ELSEVIER

European Journal of Pharmacology 366 (1999) 73–78

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Pharmacological characterization of histamine H_3 receptors in human saphenous vein and guinea pig ileum

Anthony F. Valentine, Charles A. Rizzo, Maria A. Rivelli, John A. Hey *

Allergy, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033, USA

Received 7 September 1998; revised 25 November 1998; accepted 1 December 1998

Abstract

Studies were performed to assess the functional activity of histamine H_3 receptors on neurogenic sympathetic end organ responses in cryopreserved human saphenous vein. (*R*)-α-methylhistamine inhibited electrical field stimulation-evoked contractile responses in a dose dependent manner ($pD_2 = 8.20$). Prazosin (1 μM) and tetrodotoxin (1 μM) blocked the electrical field stimulation-evoked contractile responses in human saphenous vein indicating a sympathetic neural origin of these contractions. The histamine H_3 antagonists thioperamide ($pA_2 = 8.41$) and clobenpropit ($pA_2 = 10.10$) produced parallel rightward shifts in the concentration response curve to (*R*)-α-methylhistamine in human saphenous vein and guinea pig ileum ($pA_2 = 8.59$ and 9.83, respectively). Pretreatment with (*R*)-α-methylhistamine (1 μM) did not alter contractions to exogenous norepinephrine in human saphenous vein. In addition, clonidine ($pD_2 = 10.28$) inhibited electrical field stimulation-evoked contractile responses in human saphenous vein which were blocked by yohimbine (30 nM, $pA_2 = 9.92$) but did not alter the (*R*)-α-methylhistamine dose response curve. These results demonstrate the presence of functional presynaptic histamine H_3 heteroreceptors on cryopreserved human saphenous vein sympathetic nerves that, upon activation, attenuate electrical field stimulation-evoked contractile responses in this vessel.

Keywords: Histamine H_3 receptor; (*R*)-α-methylhistamine; Saphenous human vein; Sympathetic neuron; Electrical field stimulation; Cryopreservation

1. Introduction

The histamine H_3 receptor, first described by Arrang et al. (1983), was found to act as an inhibitory autoreceptor on histaminergic neurons in rat brain cortex. Recent studies have identified histamine H_3 receptors in a variety of species on peripheral nerves acting as inhibitory heteroreceptors on parasympathetic (Ichinose et al., 1989; Menkveld and Timmerman, 1990) and sympathetic (Hey et al., 1992; Koss and Hey, 1993; Lou et al., 1993; Imamura et al., 1995) nerve terminals modulating neurotransmitter release. In human saphenous vein, histamine and the selective histamine H_3 receptor agonist (*R*)-α-methylhistamine attenuate electrically-evoked overflow of [^{3}H]norepinephrine indicating the presence of presynaptic histamine H_3 receptors on the sympathetic nerve fibers (Molderings et al., 1992).

Receptor binding assays, neurotransmitter release studies and functional assays have all been used to evaluate histamine H_3 receptor pharmacology in animal models. However, only neurotransmitter release studies and receptor binding assays have been able to identify histamine H_3 receptor pharmacology in human tissue. In addition, little is known about the histamine H_3 receptor because it has not yet been cloned and it is unclear whether presynaptic histamine H_3 receptors modulate end organ responses in humans as it does in other species. The importance of characterizing histamine H_3 receptor function in human vascular tissue is further underscored by recent evidence indicating possible species differences in the cardiovascular responses following histamine H_3 receptor activation in vivo (McLeod et al., 1994). Therefore, a functional model of end organ neuroeffector responses in human tissue would be of significant value to determine whether commonly used experimental models resemble human histamine H_3 receptor pharmacology.

To date, neurotransmitter release studies have been limited to using fresh human vascular tissue harvested

* Corresponding author. Tel.: +1-908-740-7219; Fax: +1-908-740-7175

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00904-2

immediately after surgery (Göthert et al., 1984; Molderings et al., 1992). This is largely due to the degeneration of nerve terminals and decline in muscle responsiveness in blood vessels following explant (Müller-Schweinitzer, 1994). While cryopreserved human vessels are suitable for postjunctionally mediated pharmacological studies (Müller-Schweinitzer et al., 1986; Müller-Schweinitzer and Ellis, 1992), it is not known whether cryopreserved vessels can be used to investigate human pharmacology via neuronally-dependent end organ responses. The ability to study neuronally mediated contractile responses in cryopreserved vessels and the role of histamine H_3 receptors on end organ responses in the human peripheral vasculature remains to be elucidated.

Therefore, the goals of the present study were: (1) to evaluate the effect of histamine H_3 receptor activation with the selective agonist (*R*)-α-methylhistamine (Arrang et al., 1987) on sympathetic contractile responses in fresh and cryopreserved human saphenous vein; (2) compare the potency of standard histamine H_3 receptor antagonists thioperamide (Arrang et al., 1987) and clobenpropit (van der Goot et al., 1992) in this human vascular smooth muscle preparation with the established functional histamine H_3 receptor guinea pig ileum model; and (3) determine whether the mechanism of prejunctional inhibition by histamine H_3 receptor activation in this system is independent of the inhibition caused by α_2-adrenoceptor activation.

2. Materials and methods

2.1. General

Segments of human saphenous vein were obtained from 19 male and 4 female donor patients (47–75 years old) that underwent coronary artery bypass surgery. Vessels were provided by Cooperative Human Tissue Network which is funded by the National Cancer Institute (University of Pennsylvania Medical Center, Philadelphia, PA, USA), Anatomical Gift Foundation (Woodbine, GA, USA) and Hackensack Medical Center Institute for Biomedical Research (Hackensack, NJ, USA) and shipped in physiologic media or heparinized blood on wet ice within 48 h of removal.

2.2. Cryopreservation of human saphenous vein

Segments of saphenous vein 3–7 mm in diameter were dissected free of loose connective tissue and cut into ring segments 5 mm in length before cryopreservation according to Müller-Schweinitzer and Ellis (1992). Briefly, saphenous vein ring preparations were placed into 1.8 ml Nunc liquid nitrogen storage ampoules containing 1.8 M dimethyl sulfoxide (DMSO) and 0.1 M sucrose in heat inactivated fetal calf serum. After 20 min at 4°C, the vials were placed in a 11 × 14 cm Nalgene cryofreezing cylindrical container and frozen at a mean cooling rate of approximately 1°C/min in a freezer maintained at −70°C. The ampoules were stored at this temperature until use and thawed within 2.0–2.5 min using a drybath heating unit. After thawing, the vessel rings were placed in a petri dish containing a modified Krebs–Henseleit solution (K–H) with the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.55, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.9 and glucose 11.1 (pH = 7.4) gassed with 95% O_2–5% CO_2 at 37°C. Comparative studies using fresh saphenous vein were begun within 1 h of delivery and not subjected to the cryopreservation protocol.

2.3. Electrical field stimulation-evoked contractile responses in human saphenous vein

Saphenous vein ring segments were mounted on stainless steel hooks and positioned between stainless steel electrodes in 25.0 ml organ baths (Radnoti, Monrovia, CA, USA) containing K–H buffer (pH 7.4) and aerated with 95% O_2–5% CO_2 at 37°C. Chlorpheniramine (1 μM) and cimetidine (1 μM) were added to the buffer to block H_1 and H_2 mediated effects, respectively. Ring segments were given 1.0 g of initial passive tension and isometric tensions were recorded using force displacement transducers (FT-03, Grass Instruments, Quincy, MA, USA) connected to an eight channel physiograph (K2G, Astro-Med, West Warwick, RI, USA) for continuous recording. During an equilibration period of 2–3 h, vessels were washed and responsiveness was tested with multiple challenges of exogenously administered norepinephrine (100 μM) and potassium chloride (KCl, 60 mM). Only vessel rings that responded to norepinephrine and KCl were used in these experiments. After a 30 min equilibration period, repetitive, transient contractions were induced with electrical field stimulation (16 Hz, 1 ms pulse, 30 s continuous, 12–20 V) generated by a Grass S88 Stimulator and distributed to organ bath electrical field stimulation electrodes using a Stimulus Distributor (Buxco Electronics, Sharon, CT, USA). Voltage was adjusted accordingly to produce contractile responses ranging from 0.05–0.20 g of tension. Vessel rings were washed one final time followed by a 1 h antagonist pretreatment before initiating electrical field stimulation-induced contractions at 15 min intervals. In prejunctional histamine H_3 studies, rising cumulative additions of (*R*)-α-methylhistamine were added at 10 min intervals before the following electrical field stimulation response. In all prejunctional studies, prazosin (1 μM) was added before the final electrical field stimulation contraction to obtain a maximal inhibition for normalizing prejunctional inhibitory responses. Only tissues that displayed responsiveness to prazosin blockade were used for these studies. Also, the effect of (*R*)-α-methylhistamine on exogenous norepinephrine (0.1 μM–100 μM) induced

postjunctional contractile responses was evaluated in separate cryopreserved human saphenous veins.

2.4. Electrical field stimulation-evoked tension responses in guinea pig ileum

All animal experiments were approved by the Animal Care and Use committee of the Schering-Plough Research Institute, an AAALAC (American Association for Accreditation of Laboratory Animal Care) accredited facility. Freshly dissected ileum preparations were obtained from 350–500 g male Charles River Hartley strain guinea pigs euthanized with carbon dioxide.

Approximately 20 cm segments of ileum, starting 10 cm from the ileocecal junction, were removed from each animal and placed in room temperature Tyrode's buffer (mM): NaCl 138, KCl 2.7, $MgCl_2$ 1.0, $CaCl_2$ 0.9, NaH_2PO_4 0.4, $NaHCO_3$ 11.9 and glucose 5.6 (pH 7.4) aerated with 95% O_2–5% CO_2. Two centimeter longitudinal whole ileum segments were prepared and mounted between stainless steel electrodes in 25.0 ml organ baths (Radnoti) containing Tyrode's buffer and were continuously aerated with 95% O_2–5% CO_2 at 37°C. Chlorpheniramine (1 μM) was added to the buffer solution to block H_1 receptor mediated contractions. Tissues were given an initial passive tension of 0.3 g and isometric tensions were recorded using Grass FT-03 force displacement transducers connected to an eight channel Grass physiograph (model 7) for continuous recording. After a 60 min equilibration period, repetitive, transient contractions were induced with electrical field stimulation generated by a Grass S88 Stimulator amplified and distributed to organ bath field stimulating electrodes using a Buxco Electronics Stimulus Distributor (electrical field stimulation −25 Hz, 8 V, 1 s train/min, 1 ms pulse duration). Pulse duration and voltages were reduced in a stepwise fashion to elicit 80% of a maximal contractile response. Histamine H_3 receptor antagonists were added 5 min prior to the rising cumulative additions of (*R*)-α-methylhistamine (1 nM–100 μM) at 1 min intervals before each consecutive stimulus train.

2.5. Materials

L-(−)-Norepinephrine bitartrate, clonidine HCl, prazosin HCl, yohimbine HCl, (*R*)-α-methylhistamine 2HCl, thioperamide maleate and clobenpropit 2HBr were purchased from Research Biochemicals International, Natick, MA, USA. Chlorpheniramine maleate and cimetidine were supplied by the Chemical Research Department, Schering-Plough Research Institute, Kenilworth, NJ, USA. Tetrodotoxin was obtained from Sigma, St. Louis, MO, USA. All compounds were prepared as concentrated stock solutions before addition to the assay buffer. Prazosin, yohimbine, thioperamide and clobenpropit were prepared as 10 mM stock solutions in DMSO and subsequently diluted in deionized water. Final concentration of DMSO in the bath did not exceed 0.01%. All other drugs were dissolved and diluted in deionized water.

2.6. Data analysis

Values are expressed as mean ± S.E.M. The agonist concentration required to give 50% of a maximal inhibitory response (EC_{50}) was determined for each concentration–response curve. Potencies are expressed as pD_2-values defined as the negative logarithm of the EC_{50} for the agonist used. Antagonist potency was assessed by constructing a Schild plot from three different concentrations and a pA_2-value was obtained (Arunlakshana and Schild, 1959) or expressed as apparent pA_2 estimates (Furchgott, 1955).

3. Results

3.1. Histamine H_3 receptor modulation of electrical field stimulation-induced contractile responses in human saphenous vein

In cryopreserved human saphenous vein, electrical field stimulation-induced contractions were inhibited by the selective histamine H_3 receptor agonist (*R*)-α-methylhistamine ($pD_2 = 8.20 \pm 0.25$, $n = 36$; Fig. 1A) in a dose dependent manner. In fresh human saphenous vein, electrical field stimulation-induced contractions were also dose dependently inhibited by (*R*)-α-methylhistamine ($pD_2 = 8.03 \pm 0.21$, $n = 10$; Fig. 1A). Potency estimates for (*R*)-α-methylhistamine in fresh and cryopreserved human saphenous vein were quantitatively similar. The electrical field stimulation-induced contractions in cryopreserved saphenous vein were inhibited by prazosin (1 μM, 60% ± 10%, $n = 5$). In addition, the sodium channel blocker tetrodotoxin (1 μM) inhibited electrical field stimulation-induced contractile responses (61% ± 10%, $n = 6$; Fig. 2) confirming a neuronal origin. The electrical field stimulation-evoked contractile responses in fresh human saphenous vein were inhibited by prazosin (1 μM) and tetrodotoxin (1 μM) to a similar extent (data not shown). In a separate study, the tensions generated by electrical field stimulation-evoked contractile responses in human saphenous vein from three individual patients before and after cryopreservation were 130 mg ± 20 ($n = 17$) vs. 90 mg ± 10 ($n = 26$), respectively.

The histamine H_3 receptor antagonists thioperamide (0.03, 0.1 and 1.0 μM) and clobenpropit (0.3, 1.0 and 3.0 nM) demonstrated average pA_2 estimates of 8.41 and 10.10, respectively, using Schild plot analysis (Fig. 3A and B). In supporting studies, the α_2 adrenoceptor agonist clonidine inhibited electrical field stimulation-induced contractions in cryopreserved human saphenous vein ($pD_2 = 10.28 \pm 0.19$, $n = 4$). Pretreatment with the α_2-adrenoceptor antagonist yohimbine (30 nM) caused a significant

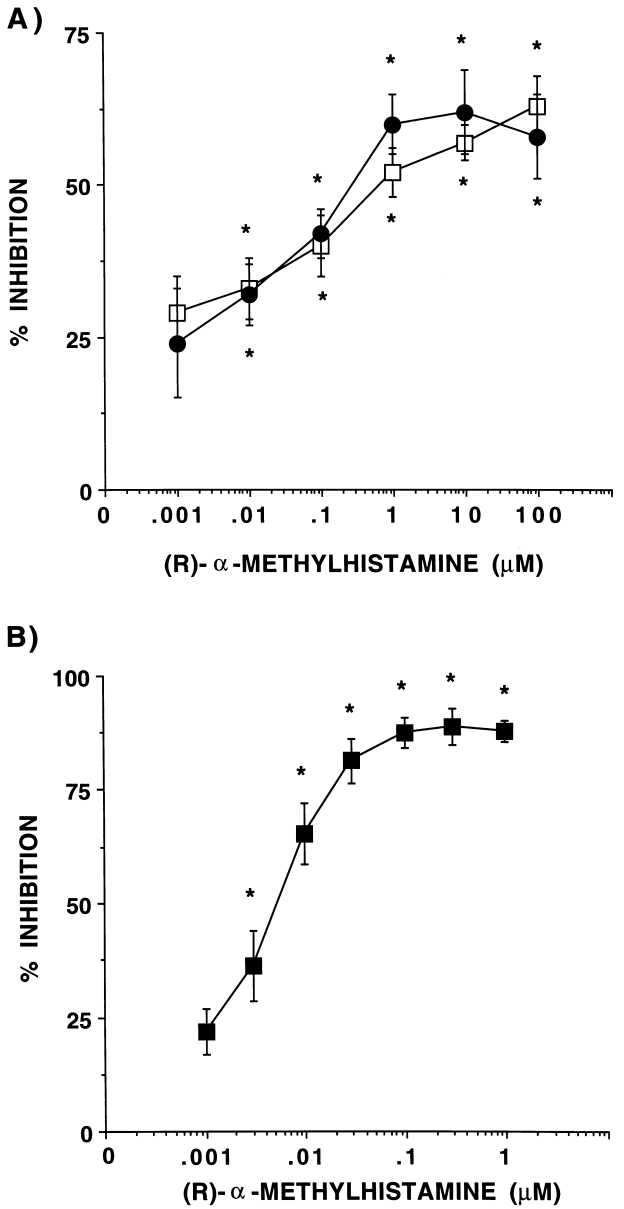

Fig. 1. (*R*)-α-methylhistamine inhibition of prazosin sensitive electrical field stimulation-evoked contractile responses in (A) fresh (-●-, $n = 10$) and cryopreserved (-□-, $n = 36$) human saphenous vein. (*R*)-α-methylhistamine inhibition of atropine sensitive electrical field stimulation-evoked contractile responses in (B) guinea pig ileum (-■-, $n = 13$). Values are expressed as mean ± S.E.M. * Significantly different from control response, $P < 0.05$.

rightward shift in the clonidine dose response ($pA_2 = 9.92 \pm 0.32$, $n = 4$) but did not shift the inhibitory response of the histamine H_3 receptor agonist (*R*)-α-methylhistamine (30 nM, $n = 4$). In postjunctional studies, exogenous administration of norepinephrine in the absence and presence of (*R*)-α-methylhistamine (1 μM) did not alter postjunctionally mediated contractile responses in cryopreserved human saphenous vein ($pD_2 = 6.51 \pm 0.29$ vs. 6.50 ± 0.24, $n = 3$).

3.2. Histamine H_3 receptor modulation of electrical field stimulation-induced contractile responses in guinea pig ileum

Electrical field stimulation-evoked contractions in isolated guinea pig ileum were dose dependently inhibited by (*R*)-α-methylhistamine ($pD_2 = 8.13 \pm 0.06$, $n = 13$, Fig. 1B). In addition, average pA_2 estimates for the histamine H_3 receptor antagonists thioperamide (0.01, 0.03, 0.1 and 1.0 μM) and clobenpropit (1.0, 3.0, and 10.0 nM) using Schild plot analysis were 8.59 and 9.83, respectively (Fig. 3A and B). The electrical field stimulation-evoked contractile responses in guinea pig ileum were inhibited by at-

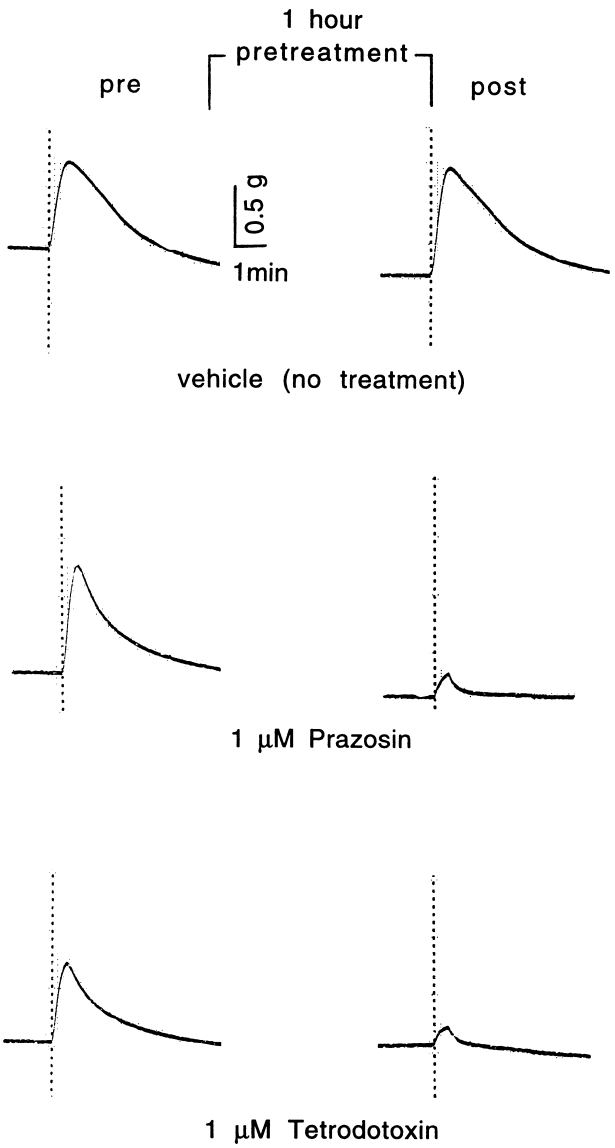

Fig. 2. Representative polygraph trace of electrical field stimulation-induced contractile responses in cryopreserved human saphenous vein pre- and post-treatment.

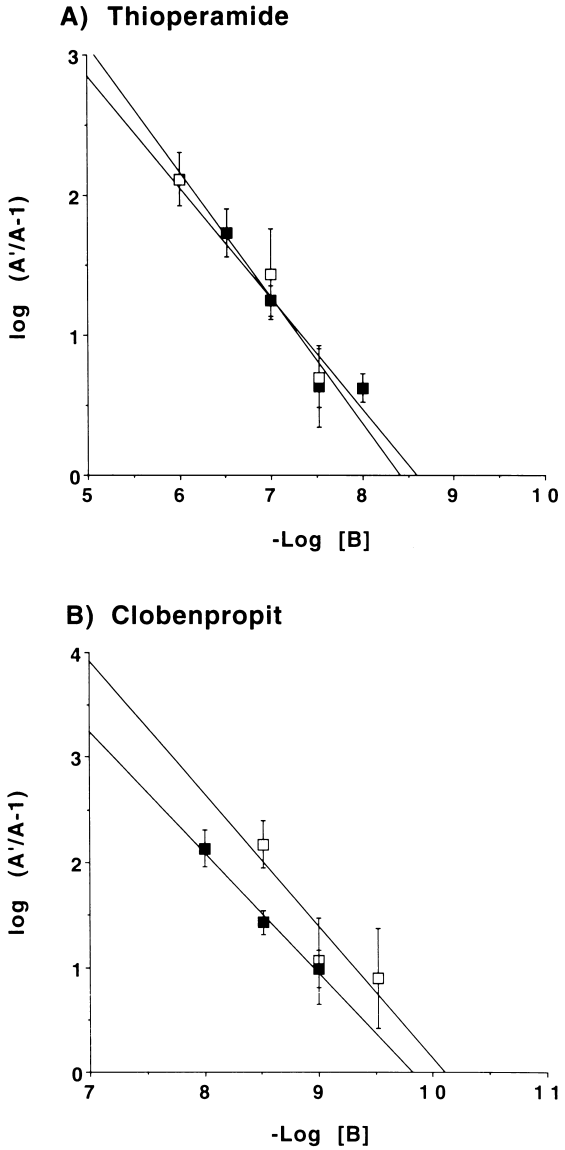

Fig. 3. Schild plot analysis of histamine H_3 receptor antagonists thioperamide (Panel A) and clobenpropit (Panel B). Panel A illustrates the Schild plot of thioperamide in cryopreserved human saphenous vein (-□-, $n = 4–6$; $pA_2 = 8.41$, slope $= -0.901$, $r = 0.98$) and in guinea pig ileum (-■-, $n = 7$; $pA_2 = 8.59$, slope $= -1.097$, $r = 1.00$, $n = 7$). Panel B shows the Schild plot of clobenpropit in cryopreserved human saphenous vein (–□-, $n = 4–6$; $pA_2 = 10.10$, slope $= -1.262$, $r = 0.91$) and in guinea pig ileum (-■-, $n = 5–6$; $pA_2 = 9.83$, slope $= -1.149$, $r = 0.99$).

ropine (0.1 μM) under these stimulus conditions (data not shown).

4. Discussion

The present results demonstrate the presence of functional presynaptic histamine H_3 receptors on sympathetic nerve terminals in human saphenous vein. Activation of prejunctional H_3 receptors attenuated the electrical field stimulation-evoked sympathetic contractile responses most likely by inhibiting the release of norepinephrine from sympathetic nerve terminals. These findings are in agreement with neurotransmitter release studies using fresh human saphenous vein which showed an inhibition of electrical field stimulation-evoked NE release by (*R*)-α-methylhistamine (Molderings et al., 1992). Also, the agonist pD_2-values for (*R*)-α-methylhistamine that were obtained in our studies with fresh and cryopreserved human saphenous vein ($pD_2 = 8.03$ and 8.20, respectively) closely resemble values reported by other investigators in guinea pig trachea (Ichinose et al., 1989), guinea pig ileum (Menkveld and Timmerman, 1990), mouse brain cortex (Kathmann et al., 1993) and guinea pig pulmonary artery (Rizzo et al., 1995).

Furthermore, these findings demonstrate that pretreatment with the histamine H_3 receptor antagonists thioperamide or clobenpropit caused a rightward shift in the (*R*)-α-methylhistamine dose–response curve. Antagonist potency (pA_2) estimates obtained for thioperamide (8.41) and clobenpropit (10.10) in the cryopreserved human saphenous vein were similar to estimates we obtained in guinea pig ileum (8.59 and 9.83, respectively).

While the isolated guinea pig ileum is a parasympathetically driven (i.e., atropine sensitive) electrical field stimulation effector response model whereby activation of prejunctional histamine H_3 receptors attenuates acetylcholine release and depresses electrical field stimulation-induced contractions (Trzeciakowski, 1987; Menkveld and Timmerman, 1990; Schlicker et al., 1994), electrical field stimulation-evoked contractile responses in human saphenous vein appears to be of a predominantly sympathetic origin. However, the present findings demonstrate that neuronally mediated end organ responses in guinea pig ileum and human saphenous vein are attenuated upon activation of presynaptic histamine H_3 receptors in a similar manner. Hence, this demonstrates that the guinea pig ileum is predictive of histamine H_3 receptor pharmacology in this human vascular smooth muscle.

The present results also show that prejunctional inhibition of norepinephrine release by histamine H_3 receptor activation occurs independently of prejunctional α_2-adrenoceptor mediated inhibition in human saphenous vein. In support, the potency estimate for (*R*)-α-methylhistamine was shifted with the histamine H_3 receptor antagonists but was not altered by yohimbine (30 nM) while the potency estimate of the α_2-adrenoceptor agonist clonidine ($pD_2 = 10.28$) was shifted to the right after pretreatment with yohimbine ($pA_2 = 9.92$). Thus, agonist-induced activation of the histamine H_3 prejunctional inhibitory mechanism appears to act independently of α_2-adrenergic inhibition and can be differentiated pharmacologically in this vessel. Our findings are in agreement with results obtained in an earlier study using human saphenous vein (Molderings et al., 1992) which found no interaction between H_3 receptor and α_2-adrenoceptor mediated inhibitory actions. How-

ever, interactions of the histamine H_3 receptor with other prejunctional inhibitory receptors were not part of this study and cannot be ruled out.

These findings also demonstrate that histamine H_3 receptor modulation of sympathetic nerve terminal release is maintained in explanted human saphenous veins after cryopreservation. This is supported by the ability of (*R*)-α-methylhistamine to attenuate prazosin-sensitive electrical field stimulation-evoked contractile responses in fresh and cryopreserved human saphenous vein. In addition, tetrodotoxin abolished the prazosin sensitive portion of the electrical field stimulation-evoked contractile response in fresh and cryopreserved human saphenous vein under identical conditions suggesting a similar sensitivity to electrical field stimulation-evoked neurotransmitter release. Taken together, these results are in agreement with other investigators that have shown functional adrenergic neurotransmission was unaltered following cryopreservation of canine saphenous vein (Müller-Schweinitzer and Tapparelli, 1987) and rabbit ear artery (Thompson et al., 1989).

While the sensitivity and magnitude of inhibition by (*R*)-α-methylhistamine on electrical field stimulation-evoked contractile responses in fresh and cryopreserved saphenous vein was not significantly different, we observed about a 30% reduction in the amplitude of electrical field stimulation-evoked contractile responses in cryopreserved human saphenous vein relative to fresh tissue. A similar phenomenon has been described by Müller-Schweinitzer et al. (1986) which was characterized by reduced contractility without effecting sensitivity to a variety of postjunctional mediators after cryopreserving human peripheral veins.

In summary, our results demonstrate the presence of functional histamine H_3 receptors on sympathetic nerve terminals in cryopreserved human saphenous vein. Activation of these prejunctional histamine H_3 heteroreceptors with exogenously applied (*R*)-α-methylhistamine modulates sympathetic responses in this vessel. These studies also demonstrate that prejunctional inhibition by histamine H_3 receptor activation is independent of α_2 autoreceptors located on sympathetic nerves in cryopreserved human saphenous vein.

Acknowledgements

The authors wish to thank Dr. William Kreutner and Dr. Robert W. Egan for their review of the manuscript and useful comments.

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