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Modulation by estradiol of rabbit atrial chronotropic response to histamine

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Summary

The chronotropic response (△ rate) to histamine of atria from estradiol 17-β (E_2; 0.1 mg/kg)-treated (14 days) rabbit was significantly greater compared to those from control rabbits. However, the maximum response of atria from rabbits treated with a higher dose of E_2 (1.0 mg/kg) was not significantly different from control. Cimetidine (2.8×10^{-7} M) inhibited the theoretical maximum increase in rate to histamine in all 3 groups, control 25 %, E_2 (0.1 mg) 42 % and E_2 (1.0 mg) 35 %.

Key words: estrogen, rabbit, atrial rate, histamine, cimetidine

Introduction

It is evident that estrogens can cause changes in certain aspects of the cardiovascular function (2). Estrogens have been shown to modulate histamine receptors at the hypothalamic level (9). The female sex hormones, particularly estrogens, are suspected of altering histamine metabolism (5, 7). Cardiac histamine receptors may have important functions in cardiovascular physiology (6). These studies suggest that estrogens probably influence cardiac histamine receptors also. The present study investigates the effect of estradiol 17-β pretreatment on cardiac histamine receptor function in isolated rabbit atrial pairs. A preliminary report of this work has appeared in abstract form (1).

Materials and methods

Twelve immature (average body weight 1.0 ± 0.04 kg) female New Zealand rabbits were divided into 3 equal groups. Two groups received estradiol 17-β (E_2) dissolved in corn oil at a dose of 0.1 or 1.0 mg/kg daily subcutaneously for 14 days. The control group received oil (0.1 ml/kg). The rabbits were fed a commercial diet (Thriftymaster™ Rabbit Pellets, Acco Feeds, Abilene, TX). Tap water was available *ad libitum*. The rabbit room temperature was 26 °C and the light/dark period was controlled with 14 h of light. At the end of 14 days, treatment (75 days after birth) each rabbit was decapitated, the chest was opened along the midline, and the whole heart was removed and immersed in Tyrode's solution which was gassed with 95 % O_2 and 5 % CO_2. The atrial pair was dissected free of all extraneous tissue and a thread passed through the tip of each auricular apex.

The atria were mounted in a 50-ml tissue bath, with one atrium tied to a glass hook and the other to a Statham Strain gauge (Model UC-3). The average control tension

for atria was 0.6 ± 0.03 g. The tissue was allowed to equilibrate for 60–90 min before experimental procedure began. Signals from transducers were recorded on a Beckman Biomedical Dynograph R411 recorder. Drugs were dissolved in deionized water and dispensed with a Finnpipette in a volume of 500 µl or less, near the base of the bath. The concentrations of drugs are specified below.

The gas mixture was bubbled into the tissue bath chamber through a sintered-glass base and served as a source of oxygenation, regulation of pH and rapid mixing of the drug solutions with the bathing medium. The pH of the bath was 7.4 and the temperature 36 °C. The method has been described in detail before (6). The drugs used and their sources are: estradiol 17-β and histamine diphosphate (Sigma Chemical Co.) and cimetidine hydrochloride (Tagamet™, Smith Kline and French Lab. Co.).

Cumulative histamine doses (1.4, 2.2, 4.5, 6.7, 9.0 and 18.0×10^{-6} M) were added to the bath at 10 min interval and the rate recorded (1-min counts were made before each addition and 10 min after the last addition). After the initial histamine dose response testing, the baths were completely emptied by suction and refilled three times. Retesting did not start until the atrial rate had returned to the control value. At this time, cimetidine (2.8×10^{-7} M) was added to the bath, a waiting period of 25 min was observed before the same concentrations of histamine were added as before.

Statistical calculations were done on a Texas Instruments Programmable 59 calculator equipped with applied statistics software. Scatchard plots of the data from the histamine dose response curves before and after cimetidine were calculated by dividing the chronotropic response (△ rate; beats/min) change in rate above the spontaneous atrial rate by at each concentration of histamine (fig. 1).

Result

The effect of estradiol 17-β (E_2) pretreatment on the chronotropic response to histamine is given in table 1 and figure 1 (B). Scatchard plots were drawn by using a statistically determined regression line, and the maximum increase in rate (R_{max}) was found to be 130 ± 3.1, 174 ± 1.6 and 141 ± 9.9 beats/min for atria ± SEM from oil, 0.1 and 1.0 mg/kg E_2-treated animals, respectively (fig. 1 A). E_2 (0.1 mg/kg) treatment produced increase in R_{max} in response to histamine that was significantly greater than control atria, but not at the higher dose of E_2 (1.0 mg/kg) compared to control. Cimetidine reduced the maximum response to histamine differently in the 3 groups. The inhibition in control group was 25 % whereas it was 42 % and 35 % in 0.1 and 1.0 mg/kg E_2-treated groups, respectively. The dose of histamine which would cause a half maximum increase in rate ($D_{½max}$) was also increased significantly by E_2-treatments before cimetidine was added. But there was no significant difference in $D_{½max}$ after cimetidine in the atria from higher (1.0 mg/kg) dose of E_2-treated animals (table 1). Cimetidine-induced reduction in R_{max} in response to the same doses of histamine was 116 ± 2.6, 126 ± 6.8 and 87 ± 6.4 beats/min for atria from oil, 0.1 and 1.0 mg/kg E_2-treated animals, respectively. The slopes and correlation coefficient (r) for these three were calculated and found to be: −1.27 and (r = −0.97) for oil, −0.97 and (r = −0.96) for 0.1 mg/kg E_2, −1.33 and (r = −0.97) for 1.0 mg/kg E_2. The mathematical basis for these kinds of calculations using histamine and rabbit atria have been published (4). The

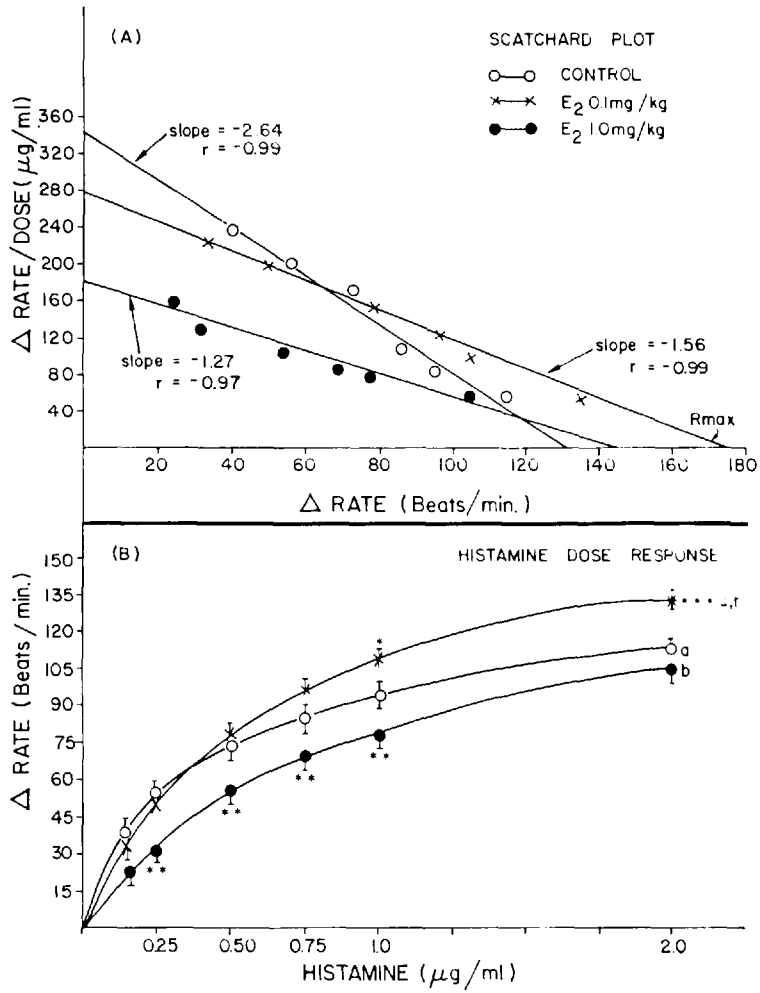

Fig. 1. (A) Scatchard analysis of the chronotropic response to histamine of atria from estradiol 17-β (E_2)-pretreated rabbits, data from histamine dose response curve. R_{max} is the maximum calculated response and can be read from horizontal axis. The slope yields $-1/Km$.
(B) Rabbit atrial chronotropic response to histamine (1.4 to 18×10^{-6} M) from E_2-pretreated rabbits. **$P < 0.01$; ***$P < 0.001$ from control (oil). Means bearing the same superscript are significantly different. Vertical bar represents SEM of 4 atria in each group.

mean frequency of atria (beats/min) before adding drugs were 125 ± 4 (control); 127 ± 7 (E_2 0.1 mg) and 131 ± 6 (E_2 1.0 mg).

Fourteen days of E_2 treatment significantly ($P < 0.001$) increased the uterine weight from control value of 0.59 ± 0.05 g (mean ± SEM) to 3.95 ± 0.7 and 5.73 ± 0.3 in 0.1 and 1.0 mg/kg E_2-treated groups, respec-

tively. There was no significant difference in body weight gain after E_2 treatments (data not shown).

Discussion

The present study demonstrates that estradiol 17-β (E_2) pretreatment can induce alterations in the histamine response of atria from immature rabbit. The increase in Δ rate after histamine in rabbits treated with low dose of E_2 (0.1 mg/kg) could be due to increased number of histamine receptors in the atria (fig. 1). Rabbit atria contain both H_1 and H_2 chronotropic histamine receptors (6). Using an antagonist of histamine, cimetidine which competes with the H_2 type receptor, we found that inhibition of maximum rate by cimetidine was more in the two E_1-treated groups compared to oil-treated control, and in all cases the inhibition was non-competitive (table 1). We interpret these results as an indication of possible change in the ratio of H_1 and H_2 type receptors for histamine. The $-1/Km$ value which gives the calculated dose of histamine required to obtain half the maximum chronotropic response ($D_{½max}$) was also higher in low dose of E_2 (0.1 mg/kg)-treated rabbits compared to control, suggesting a reduced affinity of histamine for the receptors. The percent increase in $D_{½max}$ after cimetidine is not very different at the low dose of E_2 from that in the control but is markedly reduced after the higher dose of E_2 (1.0 mg/kg) treatment, again suggesting an alteration in H_1 and H_2 receptor ratio (table 1).

The histamine content of rat uterus after estrogen treatment is reported to be decreased (8). Estrogen treatment (83 μg/kg) has also been shown to reduce the tissue store and increase the releasable fraction of histamine from guinea pig atria (3). Based on these findings, we speculate that a low dose (0.1 mg/kg) of E_2 probably decreases histamine content of rabbit atria, and the apparent increase in the number of histamine receptors in this group may be a compensatory response to diminished histamine output. The presence of estrogen receptor in the atrium of the rat have been reported (10).

Table 1. The chronotropic response to histamine of isolated atria from immature rabbit pretreated with estradiol 17-β (E_2) for 14 days. Data from Scatchard plots. $D_{½max}$ is the dose of histamine necessary to obtain the theoretical half the maximum response. Mean ± SEM of 4 atria in each group.

Cimetidine (2.8×10^{-7} M)	Maximum Δ rate (beats/min)			Histamine dose ($\times 10^{-6}$ M) for $D_{½max}$		
	Before	After	% Change	Before	After	% Change
Control (oil)	112 ± 3^{a}	$84 \pm 5^{**}$	25	3.4	6.4	47
E_2 0.1 mg/kg	$132 \pm 1^{a,b}$	$76 \pm 5^{***}$	42	5.8	9.3	38
E_2 1.0 mg/kg	104 ± 4^{b}	$68 \pm 3^{***}$	35	7.1	6.8	–4

$^{**}p < 0.01$; $^{***}p < 0{,}001$. Significantly different from control. Means bearing the same superscript are significantly different.

Our observation may be physiologically relevant since estrogen-containing contraceptive drugs are widely used.

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