ELSEVIER

European Journal of Pharmacology 419 (2001) 55–59

ejp

www.elsevier.nl/locate/ejphar

Histamine H_3 receptor-mediated inhibition of sympathetically evoked mydriasis in rats

Yongxin Yu [a], Masahiro Kawarai [b], Michael C. Koss [a,*]

[a] *Department of Cell Biology, University of Oklahoma College of Medicine, PO Box 26901, Oklahoma City, OK 73190, USA*
[b] *Department of Biosciences, Teikyo University of Science and Technology, Yamanashi, Japan*

Received 18 January 2001; received in revised form 19 March 2001; accepted 6 April 2001

Abstract

This study was designed to determine if the histamine H_3 receptor agonist *R*-α-methylhistamine would play a role in modulation of sympathetically evoked mydriasis in anesthetized rats, and if so, to ascertain the specific receptor subtype(s) involved. Reproducible frequency–response curves of pupillary dilation were generated by stimulation of the cervical preganglionic sympathetic nerve (1–32 Hz). Systemic administration of *R*-α-methylhistamine (0.3–3.0 mg kg^{-1}) produced a dose-related inhibition of the evoked mydriasis. The greatest inhibition was seen at lower frequency levels, with about 43% depression observed at 2 Hz. The specific histamine H_3 receptor antagonist, clobenpropit (3.0 mg kg^{-1}, i.v.), blocked the inhibitory effect of *R*-α-methylhistamine, whereas neither the histamine H_2 receptor antagonist, cimetidine (5.0 mg kg^{-1}, i.v.), nor the histamine H_1 receptor antagonist, chlorpheniramine (0.5 mg kg^{-1}, i.v.), was effective. The histamine H_2 receptor agonist, dimaprit (10 mg kg^{-1}, i.v.), was also without effect on the evoked mydriasis. *R*-α-methylhistamine (3.0 mg kg^{-1}) did not inhibit phenylephrine-induced mydriasis. These results support the conclusion that *R*-α-methylhistamine produces inhibition of sympathetically evoked mydriasis via histamine H_3 receptor stimulation, presumably by an action on presynaptic histamine H_3 receptors. © 2001 Published by Elsevier Science B.V.

Keywords: Histamine H_3 receptor; *R*-α-methylhistamine; Clobenpropit; Mydriasis; Iris, rat; Sympathetic nerve stimulation

1. Introduction

Initially discovered as autoreceptors in the brain (Arrang et al., 1983), histamine H_3 receptors were also found to act as heteroreceptors in the periphery, negatively modulating the release of a variety of transmitters including noradrenaline, acetylcholine and neuropeptides (for reviews, see Hill et al., 1997; Malinowska et al., 1998). The presence of histamine H_3 receptors has been confirmed in recent cloning studies (Lovenberg et al., 2000).

In the peripheral sympathetic system, presynaptic histamine H_3 receptors have been identified in different species and tissues. For example, Ishikawa and Sperelakis (1987) first observed in guinea pig mesenteric artery, in vitro, that perivascular nerve terminals are endowed with these receptors, which, upon activation, depress sympathetic neurotransmission. Similar results were also obtained in human saphenous vein (Molderings et al., 1992) and pig retina (Schlicker et al., 1990). In vivo, activities of these receptors have been demonstrated in the resistance vessels of guinea pigs and rabbits (McLeod et al., 1994). It is also clear that histamine H_3 receptors inhibit sympathetically evoked pupillary dilations, nictitating membrane contractions and sympathetic–cholinergic sudomotor responses by a presynaptic mechanism (Koss and Hey, 1992, 1993; Koss, 1994) in cats.

In rats, however, the situation seems different. It was reported that histamine H_3 presynaptic receptors are involved in the rat cardiovascular system (Malinowska and Schlicker, 1991, 1993; Smit et al., 1997), as sympathetically evoked vasopressor responses are selectively diminished by the histamine H_3 receptor agonist *R*-α-methylhistamine (Arrang et al., 1987). In contrast, McLeod et al. (1994) showed clearly that *R*-α-methylhistamine decreases systemic arterial blood pressure in both guinea pigs and rabbits, while having no effect in rats. Similar studies in anesthetized and pithed rats also do not support the involvement of these receptors (Hegde et al., 1994). In addition, it has even been reported that presynaptic histamine H_2 receptors, rather than histamine H_3 receptors,

* Corresponding author. Tel.: +1-405-271-2107; fax: +1-405-271-3415.
E-mail address: Michael-koss@ouhsc.edu (M.C. Koss).

0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00977-3

are present in the hindquarter vasculature (Holcslaw and Lassiter, 1987) and vas deferens (Poli et al., 1994) of rats.

In view of these controversies, this study was undertaken to determine if *R*-α-methylhistamine would play a role in modulation of sympathetically evoked mydriasis in anesthetized rats, as it does in cats, and if so, to ascertain the specific receptor subtype(s) involved. Our results support the conclusion that *R*-α-methylhistamine produces inhibition of sympathetically evoked mydriasis via histamine H_3 receptor stimulation, presumably by stimulation of presynaptic histamine H_3 receptors.

2. Methods

2.1. General

Male Sprague–Dawley rats (300–450 g) were anesthetized with pentobarbital (60 mg kg^{-1}, i.p.). The trachea was intubated and a femoral artery and vein were cannulated for monitoring blood pressure (Statham P23 pressure transducer) and for i.v. drug administration, respectively. One cervical preganglionic sympathetic trunk was carefully separated from the vagus and crushed proximally. Rectal temperature was maintained at approximately 37°C with a Deltaphase isothermal pad. Pupillary diameter was measured using a ruler with a 0.1-mm scale under an Olympus surgical microscope with a green light filter. Heart rate was derived from the pressure wave using a Grass tachograph (7P4D).

2.2. Sympathetic nerve stimulation

A bipolar electrode was placed under the separated cervical sympathetic nerve (proximal to superior cervical ganglion) and covered with mineral oil. The stimuli, generated by a Grass S88 stimulator, consisted of 10-s trains of 3–5 V pulses (width 2 ms, frequency 1–32 Hz), which elicited repeatable pupillary dilations over time up to 1 h. Each pupillary response was allowed to recover fully before the next higher frequency of stimulation was performed. Stimulations were performed approximately 15 min after i.v. drug or saline administration.

2.3. Drugs and statistics

R-(−)-α-methylhistamine dihydrochloride, cimetidine, (±)-chlorpheniramine maleate and L-phenylephrine hydrochloride were supplied by Sigma. Clobenpropit dihydrobromide and dimaprit dihydrochloride were provided from Research Biochemical International (RBI). All solutions of drugs were prepared in physiological saline. Doses represent the respective salts and were chosen according to previous studies (Kathmann et al., 1993; Malinowska and Schlicker, 1993; Mcleod et al., 1994; Godlewski et al., 1997a). Values were reported as means ± S.E.M. Statistical significance was determined using a one-way analysis of variance (ANOVA) and Student's paired *t*-test. *P*-values of less than 0.05 were considered significant.

3. Results

3.1. Responses to sympathetic nerve stimulation

As shown in Fig. 1, electrical stimulation of decentralized preganglionic cervical sympathetic nerve produced reproducible frequency–response curves of pupillary dilation at 1–32 Hz in anesthetized rats. None of the histamine agonists or antagonists used in this study had any observable effect on the resting pupillary size. In addition, none of the histamine antagonists (chlorpheniramine, cimetidine and clobenpropit) per se caused any significant shift of the frequency–response curves (data not shown). The contralateral pupillary size was recorded in a group of rats and no changes were observed during the nerve stimulation, indicating lack of reflex effects.

3.2. Graded inhibitory effect of R-α-methylhistamine on sympathetically evoked mydriasis

The selective histamine H_3 receptor agonist *R*-α-methylhistamine was administered intravenously to anesthetized rats with the effect on the evoked frequency–response curves observed. Three cumulative doses (0.3, 1.0 and 3.0 mg kg^{-1}) were administered with approximately 15-min intervals between subsequent frequency–response curves. As illustrated in Fig. 2, *R*-α-methylhistamine produced a significant dose-related inhibition of mydriasis evoked by preganglionic sympathetic nerve stimulation. The maximal depression elicited by *R*-α-methylhistamine (3.0 mg kg^{-1}) was seen at lower frequencies of stimula-

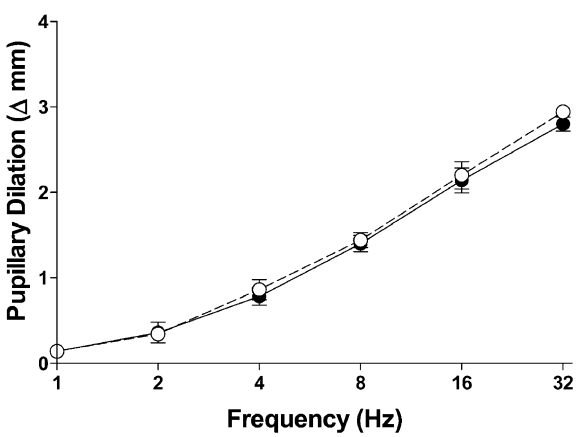

Fig. 1. Frequency–response curves for electrical stimulation of the decentralized preganglionic cervical sympathetic nerve to the iris dilator muscle in pentobarbital anesthetized rats before (clear circles) and 1 h after (solid circles) intravenous administration of saline (0.5 ml). Values represent means ± S.E.M. of evoked pupillary dilation for five animals. Note the stability of the pupillary response curves over time.

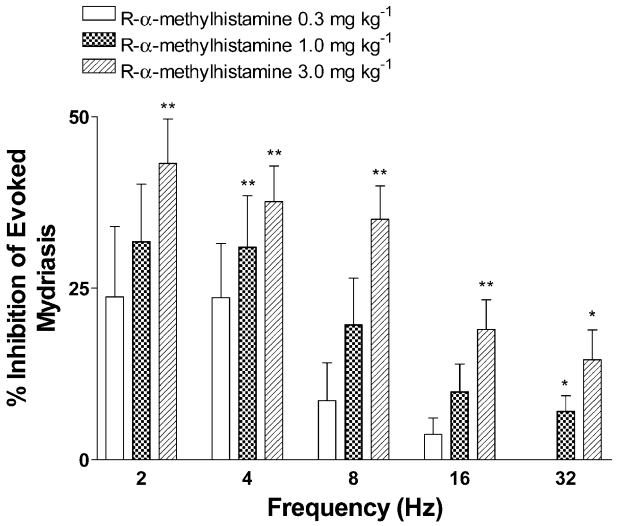

Fig. 2. Effects of intravenous administration of *R*-α-methylhistamine on sympathetically evoked mydriatic responses in anesthetized rats. Values represent percentage inhibition of mydriasis expressed as means ± S.E.M. Three cumulative doses of *R*-α-methylhistamine (0.3, 1.0 and 3.0 mg kg^{-1}) were used with a 15-min time interval between subsequent frequency–response curves. The percentage values were calculated by comparing with the pretreatment response as 100%. Note that *R*-α-methylhistamine produced a dose-related inhibition of the evoked mydriasis. $^{*}P < 0.05$; $^{**}P < 0.01$; $n = 4$–9; 0.3 mg kg^{-1}, clear bars; 1.0 mg kg^{-1}, dotted bars; 3.0 mg kg^{-1}, hatched bars.

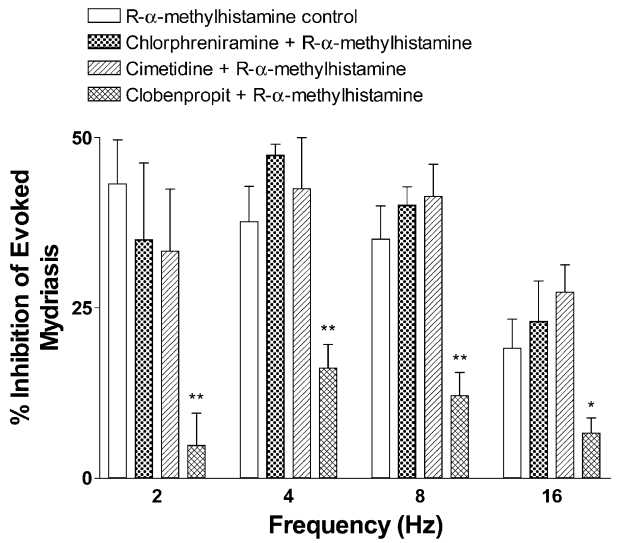

Fig. 3. Inhibition of evoked pupillary responses produced by intravenously administered histamine H_3 receptor agonist *R*-α-methylhistamine (3.0 mg kg^{-1}) in anesthetized rats pretreated with the histamine H_1 receptor antagonist, chlorpheniramine (0.5 mg kg^{-1}), the histamine H_2 receptor antagonist, cimetidine (5.0 mg kg^{-1}), or the histamine H_3 receptor antagonist, clobenpropit (3.0 mg kg^{-1}). Note that only the histamine H_3 receptor antagonist clobenpropit prevented the inhibitory action of *R*-α-methylhistamine. Values represent means ± S.E.M. $^{*}P < 0.05$; $^{**}P < 0.01$; Control group, clear bars, $n = 8$; Chlorpheniramine group, dotted bars, $n = 5$; Cimetidine group, hatched bars, $n = 5$; Clobenpropit group, cross-hatched bars, $n = 7$.

tion, with about 43% inhibition observed at 2 Hz. Further increase of its dose to 10 mg kg^{-1} did not cause an additional inhibitory effect (data not shown). Note that each dose of *R*-α-methylhistamine produced proportionally less inhibition of evoked responses as the frequency of stimulation was increased.

3.3. Histamine H_3 receptors mediate the inhibitory effect by R-α-methylhistamine

As shown in Fig. 3, a single dose of *R*-α-methylhistamine (3 mg kg^{-1}) was administered to anesthetized rats pretreated intravenously with the histamine H_3 receptor antagonist, clobenpropit (3 mg kg^{-1}), the histamine H_2 receptor antagonist, cimetidine (5 mg kg^{-1}) or the histamine H_1 receptor antagonist, chlorpheniramine (0.5 mg kg^{-1}). Only the histamine H_3 receptor antagonist, clobenpropit, blocked the inhibitory effect of *R*-α-methylhistamine, whereas neither histamine H_1 nor histamine H_2 receptor antagonist was effective.

3.4. Histamine H_2 receptor agonist has no effect on the evoked mydriasis

In four anesthetized rats, a single dose of the histamine H_2 receptor agonist dimaprit (10 mg kg^{-1}) was administered intravenously with the frequency–response curves recorded. Dimaprit elicited no obvious effect on sympathetically evoked mydriasis (Fig. 4).

3.5. R-α-methylhistamine does not inhibit phenylephrine-induced mydriasis

To determine whether *R*-α-methylhistamine might exert an inhibitory effect at postsynaptic sites, three doses of the α_1-adrenoceptor agonist, phenylephrine (3, 10 and 33 μg kg^{-1}), were injected intravenously before and 15 min after *R*-α-methylhistamine (3.0 mg kg^{-1}, i.v.) administration. Small (less than 1 mm), but highly consistent, mydriatic

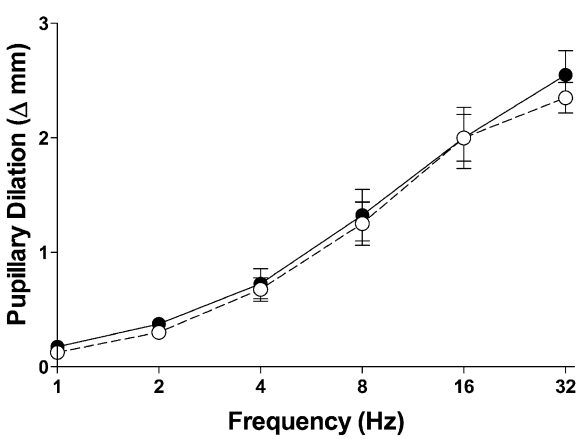

Fig. 4. Sympathetically evoked mydriasis before (clear circles) and after (solid circles) a single dose of the selective histamine H_2 receptor agonist, dimaprit (10 mg kg^{-1}, i.v.), in four anesthetized rats. Values represent means ± S.E.M. Note that dimaprit had no significant effect on the evoked mydriatic responses.

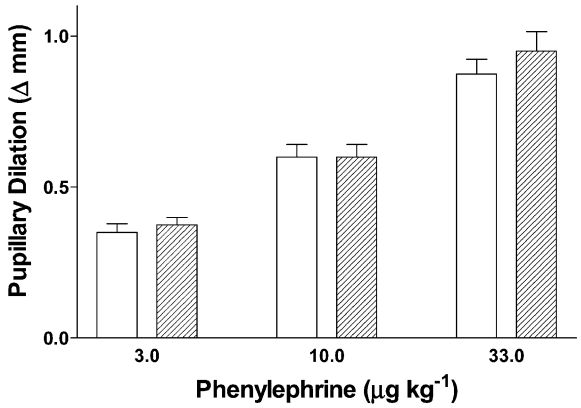

Fig. 5. Pupillary dilations induced by phenylephrine (3, 10 and 33 μg kg^{-1}, i.v.) before (clear bars) and 15 min after (hatched bars) *R*-α-methylhistamine (3.0 mg kg^{-1}, i.v.) administration in four anesthetized rats. Values represent means ± S.E.M. Note that *R*-α-methylhistamine did not block phenylephrine-induced mydriasis.

responses were elicited in response to phenylephrine. As can be seen, *R*-α-methylhistamine did not alter phenylephrine-induced mydriasis. Due to marked cardiovascular effects, higher doses of phenylephrine were not examined (Fig. 5).

4. Discussion

Our present data show that the selective histamine H_3 receptor agonist, *R*-α-methylhistamine, dose-dependently inhibited sympathetically evoked mydriasis. This inhibitory effect was significantly blocked by the selective histamine H_3 receptor antagonist, clobenpropit. In contrast, neither the histamine H_2 receptor antagonist, cimetidine, nor the histamine H_1 receptor antagonist, chlorpheniramine, blocked the effect of *R*-α-methylhistamine. These results support the presence of histamine H_3 receptors in the rat iris dilator muscle. The histamine H_2 receptor agonist, dimaprit, did not exert any inhibitory effect on the evoked mydriasis, suggesting that histamine H_2 receptors can be excluded.

A fourth histamine receptor subtype has been cloned recently (Oda et al., 2000). Different from histamine H_3 receptors, the distribution of this novel histamine H_4 receptor seems to be restricted to cells of the immune system. In vitro studies suggested that *R*-α-methylhistamine causes activation of this receptor subtype (Oda et al., 2000). However, clobenpropit also shows an agonistic activity (ED_{50} = 8.22 nM) on histamine H_4 receptors, in contrast to its antagonistic effect on histamine H_3 receptors, suggesting that it is not likely that histamine H_4 receptors are directly involved in our model system.

Postsynaptic histamine H_3 receptors have been reported in the rabbit middle cerebral artery (Ea-Kim and Oudart, 1988) and rat aorta (Djuric and Andjelkovic, 1995). Activation of these receptors by *R*-α-methylhistamine relaxes precontracted vessels, which can be antagonized by selective histamine H_3 antagonists. However, in our system, a postsynaptic location of histamine H_3 receptors is unlikely, as *R*-α-methylhistamine did not alter resting pupil size. Furthermore, *R*-α-methylhistamine (3 mg kg^{-1}), which significantly inhibits electrically evoked mydriasis, did not antagonize phenylephrine-induced mydriasis. Thus, a presynaptic location of histamine H_3 receptors is most probable. Due to technical difficulties in rats, only preganglionic stimulations were performed in our study. A ganglionic location of this receptor could not be totally excluded. However, as found in our previous studies (Koss and Hey, 1992, 1993), *R*-α-methylhistamine acts primarily on the postganglionic sympathetic nerve terminals.

It should be noticed that the inhibitory effect of *R*-α-methylhistamine was frequency-related, i.e. with an increase of stimulation frequency, there was less inhibition of the evoked mydriasis. The maximal inhibition elicited by *R*-α-methylhistamine (3.0 mg kg^{-1}) was seen at lower frequencies, with about 43% depression observed at 2 Hz. These results are comparable to the data from other groups in this species (Malinowska and Schlicker, 1991; Godlewski et al., 1997a,b; Smit et al., 1997), with a range from 20% to 43%, as found in our present study. This inverse frequency–efficacy relationship has been discussed as a general characteristic of presynaptic receptors (Duckles and Budai, 1990).

The physiological significance of presynaptic histamine H_3 receptors is still unclear. Tonic activation of these receptors by tissue histamine has been observed in rat vasculature (Godlewski et al., 1997a; Acuña et al., 1998). In guinea pig cardiac tissues, histamine H_3 receptors appear to be quiescent under normal situations or even during adrenergic nerve stimulation, while they are clearly active and play an important modulatory role in ischemic conditions, due to greatly increased histamine spillover (Levi and Smith, 2000). In our model, systemic administration of clobenpropit 3.0 mg kg^{-1} did not enhance the evoked mydriasis. Thus, it seems unlikely that endogenous histamine is tonically activating these receptors. It is not known if such an effect might appear under pathological situations such as ocular inflammation.

In conclusion, this is the first report to show that *R*-α-methylhistamine produces inhibition of sympathetically evoked mydriasis via histamine H_3 receptor stimulation in rats. The mechanism of action supports a prejunctional location of histamine H_3 receptors, most probably on the postganglionic sympathetic nerve terminals. This study adds to the growing body of evidence that presynaptic histamine H_3 receptors are functionally involved in the peripheral sympathetic system of rats.

Acknowledgements

This work was supported by NIH Grant EY09344. The authors are grateful for the expert technical assistance provided by Ms. Linda Hess.

References

Acuña, Y., Mathison, Y., Campos, H.A., Israel, A., 1998. Thioperamide, a histamine H_3-receptor blocker, facilitates vasopressor response to footshocks. Inflammation Res. 47, 109–114.

Arrang, J.M., Garbarg, M., Schwartz, J.C., 1983. Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. Nature 302, 832–837.

Arrang, J.M., Garbarg, M., Lancelot, J.C., Lecomte, J.M., Pollard, H., Robba, M., Schunack, W., Schwartz, J.C., 1987. Highly potent and selective ligands for histamine H_3-receptors. Nature 327, 117–123.

Djuric, D.M., Andjelkovic, I.Z., 1995. The evidence for histamine H_3 receptor-mediated endothelium-dependent relaxation in isolated rat aorta. Mediators Inflammation 4, 217–221.

Duckles, S.P., Budai, D., 1990. Stimulation Intensity as critical determinant of presynaptic receptor effectiveness. Trends Pharmacol. Sci. 11, 440–443.

Ea-Kim, L., Oudart, N., 1988. A highly potent and selective H_3 agonist relaxes rabbit middle cerebral artery, in vitro. Eur. J. Pharmacol. 150, 393–396.

Godlewski, G., Malinowska, B., Buczko, W., Schlicker, E., 1997a. Inhibitory H_3 receptors on sympathetic nerves of pithed rat: activation by endogenous histamine and operation in spontaneously hypertensive rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 261–266.

Godlewski, G., Malinowska, B., Schlicker, E., Bucher, B., 1997b. Identification of histamine H_3 receptors in the tail artery from normotensive and spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 29, 801–807.

Hegde, S.S., Chan, P., Eglen, R.M., 1994. Cardiovascular effects of *R*-α-methylhistamine, a selective histamine H_3 receptor agonist, in rats: lack of involvement of histamine H_3 receptors. Eur. J. Pharmacol. 251, 43–51.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R., Haas, H.L., 1997. International union of pharmacology: XIII. Classification of histamine receptors. Pharmacol. Rev. 49, 253–278.

Holcslaw, T.L., Lassiter, D., 1987. Evidence for presynaptic inhibitory histamine (H_2) receptors in the rat hindquarter vasculature. J. Cardiovasc. Pharmacol. 9, 651–660.

Ishikawa, S., Sperelakis, N., 1987. A novel class (H_3) of histamine receptors on perivascular nerve terminals. Nature 327, 158–160.

Kathmann, M., Schlicker, E., Detzner, M., Timmerman, H., 1993. Nordimaprit, homodimaprit, clobenpropit and imetit: affinities for H_3 binding sites and potencies in a functional H_3 receptor model. Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 498–503.

Koss, M.C., 1994. Histamine H_3 receptor activation inhibits sympathetic–cholinergic responses in cats. Eur. J. Pharmacol. 257, 109–115.

Koss, M.C., Hey, J.A., 1992. Activation of histamine H_3 receptors produces presynaptic inhibition of neurally evoked cat nictitating membrane responses in vivo. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 208–212.

Koss, M.C., Hey, J.A., 1993. Prejunctional inhibition of sympathetically evoked pupillary dilation in cats by activation of histamine H_3 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 141–145.

Levi, R., Smith, N.C.E., 2000. Histamine H_3-receptors: a new frontier in myocardial ischemia. J. Pharmacol. Exp. Ther. 292, 825–830.

Lovenberg, T.W., Pyati, J., Chang, H., Wilson, S.J., Erlander, M.G., 2000. Cloning of rat histamine H_3 receptor reveals distinct species pharmacological profiles. J. Pharmacol. Exp. Ther. 293, 771–778.

Malinowska, B., Schlicker, E., 1991. H_3 receptor-mediated inhibition of the neurogenic vasopressor response in pithed rats. Eur. J. Pharmacol. 205, 307–310.

Malinowska, B., Schlicker, E., 1993. Identification of endothelial H_1, vascular H_2 and cardiac presynaptic H_3 receptors in the pithed rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 55–60.

Malinowska, B., Godlewski, G., Schlicker, E., 1998. Histamine H_3 receptors—general characterization and their function in the cardiovascular system. J. Physiol. Pharmacol. 49, 191–211.

McLeod, R.L., Gertner, S.B., Hey, J.A., 1994. Species differences in the cardiovascular responses to histamine H_3 receptor activation. Eur. J. Pharmacol. 259, 211–214.

Molderings, G.J., Weißenborn, G., Schlicker, E., Likungu, J., Göthert, M., 1992. Inhibition of noradrenaline release from the sympathetic nerves of the human saphenous vein by presynaptic histamine H_3 receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 346, 46–50.

Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S., 2000. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J. Biol. Chem. 275, 36781–36786.

Poli, E., Todorov, S., Pozzoli, C., Bertaccini, G., 1994. Presynaptic histamine H_2 receptors modulate the sympathetic nerve transmission in the isolated rat vas deferens; no role for H_3-receptors. Agents Actions 42, 95–100.

Schlicker, E., Schunack, W., Göthert, M., 1990. Histamine H_3 receptor-mediated inhibition of noradrenaline release in pig retina discs. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 497–501.

Smit, J., Coppes, R.P., Van Tintelen, E.J.J., Rofflel, A.F., Zaagsma, J., 1997. Prejunctional histamine H_3-receptors inhibit electrically evoked endogenous noradrenaline overflow in the portal vein of freely moving rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 256–260.