Jugoslov Med Biohem 2005; 24 (4): 265 DOI:10.2298/JMB0504265R

UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 24: 265–269, 2005

Originalni naučni rad
Original paper

EFFECTS OF PHENYLETHYLAMINE ON ISOLATED UTERUS IN RATS TREATED WITH ESTROGEN

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Summary: It is well known that histamine produces relaxation of precontracted rat uterus in oestrogenized animals. This effect of histamine was caused by the stimulation of H_2 histamine receptors. Our results confirm these findings (histamine relaxation effect is $EC_{50} = 40.52 \pm 1.1 \times 10^{-6}$ mol/L). Phenylethylamine also produces relaxation of precontracted rat uterus in oestrogenized animals ($EC_{50} = 40.98 \pm 1.1 \times 10^{-6}$ mol/L, $p < 0.001$). This effect of phenylethylamine is abolished by means of ranitidine, a selective H_2 histamine receptors antagonist. Actually, H_2 blockade has shifted concentration-dependent phenylethylamine curve to the right, i.e. produced inhibition of phenylethylamine induced relaxation ($EC_{50} = 61.42 \pm 1.2 \times 10^{-6}$ mol/L, $p<0.001$). Since phenylethylamine is described as a potent inhibitor of histamine N-methyl-transferase (HMT), it is possible that modulation of histamine catabolism may lead to H_2 mediated relaxation of precontracted rat uterus in oestrogenized animals.

Key words: histamine, phenylethylamine, uterus, estrogen

Introduction

Histamine effects on the smooth muscle of a rat uterus are already known. Namely, histamine produces relaxation of precontracted rat uterus (1). Those effects are mediated via H_1 and H_2 histamine receptors (2). However, ovarian steroid hormones have a significant influence on applied histamine effects on precontracted rat uterus, i.e. H_1 relaxant response to histamine was present only in progesterone dominant rat uteri (2).

Also, it has been reported that ovarian hormones treatment can increase the number of rat uterine mast cells (3). Nevertheless, there is still no reliable evidence of the influence of ovarian steroid hormones on histamine catabolism in the rat uterus. Special interest can be focused on histamine N-methyl-transferase (HMT), enzyme responsible for rapid inactivation of histamine by methylation of ring tele-nitrogen in histamine (4–6). Furthermore, there is strong evidence that at least a few of histamine methylation products show higher selectivity to different classes of histamine receptors in various tissues, but at the same time have rather week biological activity (7, 8).

The aim of this study was to evaluate possible effects of phenylethylamine, a potent inhibitor of HMT, on the isolated uterus motility in rats pretreated with estrogen.

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Materials and Methods

Preparation of isolated rat uterus horns

Young female albino rats of the Wistar strain, weighing between 250 g and 300 g, pretreated with diethylstilbestrol (5 mg/kg i.p., 24 hours before sacrifice), were used in this study. Rats were killed by cervical dislocation (according to Schedule 1 of the Animals, Scientific procedures, Act 1986, UK) and exsanguinated. Each experiment was conducted on isolated preparations from five different animals. After laparatomy, both uterine horns were rapidly removed, cut along the longitudinal axis and placed in organ bath.

Experimental design

Each isolated preparation was mounted in the 10 mL organ bath with constant flow (5 mL/min) of De Jalon's solution (NaCl 154 mmol/L, KCl 5.6 mmol/L, $CaCl_2$ 0.4 mmol/L, KH_2PO_4 1.18 mmol/L, $NaHCO_3$ 5.95 and glucose 2.5 mmol/L) maintained at 31±1 °C to avoid spontaneous contractions. The bath was aerated continuously with 95% O_2 and 5% CO_2. One end of the isolated uterine horn was fixed to the organ bath, and the other was fixed to a force-displacement transducer (IT-1 sensor, EMKA Technologies) coupled with tension amplifier and chart recorder.

All preparations were loaded with 1 g weight and allowed to equilibrate 30 minutes. At the end of the equilibration period a submaximal plateau contraction of preparation was obtained by adding KCl (60 mmol/L).

The first set of experiments consisted of recording the uterine horn preparations (precontracted with KCl) responses to histamine (1, 2.6, 26, 260 × 10^{-6} and 2.6 × 10^{-3} mol/L, 2 minute each concentration) in animals pretreated with diethylstilbestrol, as well as uterine response to same doses of histamine in the presence of S(+)-chlorpheniramine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 5 × 10^{-7} mol/L) or ranitidine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 1 × 10^{-6} mol/L). The second set of experiments consisted of recording the uterine horn preparations (precontracted with KCl) responses to phenylethylamine (13, 26, 52, 80 and 130 × 10^{-6} mol/L, 2 minute each concentration) in animals pretreated with diethylstilbestrol, as well as uterine response to same doses of phenylethylamine in the presence of S(+)-chlorpheniramine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 5 × 10^{-7} mol/L) or ranitidine (permanent perfusion for 5 minutes before agonist use and during agonist's action, with final concentration of 1 × 10^{-6} mol/L). Next concentration of histamine or phenylethylamine on the same preparation was applied only after a period of 15 min. All drugs were applied to organ bath using micro infusion pump with constant flow of 125 μL/min.

Relaxant responses were measured as changes in isometric tension and converted into a percentage of the reference maximum relaxations for each group of experiments.

Chemicals

Drugs used in these experiments were histamine dihydrochloride, ranitidine hydrochloride, diethylstilbestrol, (Sigma Chemical Co, USA), S(+)-chlorpheniramine maleate (RBI, USA), phenylethylamine (Calbiochem, GB) and KCl (Zorka Šabac, Serbia). The drugs were prepared on the day of experiment in NaCl 154 mmol/L (Zorka Šabac, Serbia). Concentrations reported are expressed as final concentrations within the organ bath.

Statistical analysis

Each concentration was assayed on isolated preparatus from five different animals. Concentration-response curves were constructed using linear regression according to least-squares analysis (9, 10). Effective concentration of agonists that produced 50% of maximal response and response duration (EC_{50}) was calculated for each agonist together with its confidence limits (1.96 × standard error). The Student *t* test was used for the comparison of maximal responses (expressed as mean ± SEM) for groups 1 and 2. The results were considered statistically significant when $p \leq 0.05$.

Results

The effects of histamine on the isolated uterus horns from oestrogenised rats

Histamine (1 × 10^{-6} mol/L to 2.6 × 10^{-3} mol/L) produced concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats (EC_{50} = 40.52 ± 1.1 × 10^{-6} mol/L, $p < 0.001$). S(+)-chlorpheniramine (5 × 10^{-7} mol/L), H_1 receptors antagonist, did not affect isolated uterus response to histamine (EC_{50} = 39.41 ± 1.3 × 10^{-6} mol/L). Ranitidine (1 × 10^{-6} mol/L), H_2 receptors antagonist, shifted concentration-dependent relaxation curve of histamine to the right (EC_{50} = 130.41 ± 1.5 × 10^{-6} mol/L, $p < 0.001$) (*Figure 1*).

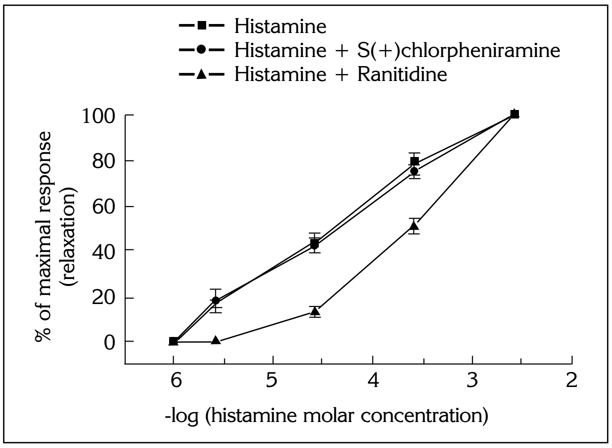

Figure 1 Histamine (1 × 10^{-6} mol/L to 2.6 × 10^{-3} mol/L) caused concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats. S(+)-chlorpheniramine (5 × 10^{-7} mol/L) did not affect isolated uterus response to histamine. Ranitidine (1 × 10^{-6} mol/L) shifted concentration-dependent relaxation curve of histamine to the right.

The effects of phenylethylamine on the isolated uterus horns from oestrogenised rats

Phenylethylamine (13 × 10^{-6} mol/L to 130 × 10^{-6} mol/L) produced concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats (EC_{50} = 40.98 ± 1.1 × 10^{-6} mol/L, $p < 0.001$). S(+)-chlorpheniramine (5 × 10^{-7} mol/L), H_1 receptors antagonist, did not affect isolated uterus response to phenylethylamine in oestrogenized rats (EC_{50} = 38.89 ± 1.1 × 10^{-6} mol/L). On the other hand, ranitidine (1 × 10^{-6} mol/L), H_2 receptors antagonist, has shifted concentration-dependent relaxation curve of phenylethylamine to the right (EC_{50} = 61.42 ± 1.2 × 10^{-6} mol/L, $p < 0.001$) (*Figure 2*).

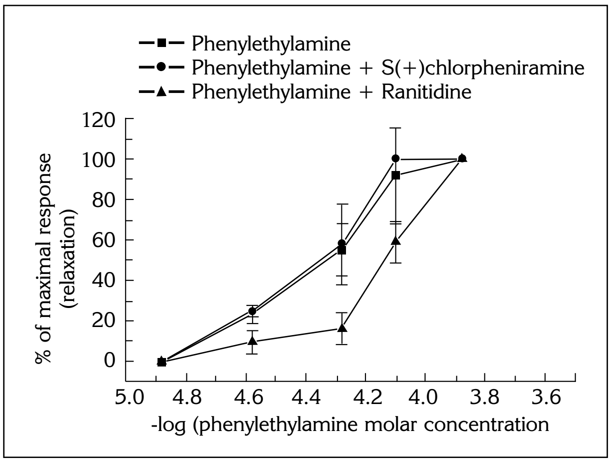

Figure 2 Phenylethylamine (13 × 10^{-6} mol/L to 130 × 10^{-6} mol/L) produced concentration-dependent relaxation of isolated uterus horns (precontracted with KCl) from oestrogenized rats. S(+)-chlorpheniramine (5 × 10^{-7} mol/L) did not affect isolated uterus response to phenylethylamine in oestrogenized rats. Ranitidine (1 × 10^{-6} mol/L) shifted concentration-dependent relaxation curve of phenylethylamine to the right.

In the presence of the highest concentration of phenylethylamine, oestrogenised isolated rat uterus horns showed maximal relaxation (16.83 ± 6.12% of KCl-induced contraction) (*Figure 3*).

The maximal relaxation (60.57 ± 3.55% of KCl-induced contraction) in this experimental group was reached with the highest concentration of histamine (*Figure 3*).

Discussion

Our results show that histamine induced relaxation of isolated precontracted rat uterus in animals treated with estrogen. The relaxation effect of histamine is concentration-dependent (EC_{50} = 40.52 ± 1.1 × 10^{-6} mol/L).

In animals treated with estrogen, histamine induced relaxation is mediated only via H_2 histamine receptors because H_1 antagonist failed to prevent this effect (EC_{50} = 40.52 ± 1.1 × 10^{-6} mol/L vs. EC_{50} = 39.41 ± 1.3 × 10^{-6} mol/L with H_1 blockade, $p > 0.05$). The histamine-induced relaxation of rat uterus in animals treated with estrogen is prevented by H_2 histamine antagonist (EC_{50} = 40.52 ± 1.1 × 10^{-6} mol/L vs. EC_{50} = 130.41 ± 1.5 × 10^{-6} mol/L with H_2 blockade, $p < 0.001$).

Effects of histamine on isolated rat uterus (precontracted with KCl) have already been described (1, 2). Our results indicate that histamine produces relaxation of KCl-precontracted isolated rat uterus via stimulation of H_2 histamine receptors. This is in accordance with similar published results (2, 8) supporting the conclusion that histamine produces H_2 relaxation of KCl-precontracted uterus in estrogen pretreated animals.

Phenylethylamine produced concentration-dependent relaxation of isolated rat uterus (precontracted with KCl) in oestrogenized rats (EC_{50} = 40.98 ± 1.1 × 10^{-6} mol/L, $p < 0.001$). In addition, it is clear that H_2 blockade has shifted concentration-dependent phenyl ethylamine curve to the right, i.e. produced inhibition of phenylethylamine-induced relaxation (EC_{50} = 61.42 ± 1.2 × 10^{-6} mol/L, $p < 0.001$).

The relaxation effect of phenylethylamine is weak comparing to the effects of exogenous histamine (*Figure 3*).

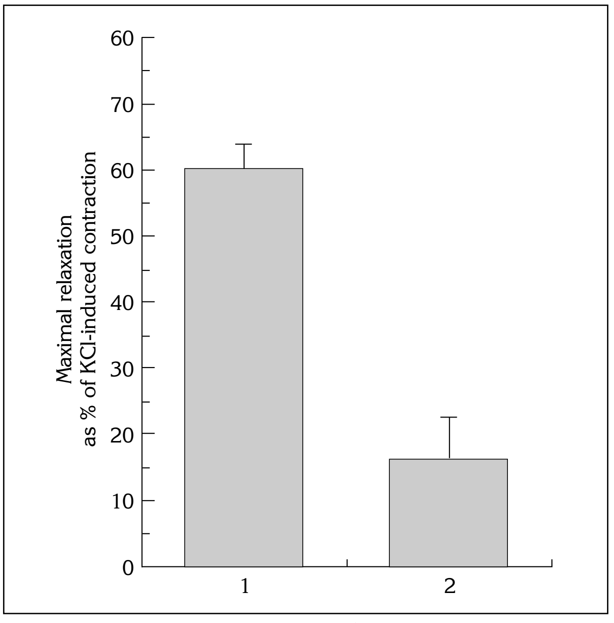

Figure 3 Maximal relaxation of isolated uterus horns (KCl-induced contraction). 1 – histamine in estrogen pretreated rats and 2 – phenylethylamine in estrogen pretreated rats.

Phenylethylamine-induced relaxation of oestrogenized rat's uterus could be the consequence of direct stimulation of H_2 histamine receptors. Phenylethylamine is described in literature as a potent inhibitor of HMT, i.e. it affects histamine metabolic turnover in various tissues by methylation of ring telenitrogen in histamine (4–6). It is also known that some of histamine methylation products show higher selectivity in different classes of histamine receptors in various tissues, but at the same time, they have rather weak biological activity (7, 8). Our results suggest that blockade of methylation (degradation) of endogenous histamine molecule in rat uterus could lead to H_2 mediated relaxation in rats treated with estrogen. It is also known that tissue histamine content and mast cell density are greater during diestrus and in mice treated with progesterone (3). Further investigations have to clarify whether the H_2 relaxation of precontracted rat uterus is the consequence of direct phenylethylamine binding to these receptors, or it is a consequence of HMT inhibition, and consequent alteration of endogenous histamine catabolism. Since there is no evidence in literature for direct phenylethylamine binding to histamine receptors, though there is strong evidence for phenylethylamine inhibition of HMT in various tissues, it seems reasonable at the moment, to accept the second hypothesis. If it is true, then the modulation of rapid turnover pool of histamine in uterus leads to a variation of biological effects of this endogenous amine. In course of this idea, the alteration of histamine metabolism could lead to various mechanisms of its actions with different consequences. So, not only does the concentration of histamine itself affect the tissue, but also variation in histamine metabolic pathways could lead to different (sometimes opposite) final effects via stimulation of histamine receptors pool.

In the end, it is very important to emphasize that we performed our experimental protocol with continuous perfusion of preparation in organ bath. This allowed us to avoid accumulation of products of metabolic degradation, because the tissue was continuously washed out. Administration of drugs in the organ bath was performed by means of micro infusion pump (125 μL/min) – small volume that cannot affect environmental conditions in the organ bath (10 mL volume, and perfusion rate of 5 mL/min). Previous experiments were predominantly performed in classic organ bath without constant washing-out of tissue, where the accumulation of degradation product could 'mask' some effects.

EFEKTI FENILETILAMINA NA ESTROGENOM TRETIRANI IZOLOVANI UTERUS PACOVA

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Kratak sadržaj: Dobro je poznato da histamin dovodi do relaksacije prekontrahovanog uterusa u životinja tretiranih estrogenom. Ovaj efekat histamina nastaje kao posledica stimulacije H_2 histaminskih receptora. Naši rezultati potvrđuju ova saznanja (u prisustvu histamina efekat relaksacije je imao vrednost EC_{50} = 40,52 ± 1,1 × 10^{-6} mol/L). Takođe, feniletilamin izaziva relaksaciju prekontrahovanog uterusa kod pacova koji su bili tretirani estrogenom (EC_{50} = 40,98 ± 1,1 × 10^{-6} mol/L, $p < 0{,}001$). Ranitidin, selektivni antagonist H_2 histaminskog receptora, umanjuje ovaj efekat feniletilamina. Blokada H_2 histaminskih receptora ranitidinom je tako pomerila udesno koncentraciono-zavisnu krivu feniletilamina tj, dovela je do inhibicije feniletilaminom izazvane relaksacije (EC_{50} = 61,42 ± 1,2 × 10^{-6} mol/L, $p<0{,}001$). S tim u vezi, feniletilamin se opisuje kao potentni inhibitor histamin-metil-transferaze (HMT) tako da modulacija katabolizma histamina možda dovodi do relaksacije prekontrahovanog uterusa pacova, tretiranog estrogenom, preko histaminskih H_2 receptora.

Ključne reči: histamin, feniletilamin, uterus, estrogen

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Received: December 20, 2004

Accepted: June 18, 2005