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Short communication

Histamine H_3 receptor desensitization in the guinea-pig ileum

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Abstract

Histamine H_3 receptor ligands are usually tested in guinea-pig intestine preparations. A possible desensitization of agonist-induced twitch inhibition was studied in longitudinal muscle-myenteric plexus from ileal segments. A cumulative concentration–response curve for *R*-α-methylhistamine was made; when a second curve was made 30 min afterwards, a marked decrease of pD_2 and a more modest decrease of E_{max} were observed without changes in tissue sensitivity to electrical stimulation or morphine inhibition. At 120 min, pD_2 and E_{max} were not different from those for the first curve. Receptor desensitization seems homologous and reversible and could interfere with repetitive testing of histamine H_3 receptor ligands. © 1998 Elsevier Science B.V.

Keywords: Histamine H_3 receptor; *R*-α-methylhistamine; Receptor desensitization; Ileum, guinea-pig

1. Introduction

Studies on histamine release from brain neurons performed in the early eighties led to the description of a new histamine autoreceptor which was termed H_3 (Arrang et al., 1983). Specific agonists and antagonists for this receptor were soon available (Arrang et al., 1987). Further work revealed that histamine H_3 receptors were also located on non-histaminergic cells, where they influenced the release of 5-hydroxytryptamine (Fink et al., 1990), norepinephrine (Schlicker et al., 1989), dopamine (Schlicker et al., 1993), and acetylcholine (Vollinga et al., 1992). This broad interaction with central and peripheral neurotransmitters has encouraged the study of the potential therapeutic effects of histamine H_3 receptor ligands. The medicinal chemistry of histamine H_3 receptors has been extensively reviewed by Leurs et al. (1995) and Stark et al. (1996).

A rapid, easy and effective method to test new drugs active at histamine H_3 receptors was obviously needed. The guinea-pig isolated intestine had been widely used as a bioassay for the study of drugs that influence histamine-mediated effects, and so it was chosen for histamine H_3 receptor studies. In fact, histamine H_3 receptor agonists can be evaluated with this test system since they inhibit electrically evoked twitches in a concentration-related manner by reducing acetylcholine release from postganglionic cholinergic neurons; moreover, histamine H_3 receptor blockade can be quantified by the reversal of twitch inhibition induced by reference agonists (Trzeciakowski, 1987; Vollinga et al., 1992). In our laboratory, we chose longitudinal muscle-myenteric plexus strips from the guinea-pig ileum for this kind of study and observed during preliminary experiments that repeated exposure to the histamine H_3 receptor agonist *R*-α-methylhistamine seemed to desensitize the preparation to the inhibitory action of the drug. This had been previously observed in duodenum (Coruzzi et al., 1991) but not in jejunum or ileum, although some evidence existed concerning an irregular response of jejunum strips during the first trials with *R*-α-methylhistamine (Vollinga et al., 1992). The present experiments were designed to confirm this effect, which could provide more information about the regulation of histamine H_3 receptor function. This effect could seriously interfere with in vitro studies of new ligands of this receptor. The possible modifications of heterologous mechanisms were also explored by testing the inhibitory effect of morphine on strips previously exposed to *R*-α-methylhistamine.

2. Materials and methods

The methods used were adapted from previous experiments performed in our laboratory (Alguacil et al., 1990).

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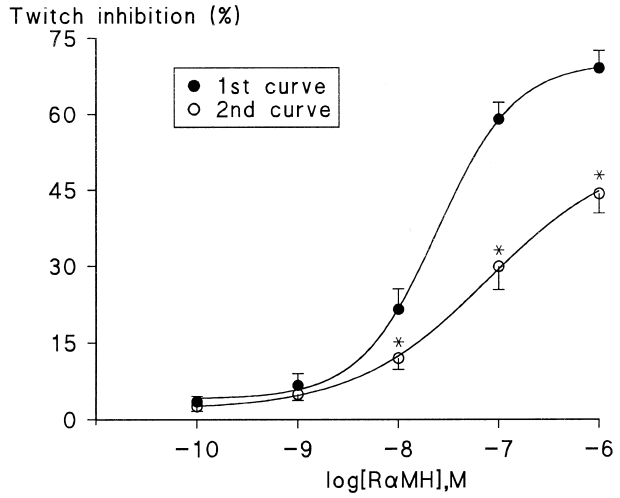

Fig. 1. Concentration–response curves of *R*-α-methylhistamine (R α MH) obtained consecutively in the same longitudinal muscle-myenteric plexus strips. The second curve was recorded 30 min after the first one. Points represent means ± S.E.M. * $P < 0.05$.

Male Dunkin–Hartley guinea-pigs (Charles River, France) weighing 300–350 g were used. Animals had free access to water and standard diet until at least 15 h before the experiments and were kept in a controlled environment (temperature 20–22°C, dark/light 12 h/12 h, humidity 45–55%). After the animals were killed by a blow on the neck and exsanguination, the ileum was excised and kept in Ringer's modified solution of the following composition (mM): NaCl, 154; KCl, 5.66; $CaCl_2$, 2.54; glucose, 2.7; $NaHCO_3$, 5.95 and choline chloride, 0.002. The last 5 cm of the terminal ileum was systematically discarded.

The longitudinal muscle with the attached myenteric plexus was obtained from 2 cm segments of ileum according to Puig et al. (1978). The strips were mounted in an organ bath at 37°C and bubbled with 95% O_2–5% CO_2 under a resting tension of 0.5 g. Twitches were recorded by isometric transducers attached to a MacLab data recording system.

After a 60 min equilibration period, field stimulation was effected with CIBERTEC stimulators through platinum electrodes, using repeated pulses (5 ms, 0.2 Hz) at supramaximal intensity (close to 0.15 mA for all tissues). Under these experimental conditions, electrically evoked twitches are atropine-sensitive (Alguacil et al., 1990). When the response reached a steady amplitude, a cumulative concentration–response curve of *R*-α-methylhistamine (RBI, USA) was recorded until the inhibitory effect of the agonist reached a maximum. The strips were then washed and the stimulation was switched off. To prevent activation of histamine H_1 receptors, concentration–response curves were always recorded in the presence of pyrilamine 0.1 μM (Sigma, Spain). Thioperamide (RBI, USA) blockade of *R*-α-methylhistamine inhibition was studied by adding different concentrations of this drug 20 min before the generation of concentration–response curves with the agonist.

The effect of *R*-α-methylhistamine exposure on subsequent concentration–response curves was studied by recording these new curves 30, 60 or 120 min after the first one. In a separate experiment, the inhibitory effect of morphine on electrically evoked twitches was examined 30 min after the generation of a concentration–response curve with *R*-α-methylhistamine and compared with the effect in naive tissues.

Concentration–effect data from each experiment were fitted to a logistic sigmoid curve. pD_2 values were obtained by extrapolation from these fittings. pA_2 of thioperamide was estimated from Schild plots.

Statistics were performed by one-way analysis of variance followed by Newman–Keuls post-hoc tests. Significance was considered at the 0.05 level.

3. Results

R-α-methylhistamine provoked a concentration-related inhibition of electrically evoked twitches, an effect which was competitively antagonized by the histamine H_3 receptor antagonist thioperamide (Schild regression slope = 0.9 ± 0.1; $pA_2 = 8.65 \pm 0.14$). Redetermination of the concentration–response curve for *R*-α-methylhistamine 30 min after the first one revealed a decreased effect of the agonist, both with regard to the maximum effect and the pD_2 (Fig. 1, Table 1). This desensitization was observed without there being any significant change in the tissue response to electrical stimulation (twitch amplitude before the first curve: 1.1 ± 0.1 g; twitch amplitude before the second curve: 1.0 ± 0.1 g; $n = 19$). The inhibitory effect

Table 1
Parameters obtained from concentration–response curves of *R*-α-methylhistamine (R α MH) after consecutive exposure to the drug

Interval between curves (min)	*n*	First curve			Second curve		
		drug	pD_2	E_{max} (%)	drug	pD_2	E_{max} (%)
30	11	Vehicle	—	—	R α MH	7.74 ± 0.10	72.97 ± 5.16
30	19	R α MH	7.74 ± 0.11	70.37 ± 2.82	R α MH	6.92[a] ± 0.18	59.46[a] ± 3.24
60	11	R α MH	7.57 ± 0.06	60.45 ± 3.06	R α MH	7.26[a] ± 0.08	50.19[a] ± 3.92
120	10	R α MH	7.81 ± 0.07	67.19 ± 2.59	R α MH	7.62 ± 0.06	60.04 ± 4.50

Results are expressed as means ± S.E.M.
[a] $P < 0.05$.

of *R*-α-methylhistamine was partially reversed 60 min after the initial concentration–response determination and did not show significant differences with respect to initial values at 120 min (Table 1). The concentration–response curve for *R*-α-methylhistamine made 30 min after vehicle was identical to that made immediately. This finding excludes a possible decrease in potency due to the delay between setting up the preparation and *R*-α-methylhistamine testing (Table 1).

Opioid inhibition of electrically induced twitches was also studied at the time when maximal desensitization to *R*-α-methylhistamine was obtained (30 min after determination of the first curve with this drug). The effect of morphine under these conditions ($pD_2 = 7.35 \pm 0.20$; $E_{max} = 92.79 \pm 2.97\%$ twitch inhibition, $n = 5$) was very similar to that observed in naive tissues ($pD_2 = 7.49 \pm 0.20$; $E_{max} = 86.91 \pm 2.95\%$ twitch inhibition, $n = 7$).

4. Discussion

The effect of *R*-α-methylhistamine was similar to that obtained by other authors. The thioperamide blockade also resembled that reported earlier, thus showing that the experimental conditions used in this study approached the settings used by other teams (Hew et al., 1990; Schlicker et al., 1994). However, we observed a marked decrease of the response to *R*-α-methylhistamine when this drug was repeatedly assayed, a finding that was not previously reported for ileal strips of the guinea-pig intestine. Poor removal of the agonist or slow dissociation from the receptor sites does not seem to explain this effect since the tissue response to electrical stimulation was identical to that obtained before exposure to *R*-α-methylhistamine. These results probably reflect a desensitization of histamine H_3 receptors after *R*-α-methylhistamine exposure. The tissue adaptation seems to be reversible since recovery of the *R*-α-methylhistamine effect was noted at 120 min. Also, the desensitization appears to be homologous since the presynaptic inhibition provided by morphine remained unaffected.

Repeated exposure of guinea-pig jejunum or longitudinal muscle-myenteric plexus strips to histamine H_3 receptor ligands has been performed previously by other authors who did not report any shift in the tissue response (Vollinga et al., 1992; Schlicker et al., 1994). Differences in the experimental protocols do not seem to be important enough to explain the lack of desensitization in those studies. Alternatively, receptor desensitization was not detected since it was not specifically studied. The present experiments provide strong evidence that exposure to increasing concentrations of *R*-α-methylhistamine can modify histamine H_3 receptor function, thus affecting the results of further studies of these receptors in the same preparation. This adaptation is in agreement with the findings of Coruzzi et al. (1991) for duodenum, although these authors reported a more sustained adaptation since it was prominent even 3 h after *R*-α-methylhistamine exposure. The faster recovery of the *R*-α-methylhistamine effect in our study could be related to the different experimental conditions, mainly the segment of the intestine selected.

Receptor desensitization could partially explain the results obtained by Vollinga et al. (1992), who discarded the first trials of histamine H_3 receptor agonists in their tissues because of *irregular* tissue responses. Consequently, it is advisable to check histamine H_3 receptor function with a parallel control group in any bioassay involving successive exposure to new putative ligands of this receptor.

Diverse mechanisms can contribute to receptor desensitization, but short-term variations in drug response are probably related to receptor uncoupling. Histamine H_3 receptors seem to belong to the family of G-protein-coupled receptors (Arrang et al., 1990), which become desensitized by phosphorylation of specific intracellular residues (Benovic et al., 1989). Desensitization, together with long-term changes such as receptor downregulation, may influence the therapeutic properties of drugs by limiting the efficacy of repeated doses (tolerance). Therefore, the effects of histamine H_3 receptor agonists should also be studied in vivo after repeated administration to check a possible decay of their pharmacological properties. The studies performed so far have not fully examined this possibility; however, Miyazaki et al. (1995) have suggested that chronic administration of *R*-α-methylhistamine to rats could modify the sensitivity of histamine H_3 receptors, thus explaining the discrepancy between their results and those reported by Smith et al. (1994) for learning deficits in the water maze test.

In summary, our results provide preliminary evidence that histamine H_3 receptor stimulation in vitro could be followed by a transient, homologous desensitization which limits the efficacy of histamine H_3 receptor agonists. Further work is needed to verify this hypothesis. The study of different periods of incubation with *R*-α-methylhistamine and the use of a second receptor agonist (i.e. immepip) are highly recommended. It is also advisable to check the relevance of this possible desensitization in vivo.

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