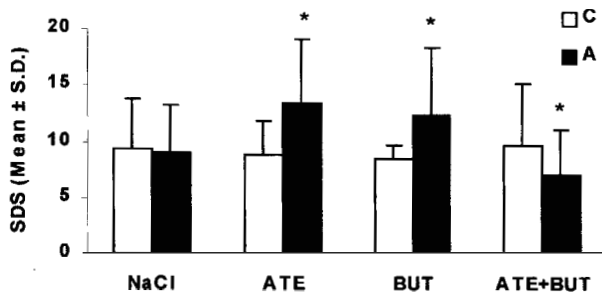


Fig. 1: Sex drive score (SDS) before drug treatment (C), and after the drug treatment (A). NaCl stands for saline, ATE for atenolol and BUT for butoxamine group. * $P < .05$, significantly different after treatment, compared to before treatment.

A decrease in intromission latency was also observed after butoxamine injection. On the other hand, administration of a mixture of atenolol and butoxamine produced inhibition of male sexual behavior (Fig. 1). There was an increase in mean inter-intromission and post-ejaculatory intervals and all the latencies except pursuit latency, after administration of the mixture. There was a

TABLE I: The latencies and intervals (median and range) of various components of male sexual behavior of four groups of animals before (C) and after (A) infusion of drugs.

Drug		PL		ML		IL		EL		PEI		MIII	
		C	A	C	A	C	A	C	A	C	A	C	A
Saline	Median	0.02	0.02	0.06	0.06	0.10	0.12	4.25	5.10	5.85	6.31	0.56	0.57
	Max.	0.13	0.10	0.24	0.21	0.39	0.36	12.4	11.7	9.15	7.27	0.95	0.94
	Min.	0.02	0.01	0.06	0.02	0.08	0.07	4.03	4.13	5.06	5.82	0.24	0.23
Atenolol	Median	0.05	0.05	0.27	0.13	0.32	0.12	6.74	2.99*	5.42	4.90	0.46	0.32*
	Max.	0.07	0.30	0.40	0.38	0.52	0.39	22.9	8.05	6.95	10.9	0.92	0.65
	Min.	0.03	0.03	0.05	0.06	0.15	0.02	3.67	1.94	4.78	4.60	0.33	0.17
Butoxamine	Median	0.06	0.04	0.22	0.13	0.38	0.18*	8.58	4.08*	7.12	6.06	0.46	0.44*
	Max.	0.07	0.48	0.31	0.58	0.95	0.65	16.2	6.65	7.34	7.59	0.74	0.61
	Min.	0.03	0.03	0.18	0.07	0.28	0.09	6.35	2.26	6.35	4.67	0.39	0.15
Atenolol + Butoxamine	Median	0.03	0.04	0.06	0.09*	0.12	0.17*	9.44	12.0*	6.10	7.5*	0.76	0.84*
	Max.	0.07	0.05	0.12	0.20	0.14	0.33	12.8	16.0	8.49	9.45	0.94	2.01
	Min.	0.03	0.03	0.05	0.08	0.06	0.11	2.46	3.10	5.60	5.60	0.29	0.45

PL, ML, IL, EL stands for latency to pursuit, mount, intromission, ejaculation respectively, PEI and MIII refer to post-ejaculatory interval and mean-interintromission intervals.

* $P < .05$ significantly different after treatment, compared to before treatment.

TABLE II: The frequency (median and range) of occurrence of different components of male sexual behavior of four groups of animals before (C) and after (A) infusion of drugs.

Drug		PF		MF		IF	
		C	A	C	A	C	A
Saline	Median	05.0	06.0	03.0	03.0	08.0	09.0
	Max.	11.0	10.0	06.5	03.0	22.5	24.0
	Min.	04.0	05.0	03.0	03.0	07.5	06.0
Atenolol	Median	06.5	12.4*	05.9	08.7	21.7	30.9*
	Max.	18.9	20.6	08.1	15.5	30.6	58.7
	Min.	05.1	10.2	04.1	03.8	10.9	15.3
Butoxamine	Median	08.1	14.6	06.2	07.3	21.1	22.7*
	Max.	10.8	17.7	06.8	09.8	26.8	66.2
	Min.	05.8	06.0	03.9	05.7	14.6	16.4
Atenolol + Butoxamine	Median	07.1	06.2*	06.4	03.9*	13.0	12.8*
	Max.	20.3	16.1	17.0	13.2	34.5	24.0
	Min.	06.3	01.7	04.2	01.7	10.6	05.8

PF, MF and IF refer to frequency of pursuit, mount and intromission respectively.

*P<.05 significantly different after treatment, compared to before treatment.

decrease in all the frequencies and SDS. Inhibition of sexual behavior was not accompanied by any motor deficit as animals actively moved around in the chamber, and spent more time in self-grooming. The spread of drug, inferred indirectly on the basis of the spread of the stain, was confined to the LS (Fig. 2).

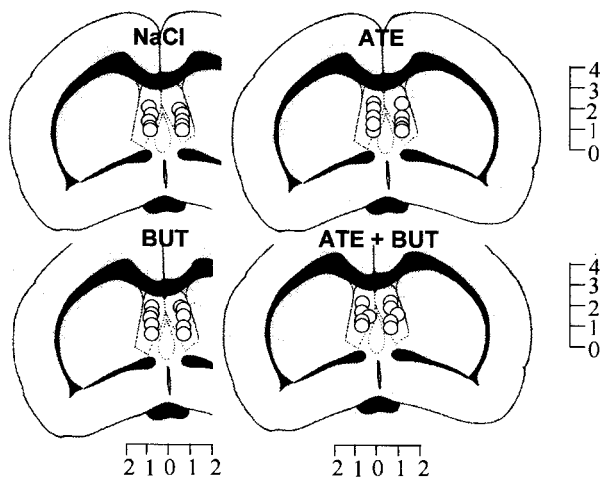


Fig. 2: Schematic representation of injection sites of NaCl, ATE, BUT and mixture of ATE and BUT in the lateral septum in four different group as per stereotaxic atlas of DeGroot (6). The circle represents the central point of the drug injection.

DISCUSSION

The results of the study showed that the administration of either atenolol or butoxamine at the LS produced decrease in ejaculation latency and mean inter-intromission interval and increase in intromission frequency resulting in activation of male copulatory behavior. However, the administration of a mixture of atenolol and butoxamine produced inhibition of the male sexual behavior. The inhibitory effect was reflected on both the motivational and performance components of sexual behavior as is evident from the increase in mount latency, ejaculation latency and mean-intromission interval respectively. Non-specific β -blocker propranolol, which blocks both β_1 and β_2 receptors, produce inhibition in male sexual behavior (2). Inhibition of sex behavior by blocking both β_1 and β_2 receptors, but not by β_1 blocker atenolol and β_2 blocker butoxamine alone, show that simultaneous blocking of both subtypes of β -receptors are necessary for inhibiting the male sexual

behavior. But, the stimulatory action of β_1 - and β_2 -blockers, when given alone, needs explanation. It could be possible that blocking either β_1 or β_2 -adrenoreceptors, leads to an unbalanced activity of the subtypes, resulting in facilitation of male sex behavior. The results obtained in this study, after application of atenolol and butoxamine in the LS are different from that reported by Smith et al, where it was shown that intracerebroventricular injections of atenolol and metoprolol produced no effect on male sexual behavior (9).

In addition to role of the β -receptor subtypes at the LS on male sexual behavior, the results of this study also provided the know-how essential for designing β -blockers, which may have no negative effects on sexual functions. Though reports on intracerebral injection of atenolol and butoxamine are scanty, the drug doses selected for this study can be justified on the basis of

available literature (7-10). Furthermore, elicitation of inhibition by the combination of the drugs, and the absence of this effect when these were administered separately, justifies the use of single dose of these drugs. A dose response study using several groups of animals and different combinations of drugs may be required before applying the results for better therapeutic strategies.

Inability of either atenolol or butoxamine to inhibit the male sexual behavior, and inhibition of the same by the mixture of atenolol and butoxamine, indicate that simultaneous blockade of both β_1 and β_2 adrenoreceptors at the LS is essential for inhibition of male sexual behavior.

ACKNOWLEDGEMENTS

The study was funded by the Indian Council of Medical Research (Grant No- 45/4/98-BMS).

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