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 H1 and H2 histamine receptors (2). However, ovarian steroid hormones have a significant influence on applied histamine effects on precontracted rat uterus, i.e. H1 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.

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 exanguinated. Each experiment was conducted on isolated preparations from five different animals. After laparotomy, 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, CaCl₂ 0.4 mmol/L, KH₂PO₄ 1.18 mmol/L, NaHCO₃ 5.95 and glucose 2.5 mmol/L) maintained at 31 ± 1 ºC to avoid spontaneous contractions. The bath was aerated continuously with 95% O₂ and 5% CO₂. 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 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 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 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).

**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 Sabac, Serbia). The drugs were prepared on the day of experiment in NaCl 154 mmol/L (Zorka Sabac, 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 (EC₅₀) 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 ≤ 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 (EC₅₀ = 40.52 ± 1.1 × 10⁻⁶ mol/L, p < 0.001). S(+)–chlorpheniramine (5 × 10⁻⁷ mol/L), H₁ receptors antagonist, did not affect isolated uterus response to histamine (EC₅₀ = 39.41 ± 1.3 × 10⁻⁶ mol/L). Ranitidine (1 × 10⁻⁶ mol/L), H₂ receptors antagonist, shifted concentration-dependent relaxation curve of histamine to the right (EC₅₀ = 130.41 ± 1.5 × 10⁻⁶ mol/L, p < 0.001) (Figure 1).

![Figure 1](histamine+runitidine.png)
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 (EC₅₀ = 40.98 ± 1.1 × 10⁻⁶ mol/L, p < 0.001). S(+)-chlorpheniramine (5 × 10⁻⁷ mol/L), H₁ receptors antagonist, did not affect isolated uterus response to phenylethylamine in oestrogenized rats (EC₅₀ = 38.89 ± 1.1 × 10⁻⁶ mol/L). On the other hand, ranitidine (1 × 10⁻⁶ mol/L), H₂ receptors antagonist, has shifted concentration-dependent relaxation curve of phenylethylamine to the right (EC₅₀ = 61.42 ± 1.2 × 10⁻⁶ mol/L, p < 0.001) (Figure 2).

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 (EC₅₀ = 40.52 ± 1.1 × 10⁻⁶ mol/L).

In animals treated with estrogen, histamine induced relaxation is mediated only via H₂ histamine receptors because H₁ antagonist failed to prevent this effect (EC₅₀ = 40.52 ± 1.1 × 10⁻⁶ mol/L vs. EC₅₀ = 39.41 ± 1.3 × 10⁻⁶ mol/L with H₁ blockade, p > 0.05). The histamine-induced relaxation of rat uterus in animals treated with estrogen is prevented by H₂ histamine antagonist (EC₅₀ = 40.52 ± 1.1 × 10⁻⁶ mol/L vs. EC₅₀ = 130.41 ± 1.5 × 10⁻⁶ mol/L with H₂ 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 H₂ histamine receptors. This is in accordance with similar published results (2, 8) supporting the conclusion that histamine produces H₂ relaxation of KCl-precontracted uterus in estrogen pretreated animals.

Phenylethylamine produced concentration-dependent relaxation of isolated rat uterus (precontracted with KCl) in oestrogenized rats (EC₅₀ = 40.98 ± 1.1 × 10⁻⁶ mol/L, p < 0.001). In addition, it is clear that H₂ blockade has shifted concentration-dependent phenylethylamine curve to the right, i.e. produced inhibition of phenylethylamine-induced relaxation (EC₅₀ = 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).
Phenylethylamine-induced relaxation of oestrogenized rat’s uterus could be the consequence of direct stimulation of H2 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 tele-nitrogen 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 H2 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 H2 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 catal-bolism. 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 meta-bolism 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 expe-riments were predominantly performed in classic organ bath without constant washing-out of tissue, where the accumulation of degradation product could ‘mask’ some effects.

References


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