Naunyn-Schmiedeberg's Arch Pharmacol (1992) 346:208–212

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Activation of histamine H_3 receptors produces presynaptic inhibition of neurally evoked cat nictitating membrane responses in vivo

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Received January 21, 1992/Accepted April 7, 1992

Summary. This study was undertaken in order to determine the potential role of prejunctional histamine H_3 receptors in an in vivo adrenergic model system. Frequency-dependent nictitating membrane responses were elicited by sympathetic nerve stimulation in anesthetized cats. Systemic administration of the selective histamine H_3 receptor agonist, (R)-α-methylhistamine (RαMeHA) produced a dose-related depression of amplitude of the evoked nictitating membrane responses with a threshold of about 10 μg/kg and maximal effect (50% depression at the lowest frequency; 0.5 Hz) seen at 100–300 μg/kg. Responses obtained with low frequency stimulation were more sensitive to depression by RαMeHA than were responses evoked with higher frequencies of stimulation. Larger doses of RαMeHA given to the same animals, failed to produce additional inhibition.

RαMeHA depressed the amplitude of nictitating membrane responses evoked by either pre- or postganglionic nerve stimulation to an equivalent degree. This depressant action of RαMeHA was antagonized by pretreatment with the specific histamine H_3 antagonist, thioperamide (3 mg/kg), but not by combined pretreatment with histamine H_1 and H_2 blockers chlorpheniramine (300 μg/kg) and cimetidine (5 mg/kg). Intravenous administration of adrenaline (1–30 μg/kg) also produced graded nictitating membrane responses that were not altered by subsequent administration of RαMeHA.

These results suggest that histamine H_3 receptors are involved in the modulation of neurally evoked noradrenaline release in the cat nictitating membrane by an inhibitory presynaptic action. The most likely site of drug action appears to be at the neuroeffector junction as no appreciable ganglionic effect of RαMeHA was observed in this in vivo model system.

Key words: Histamine H_3 receptors – Cat nictitating membrane – (R)-α-Methylhistamine – Thioperamide – Presynaptic inhibition

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Introduction

It is now generally accepted that there are at least three distinct subtypes of histamine receptors existing in both the CNS and in peripheral tissues (for reviews see Schwartz et al. 1986, 1990; Timmerman 1990). The more recently characterized histamine H_3 receptor subtype was originally discovered in brain tissue where it acts presynaptically to inhibit the release (and synthesis) of histamine (Arrang et al. 1983, 1987; Schwartz et al. 1986) as well as the release of other putative neurotransmitters such as 5-HT (Fink et al. 1990).

The availability of selective histamine H_3 receptor agonists and antagonists (Arrang et al. 1987; Van der Werf and Timmerman 1989) has fostered a number of recent investigations demonstrating presynaptic neural inhibition by histamine H_3 receptor activation in cholinergic (Trzeciakowski 1987; Tamura et al. 1988; Ichinose et al. 1989; Hew et al. 1990; Menkveld and Timmerman 1990; Poli et al. 1991; Coruzzi et al. 1991), adrenergic (Ishikawa and Sperelakis 1987; Schlicker et al. 1990; Luo et al. 1991; Malinowska and Schlicker 1991; Vassilev et al. 1991), and peptide mediated peripheral systems (Ichinose and Barnes 1989). With two exceptions (Ichinose and Barnes 1989; Malinowska and Schlicker 1991), all of the above mentioned investigations concern in vitro model systems.

The present study was undertaken to determine, in vivo, if the highly selective histamine H_3 agonist, (R)-α-methylhistamine (RαMeHA) would inhibit neurally evoked contractions of the cat nictitating membrane by a prejunctional mechanism. Questions concerning agonist dose-response relationships, potential tachyphylaxis, and site of action (pre- vs postganglionic) were also addressed in this study.

Methods

General. Adults cats of either sex were anesthetized with α-chloralose (60–80 mg/kg, i.p.). The trachea was intubated for ventilation with a Harvard respirator using room air. A femoral artery and vein were can-

nulated for recording of arterial blood pressure (Statham P 23 transducer) and for i.v. drug administration. In order to reduce artifacts due to activation of extraocular muscles the animals were immobilized by systemic administration of gallamine triethiodide (3–5 mg/kg). Body temperature was maintained at approximately 37 °C with a heating lamp placed above the animal. The preparations were fixed in a stereotaxic instrument (David Kopf) and the cervical vagosympathetic nerve trunks were sectioned. Nictitating membrane contractions were recorded using a Grass FT.03 force-displacement transducer, the initial tension set at 10 g (Westfall et al. 1969; Koss and Rieger 1976). All physiological measurements were recorded on a Grass polygraph (7D).

Sympathetic nerve stimulation. Bipolar silver electrodes were placed under the distal portion of the ligated pre- or postganglionic cervical sympathetic nerve and covered with warm mineral oil. The stimuli, generated by a Grass stimulator and isolation unit, consisted of 8 s trains of supramaximal pulses (6–12 V). We have previously demonstrated that 8 s train durations are of sufficient length to develop a maximal response at each frequency of stimulation (Koss and Rieger 1976). The pulse width was 1 ms and the frequency was varied between 0.5 Hz and 32 Hz. Each response was allowed to recover fully before the next highest frequency of stimulation was tested. At least two replicable control frequency-response curves were performed in each experiment. Stability of these responses, over time, was determined using saline controls and is consistent with previous studies using this model system (Koss and Gherezghiher 1988).

Statistics and drugs used. Values are reported as means ± SEM. Statistical differences between treatments were determined using Student's *t*-test for paired comparisons with statistical significance accepted at $p<0.05$. All drug solutions were prepared in physiological saline with the exception of thioperamide which was initially dissolved in 0.5 mol/l acetic acid (30 mg/ml) and further diluted and pH adjusted with phosphate buffer to a final concentration of 3 mg/ml. (R)-α-Methylhistamine dihydrochloride, thioperamide, chlorpheniramine maleate, and cimetidine were supplied by Schering-Plough Research, Bloomfield, N.J., USA. (−)-Adrenaline hydrochloride was obtained from Sigma Chemical Co. (St. Louis, Mo., USA). Drug dosages refer to the respective free base.

Results

Graded frequency-dependent contractions of the nictitating membrane were evoked by electrical stimulation of the ipsilateral sympathetic nerve. As shown in the composite representation (Fig. 1), stimulation produced a near linear increase in the force of nictitating membrane contraction as the frequency was increased from 0.5 to 32 Hz. Subsequent frequency-response curves (after saline) showed comparable response amplitudes at all frequencies of activation (Fig. 1). None of the drugs with affinity to histamine receptors used in this study altered basal (denervated) nictitating membrane tone nor did any of the histamine receptor antagonists significantly alter subsequently generated frequency-response curves (data not shown).

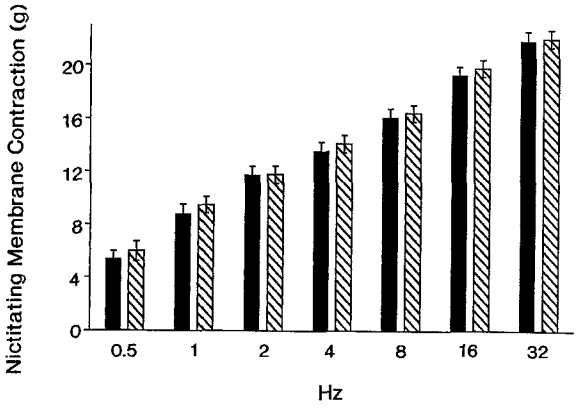

Fig. 1. Frequency-response curves for preganglionic electrical activation of the nictitating membranes in chloralose anesthetized cats before and 10 min after intravenous administration of saline. Values represent means ± SEM for 16 animals. Note stability of repeated frequency-response relationships. ■ Control; ▨ post-saline

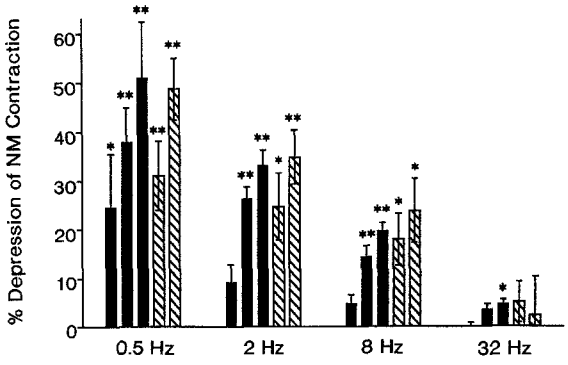

Fig. 2. Composite representation of effects of intravenous administration of (R)-α-methylhistamine (RαMeHA) on neurally evoked nictitating membrane (NM) responses. Values represent % depression at the frequencies shown for two groups of cats. Height of bars represent means ± SEM for 4 cats in each group. Group one (*solid bars*) received three sequential doses of RαMeHA (10, 30 and 100 μg/kg); the second group (*cross-hatched bars*) was given two sequential doses (100 and 300 μg/kg). Statistics based on comparison with pretreatment control responses (*$P<0.05$; **$P<0.01$)

RαMeHA dose-response curves

The specific histamine H_3 receptor agonist, (R)-α-methylhistamine (RαMeHA) was administered to separate groups of animals with the effect on preganglionic nictitating membrane frequency-response curves observed. In the first group, three doses of RαMeHA were administered (10, 30 and 100 μg/kg) with about 10 min intervals between the frequency-response curves. As illustrated (Fig. 2), RαMeHA caused a significant dose-dependent depression of response amplitude with the threshold effect seen at the 10 μg dose. Depression of evoked nictitating membrane contractile responses was most clearly seen at the lower frequencies of stimulation. A maximal effect of about 50% inhibition was seen at 100 μg/kg for 0.5 Hz. This same dose produced approximately 30% and 20% inhibition of nictitating membrane responses evoked with 2 and 8 Hz respectively.

The second group of cats (Fig. 2) were given two sequential doses of RαMeHA (100 and 300 μg/kg) with essentially the same response pattern observed. In this group, however, the 300 μg/kg dose of RαMeHA appeared to be somewhat more effective than the 100 μg/kg dose.

In other experiments, frequency-response curves were generated by pre- and postganglionic stimulation with the effects of RαMeHA at 300 μg/kg, i.v. tested (Fig. 3). At this dose level, RαMeHA produced a similar degree of

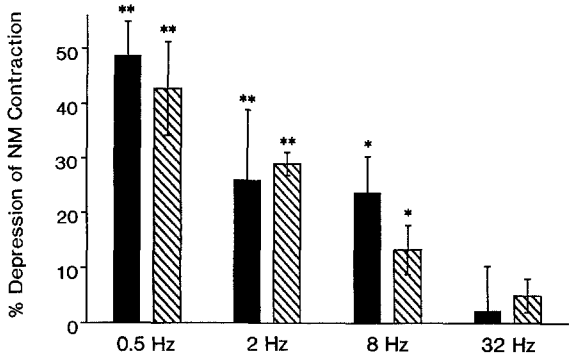

Fig. 3. Comparison of the depressant actions of (R)-α-methylhistamine (RαMeHA; 300 μg/kg, i.v.) on nictitating membrane (NM) responses evoked by electrical stimulation of pre- and postganglionic nerve fibers (n = 4 and 5 respectively). Values (means±SEM) are expressed as % depression from the control levels. Statistics based on comparison with pretreatment control responses (*$P<0.05$; **$P<0.01$). ■ Preganglionic; ▨ postganglionic

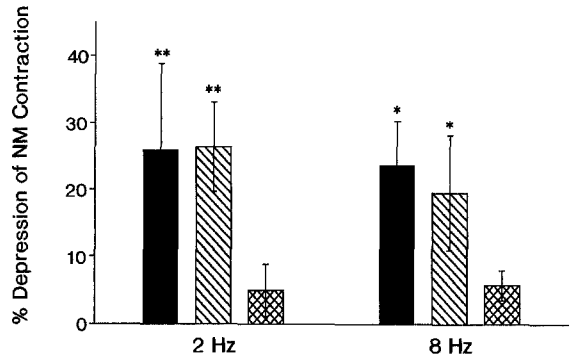

Fig. 4. Composite representation of % depression of amplitude of preganglionically evoked nictitating membrane (NM) responses produced by intravenous (R)-α-methylhistamine (RαMeHA; 300 μg/kg) in cats pretreated intravenously with H_1 and H_2 blocking drugs, chlorpheniramine (300 μg/kg) and cimetidine (5 mg/kg) or with the H_3 antagonist, thioperamide (3 mg/kg). Note the lack of significant inhibitory effect in the thioperamide pretreated group. Values represent means±SEM for 4 cats in each group. Statistics based on comparison with pretreatment control responses (*$P<0.05$; **$P<0.01$). ■ Control (RαMeHA 300 μg/kg); ▨ post H_1 & H_2 blockade; ▩ post H_3 blockade

frequency-dependent inhibition of evoked nictitating membrane responses with no significant difference observed between the two stimulation sites.

Administration of histamine receptor antagonists

RαMeHA (300 μg/kg) was administered to cats pretreated i.v. with either chlorpheniramine (300 μg/kg) and cimetidine (5 mg/kg) or with thioperamide (3 mg/kg) for blockade of histamine H_1 and H_2 or H_3 receptor respectively. As can be seen (Fig. 4), combined histamine H_1 and H_2 receptor blockade caused no antagonism of the RαMeHA inhibitory effect. In contrast, histamine H_3 receptor blockade with thioperamide largely prevented the subsequent depressant action of RαMeHA.

Direct activation with adrenaline

The final series of experiments were undertaken to determine if RαMeHA might act postjunctionally in producing depression of neurally evoked NM responses. Toward this end, (−)-adrenaline was administered intravenously in graded doses (1−30 μg/kg) before and after RαMeHA (300 μg/kg, i.v.) administration. As can be seen (Fig. 5), (−)-adrenaline produced graded dose-related nictitating membrane contractions that were not significantly altered by subsequent administration of RαMeHA.

Discussion

Histamine has long been implicated as a modulator of neurotransmitter release from peripheral adrenergic nerves causing either facilitation (Bevan et al. 1975) or inhibition (McGrath and Shepherd 1976; Powell 1979) of vascular contractions due to sympathetic nerve stimulation. Using the most selective antagonists available at the time, it was concluded that stimulation of histamine H_1 receptors increases and that activation of putative histamine H_2 receptors inhibits neuronal release of noradrenaline (Powell 1979; Suzuki and Kou 1983). Similarly, with regard to cholinergic transmission, a possible variant of the H_2 receptor has been reported to be responsible for histamine inhibition of neurally evoked guinea pig ileum contractions (Ambache and Aboo Zar 1970; Fjalland 1979).

More recently, a third histamine receptor subtype (H_3) has been characterized in brain tissue where it inhibits both histamine synthesis and release (Arrang et al. 1983). The discovery and availability of highly selective agonists (e.g., (R)-α-methylhistamine) and antagonists (e.g., thioperamide) for the H_3 receptor subtype (Arrang et al. 1987) has greatly aided in the interpretation of the putative histamine receptor subtypes involved in the above mentioned physiological responses to histamine.

In the present investigation we choose the cat nictitating membrane as a classical model to study prejunctional modulation of adrenergic transmission in vivo. Ad-

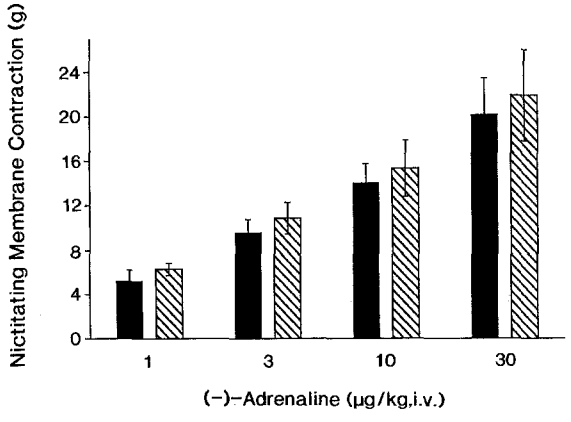

Fig. 5. Effect of (R)-α-methylhistamine (RαMeHA; 300 μg/kg, i.v.) on contractions of the nictitating membranes of four anesthetized cats produced by intravenous administration of (−)-adrenaline (1−30 μg/kg, i.v.). The second dose-response curve to (−)-adrenaline was generated 10 min after RαMeHA administration. Values represent means±SEM. ■ Control; ▨ RαMeHA 300 μg/kg

vantages of this neuroeffector system include ability to produce stable and reproducible responses (contractions) over the full frequency-response range, accessibility of pre- and postganglionic nerves, and use of a well characterized effector that contains mainly neurally activated α_1-adrenoceptors postsynaptically (Koss and Rieger 1976; Koss and Gherezghiher 1988).

Systemic administration of RαMeHA produced a consistent dose-related depression of preganglionically elicited nictitating membrane contractions. The threshold dose was approximately 10 µg/kg; the maximal effect (50% depression at 0.5 Hz) was seen at dosages in the 100–300 µg/kg range. No apparent tachyphylaxis to the inhibitory effects of RαMeHA were observed in this study as the same maximal per cent depression of evoked nictitating membrane responses was seen at the 300 µg/kg dose when given either as a single dose or when preceded by initial lower dose injections.

The present results are not entirely consistent with the effects of RαMeHA reported in a previous in vivo study (Ichinose and Barnes 1989). In their study, vagal nerve stimulation evoked non-adrenergic, non-cholinergic bronchoconstrictor responses in the guinea pig that were inhibited about 50% by RαMeHA at doses of 3–10 mg/kg. The threshold dose of RαMeHA (about 1 mg/kg) was 2 orders of magnitude higher than in the present study. These differences may be related to the use of different species and effector organs (which may demonstrate different sensitivities to RαMeHA), or may be due to differences in intensity of stimulation as the greatest effects in the present study were seen at the lower frequencies of electrical stimulation. Similar frequency dependence of inhibition of CNS histamine release has been reported previously for CNS histamine autoreceptor (Timmerman 1990) and heteroreceptor (Schlicker et al. 1991) activation. In addition, pretreatment with a higher dose of cimetidine (10 mg/kg) may have partially blocked H_3 receptors (Schwartz et al. 1990) causing an increase of the dose of RαMeHA needed to depress their responses.

On the other hand, the doses of RαMeHA used in the present study closely resemble those used by Malinowska and Schlicker (1991) in a recent study showing similar modulation of neurogenic evoked blood pressure elevations elicited by spinal cord stimulation in pithed rats. In their study, low frequency stimulations produced blood pressure elevations that were antagonized by graded injections of RαMeHA (0.01–10 µmol/kg, i.v.). RαMeHA did not reduce noradrenaline-induced vasopressor responses and inhibition of the neurally evoked responses was selectively blocked by thioperamide.

We found no evidence for a ganglionic inhibitory action of RαMeHA in this study as the degree of inhibition was comparable whether transmitter release was evoked by pre- or postganglionic nerve stimulation. This conclusion is contrary to the supposition put forth by Ichinose et al. (1989) concerning neurally induced cholinergic constriction of the guinea pig trachea as well as results showing existence of inhibitory presynaptic histamine H_3 receptors in guinea pig enteric ganglia (Tamura et al. 1988). It may well be that the presynaptic inhibitory effect of histamine reported in the superior cervical ganglion is mediated, as originally concluded, by histamine H_2 receptors (Brimble and Wallis 1973; Yamada et al. 1982; Snow and Weinreich 1987) and not by histamine H_3 receptors (see Tamura et al. 1988 and Timmerman 1990 for discussion). Of course, it is also possible that differential transmission may occur within a given autonomic ganglia depending upon which specific effector organ is studied (Koss and Rieger 1976). Our observations concerning the lack of inhibitory effect of RαMeHA on adrenaline induced nictitating membrane contractions further supports the conclusion that RαMeHA exerts its inhibition by a presynaptic action as has been shown for other autonomic systems and not by a direct action on the smooth muscle of the nictitating membrane.

These studies add to the growing body of evidence supporting an inhibitory role for presynaptic histamine H_3 receptors by demonstrating that neurally evoked nictitating membrane contractions are modulated in vivo by RαMeHA activation of prejunctional histamine H_3 receptors.

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