ATENOLOL OR BUTOXAMINE INJECTION AT THE LATERAL SEPTUM DOESN’T INHIBIT MALE SEXUAL BEHAVIOR IN RATS

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Abstract: To investigate the role of specific adrenoreceptors subtypes on sexual behavior, atenolol, butoxamine, a mixture of atenolol and butoxamine, and saline (vehicle) were injected into the lateral septum in four different groups of sexually active male rats. Application of a mixture of atenolol and butoxamine produced inhibition of copulatory activity. On the other hand, application of either atenolol or butoxamine alone did not inhibit copulatory activity. But it produced stimulation of some of the components of male sexual behavior. 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 both beta-adrenoreceptors at the lateral septum are involved in the elaboration of male sexual behavior. Stimulation of some components of sexual behavior on application of atenolol or butoxamine could be attributed to an unbalanced activity of beta-adrenoreceptors.

Key words: lateral septum sexual behavior atenolol propranolol

INTRODUCTION

The lateral septum (LS) is an important area that regulates male sexual behavior in rats (1–4). Local administration of norepinephrine (NE) at the LS facilitated male sexual behavior. Increasing the availability of endogenous NE at the LS, by injecting yohimbine, also facilitated most of the components of male sexual behavior. Inhibition of male copulatory behavior by the application of beta-antagonist, propranolol and stimulation of this behavior by beta-agonist, isoproterenol, showed that it is the beta-adrenergic system at the LS which is primarily involved in the elaboration of male sexual behavior (2). This study was carried out to identify the subtypes of beta-receptors, namely beta_1 and beta_2 adrenoreceptors, at the LS, which is involved in mating behavior in male rats.

METHODS

Twenty adult male Wistar rats, weighing 200–250 gm were used in the study. The
animals were kept in controlled temperature 
(25 ± 2°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 × 30 × 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 β₁-antagonist atenolol (4,2-
Hydroxy-3-propoxyl-phenylacetamide) and 
1 µg of β₂-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 µl/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.

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>
<thead>
<tr>
<th>Drug</th>
<th>PL Median</th>
<th>PL Max.</th>
<th>PL Min.</th>
<th>ML Median</th>
<th>ML Max.</th>
<th>ML Min.</th>
<th>IL Median</th>
<th>IL Max.</th>
<th>IL Min.</th>
<th>EL Median</th>
<th>EL Max.</th>
<th>EL Min.</th>
<th>PEI Median</th>
<th>PEI Max.</th>
<th>PEI Min.</th>
<th>M III Median</th>
<th>M III Max.</th>
<th>M III Min.</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.02</td>
<td>0.13</td>
<td>0.02</td>
<td>0.06</td>
<td>0.24</td>
<td>0.06</td>
<td>0.10</td>
<td>0.39</td>
<td>0.08</td>
<td>4.25</td>
<td>5.10</td>
<td>0.12</td>
<td>5.85</td>
<td>6.31</td>
<td>0.56</td>
<td>0.57</td>
<td>0.94</td>
<td>0.23</td>
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<tr>
<td>Atenolol</td>
<td>0.05</td>
<td>0.07</td>
<td>0.03</td>
<td>0.27</td>
<td>0.40</td>
<td>0.06</td>
<td>0.32</td>
<td>0.39</td>
<td>0.15</td>
<td>6.74</td>
<td>2.99</td>
<td>0.12</td>
<td>5.42</td>
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<td>0.46</td>
<td>0.32*</td>
<td>0.65</td>
<td>0.17</td>
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</tr>
<tr>
<td>Butoxamine</td>
<td>0.06</td>
<td>0.07</td>
<td>0.03</td>
<td>0.22</td>
<td>0.48</td>
<td>0.06</td>
<td>0.38</td>
<td>0.95</td>
<td>0.28</td>
<td>8.58</td>
<td>4.08</td>
<td>0.18*</td>
<td>7.12</td>
<td>6.06</td>
<td>0.46</td>
<td>0.44*</td>
<td>0.74</td>
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<tr>
<td>Atenolol +</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.09</td>
<td>0.04</td>
<td>0.06</td>
<td>0.12</td>
<td>0.28</td>
<td>0.09</td>
<td>9.44</td>
<td>12.0</td>
<td>0.17*</td>
<td>6.10</td>
<td>7.5*</td>
<td>0.76</td>
<td>0.84*</td>
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<td>Butoxamine</td>
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<td>0.04</td>
<td>0.03</td>
<td>0.12</td>
<td>0.05</td>
<td>0.06</td>
<td>0.14</td>
<td>0.33</td>
<td>0.11</td>
<td>12.8</td>
<td>8.49</td>
<td>0.25</td>
<td>9.45</td>
<td>8.95</td>
<td>0.94</td>
<td>0.45</td>
<td>0.29</td>
<td>0.45</td>
</tr>
</tbody>
</table>

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

*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).

**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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>PF</th>
<th>MF</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>Saline</td>
<td>05.0</td>
<td>06.0</td>
<td>03.0</td>
</tr>
<tr>
<td>Max.</td>
<td>11.0</td>
<td>10.0</td>
<td>06.5</td>
</tr>
<tr>
<td>Min.</td>
<td>04.0</td>
<td>05.0</td>
<td>03.0</td>
</tr>
<tr>
<td>Atenolol</td>
<td>06.5</td>
<td>12.4*</td>
<td>05.9</td>
</tr>
<tr>
<td>Max.</td>
<td>18.9</td>
<td>20.6</td>
<td>08.1</td>
</tr>
<tr>
<td>Min.</td>
<td>05.1</td>
<td>10.2</td>
<td>04.1</td>
</tr>
<tr>
<td>Butoxamine</td>
<td>08.1</td>
<td>14.6</td>
<td>06.2</td>
</tr>
<tr>
<td>Max.</td>
<td>10.8</td>
<td>17.7</td>
<td>06.8</td>
</tr>
<tr>
<td>Min.</td>
<td>05.8</td>
<td>06.0</td>
<td>03.9</td>
</tr>
<tr>
<td>Atenolol +</td>
<td>07.1</td>
<td>06.2*</td>
<td>06.4</td>
</tr>
<tr>
<td>Butoxamine</td>
<td>20.3</td>
<td>16.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Min.</td>
<td>06.3</td>
<td>01.7</td>
<td>04.2</td>
</tr>
</tbody>
</table>

PF, MF and IF refer to frequency of pursuit, mount and intromission respectively. *P<.05 significantly different after treatment, compared to before treatment.

**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 β₁ and β₂ receptors, produce inhibition in male sexual behavior (2). Inhibition of sex behavior by blocking both β₁ and β₂ receptors, but not by β₁ blocker atenolol and β₂ 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 $\beta_1$- and $\beta_2$-blockers, when given alone, needs explanation. It could be possible that blocking either $\beta_1$ or $\beta_2$-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 $\beta$-receptor subtypes at the LS on male sexual behavior, the results of this study also provided the know-how essential for designing $\beta$-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 $\beta_1$ and $\beta_2$ adrenoreceptors at the LS is essential for inhibition of male sexual behavior.

ACKNOWLEDGEMENTS

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REFERENCES


