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Presynaptic histamine H_1- and H_3-receptors modulate sympathetic neurotransmission in isolated guinea pig *vas deferens*

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ABSTRACT The action of (R)-α-methylhistamine (α-MeHA), a selective H_3-receptor agonist, on field stimulation induced contraction of guinea pig *vas deferens* was composed of 2 components: the "inhibition" (0.1 —100 nmon·L^{-1}) and the "enhancement" (1—10 μmol·L^{-1}). In the presence of histamine H_1 antagonist, chlorpheniramine (1 μmol·L^{-1}), α-MeHA (0.1 nmol·L^{-1}— 10 μmol·L^{-1}) showed only a concentration-dependent inhibition. Selective histamine H_3-receptor antagonist, thioperamide (1 nmol·L^{-1}— 10 μmol·L^{-1}) antagonized the inhibitory effect of α-MeHA and increased the contractile amplitude of *vas deferens* elicited by field pulses when thioperamide was used alone. α-MeHA 10 μmol·L^{-1} enhanced the contractile amplitude, which was reversed by chlorpheniramine 1 μmol·L^{-1}, but not by ranitidine (1 μmol·L^{-1}). Pyridelethylamine, an H_1-receptor agonist, facilitated concentration-dependently the contractile response of *vas deferens*. The effect was antagonized by chlorpheniramine, but not by ranitidine. Dimaprit, an H_2-receptor agonist had no effect on the field stimulation induced sympathetic response. Both α-MeHA and pyridelethylamine failed to influence the contraction of *vas deferens* elicited by direct field stimulation in smooth muscle or by exogenously applied norepinephrine. It was concluded that histamine H_1- and H_3-receptors existed in sympathetic terminals of guinea pig *vas deferens* and facilitated or inhibited the sympathetic neurotransmission.

KEY WORDS histamine receptors; methylhistamine; thioperamide; pyridelethylamine; dimaprit; ranitidine; chlorpheniramine; *vas deferens*; histamine H_1 receptor blockaders; histamine H_2 receptor blockaders

It is well known that α_2- and β_2-adrenoceptors are located on the sympathetic terminals mediating opposite effects on norepinephrine (NE) release, ie, inhibition by α_2- and facilitation by β_2-adrenoceptors[1]. The exicitatory junction potential produced by perivascular nerve stimulation in vascular smooth muscle cell or the positive inotropic action induced by electric field stimulation in right atria of guinea pig can be inhibited by histamine presynaptically. These effects are mimicked by selective histamine H_3 receptor agonist, (R)-α-methylhistamie (α-MeHA) and can be competitively antagonized by H_3 receptor antagonists, burimamide, impromidine, and thioperamidem, respectively[2-4]. Thus it is pherhaps not surprising that histamine H_3-receptors might be widely distributed on sympathetic terminals and modulate the sympathetic neurotransmission. The smooth muscle of guinea pig *vas deferens* is densely innervated with sympathetic nerve fibers and the isolated preparation has been frequently used for studying drugs supposed to interfere the sympathetic neurotransmission[5-8]. Since it has not been identified whether histamine receptors are located on the sympathetic terminals of guinea pig *vas deferens*, the purpose of present study was to investigated the distribution of different subtypes of histamine receptors on the guinea pig *vas deferens* and to assess the possible roles they played.

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MATERIALS AND METHODS

α-MeHA and thioperamide were generous gifts from Dr J M Arrang of the Unité de Neurobiologie, Centre Paul Broca de l'INSERM (Paris, France); pyridelethylamine and dimaprit were kindly supplied by Smith Kline and French Laboratory (Welwyn Garden City, UK); tetrodotoxin (TTX, Sankyo Co, Tokyo, Japan); ranitidine (Southwest No 3 Pharmaceutical

Factory, Chongqing, China); chlorpheniramine and NE (Beijing Pharmaceutical Factory, Beijing, China); desipramine (DMI) and normetanephrine (NMN, Sigma).

Guinea pigs (♂, $n = 78$, $534 \pm s$ 88 g) were stunned and bled to death. The *vas deferens* were desheathed and mounted to a jacketed organ bath at 35 ℃ and bubbled with 97 % O_2 + 3 % CO_2 in a Krebs solution: NaCl 114.0, KCl 4.5, $CaCl_2$ 2.0, KH_2PO_4 1.2, $MgSO_4$ 0.59, $NaHCO_3$ 12.5, dextrose 5.5 (mmol·L^{-1}), final pH 7.35. The *vas deferens* was stimulated via a pair of vertical platinum plate electrodes, 5 mm apart. The muscle was attached to a forcedisplacement transducer for monitoring its tension.

Presynaptic effects After a 90-min equilibration, sympathetic nerve terminals in *vas deferens* were excited by electric field stimulation with trains of 500 shocks (1 ms, 50 mA) at 30 Hz using a XF-3 stimulator and the contraction was recorded on a dual pen recorder. The field stimulation was applied every 7 min. The preparations were perfused with Krebs solution to which DMI 0.1 μmol·L^{-1} and NMN 1 μmol·L^{-1} were added to block the neuronal and extraneuronal uptakes of liberated NE and also atropine 1 μmol·L^{-1} was added to block the possible interaction with cholinergic functions. The *vas deferens* was exposed to drugs for 5 min and then excited by field pulses. One preparation was treated by only one antagonist.

Postsynaptic effects TTX 0.5 μmol·L^{-1} was added to Krebs solution to abolish the contractile responses to nerve stimulation. The field stimulation (30 ms duration, 300 shocks) was delivered which would directly excite the smooth muscle and cause a contractile response. The effects of α-MeHA or pyridelethylamine on the direct smooth muscle contraction were examined. In the presence of TTX (0.5 μmol L^{-1}), the effects of α-MeHA (1 μmol·L^{-1}) and pyridelethylamine (1 μmol·L^{-1}) on the response to exogenous NE were also scrutinized.

All values were expressed as $\bar{x} \pm s$. Statistical evaluation was accomplished by t test.

RESULTS

Actions of α-MeHA and thioperamide on contractile response to nerve stimulation In the *vas deferens* of guinea pig, the mechanical response to electric nerve stimulation was biphasic with an initial "twitch" (phase I) and a delayed slow contraction (phase II). α-MeHA 0.1 — 100 nmol·L^{-1} gave a concentration-dependent inhibitory effect on the field stimulation-induced contraction. However, when the concentration were elevated up to 1 — 10 μmol·L^{-1}, that the inhibition was lessened and the magnitude of contraction was increased (Fig 1).

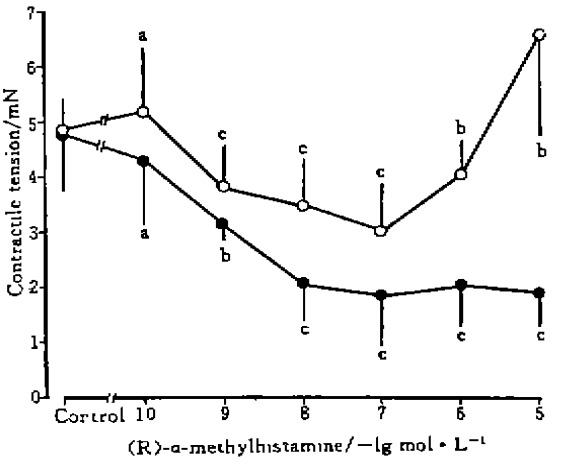

Fig 1. Effects of α-MeHA on contraction of isolated guinea pig *vas deferens* evoked by electric field stimulation in the absence (○) or presence (●) of chlorpheniramine 1 μmol·L^{-1}. $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Thioperamide reversed the inhibitory effect of α-MeHA (100 nmol·L^{-1}) concentration-dependently (Fig 2). When used alone, thioperamide (1 nmol·L^{-1} — 10 μmol·L^{-1}) increased the contractile amplitude of *vas deferens* elicited by field pulses (Fig 2).

Influences of histamine H_1- and H_2-antagonists on effects of α-MeHA In the presence of chlorpheniramine 1 μmol·L^{-1}, α-MeHA (0.1 nmol·L^{-1} — 10 μmol·L^{-1}) exhibited only a monophasic action, ie, a concentration-dependent inhibition of sympathetic response whithout increasing contractile amplitude (Fig

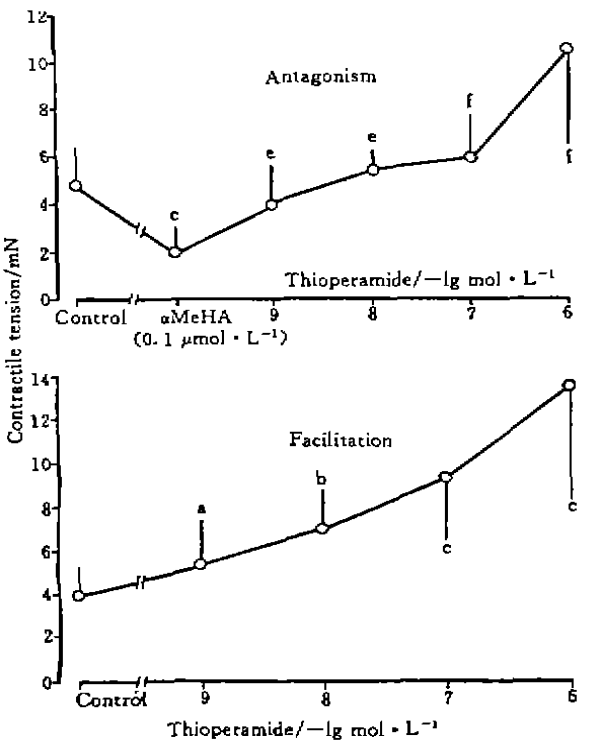

Fig 2. Influences of thioperamide (Thio) on effect of α-MeHA and on electric stimulation of guinea pig *vas deferens*. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^dP<0.05$, $^eP<0.01$ *vs* α-MeHA.

1). The EC_{50} of α-MeHA was 1.7 nmol·L^{-1}.

α-MeHA (10 μmol·L^{-1}) enhanced the contractile amplitude to 38.3 % of control tension and ranitidine failed to prevent the effect of α-MeHA. On the contrary, chlorpheniramine 1 μmol·L^{-1}, completely depressed the facilitation of sympathetic response induced by α-MeHA (10 μmol·L^{-1}) and also reversed to inhibition with a contractile response about 73.0 % of control value (Tab 1).

EFfects of histamine H_1- and H_2-agonists on field pulse-induced contraction Pyridelethylamine (1 nmol·L^{-1} — 10 μmol·L^{-1}) concentration-dependently facilitated the contractile response of *vas deferens* elicited by electric field stimulation (Fig 2) and its EC_{50} was 0.27 μmol·L^{-1}. The effect was not prevented by ranitidine (1 μmol·L^{-1}), but antagonized by chlorphenamine (1 μmol·L^{-1}) (data not shown). Dimaprit 1 nmol·L^{-1} — 10 μmol·L^{-1} did not influence the field stimulation-evoked sympathetic response of *vas deferens* (Fig 3).

Tab 1. Influences of ranitidine (Ran, 1 μmol·L^{-1}) and chlorphenamine (Chlor, 1 μmol·L^{-1}) on α-MeHA (10 μmol·L^{-1}) caused facilitation of contraction of guinea pig *vas deferens* elicited by field stimulation. $n=6$, $\bar{x}\pm s$. $^bP<0.05$ *vs* control. $^dP>0.05$, $^eP<0.05$ *vs* α-MeHA.

Group	Contractile tension/mN
Control	4.3±0.7
α-MeHA	5.8±1.3[b]
Ran + α-MeHA	5.9±1.6[d]
Chlor + α-MeHA	3.1±1.8[e]

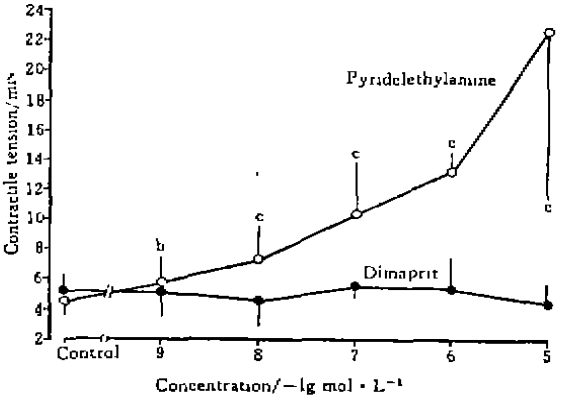

Fig 3. Effects of pyridelethylamine and dimaprit on the field pulse-induced contraction of isolated guinea pig *vas deferens*. $n=6$, $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Postjunctional effects of α-MeHA and pyridelethylamine In the presence of TTX 0.5 μmol·L^{-1}, the sympathetic responses of *vas deferens* elicited by field pulses were abolished and then direct electric stimulation of smooth muscle by delivering every 10 min 300

pulses (30 ms duration each at 30 Hz with a current strength of 50 mA) elicited a twitch response. Both α-MeHA (0.1 μmol·L^{-1}) and pyridelethylamine (0.1 μmol·L^{-1}) failed to influence the contraction of *vas deferens* induced by field pulses (Fig 5). Addition of NE 10 μmol·L^{-1} evoked the oscillatory twitch contraction by directly interacting with postjunctional α_1-adrenoceptors and neither α-MeHA (0.1 μmol·L^{-1}) nor pyridelethylamine (0.1 μmol·L^{-1}) depressed the responses to exogenous NE (Tab 2).

Tab 2. Effect of α-MeHA (0.1 μmol·L^{-1}) and pyridelethylamine (Pyr, 0.1 μmol·L^{-1}) on the contraction evoked by direct field stimulation (FS) to the smooth muscle of guinea pig *vas deferens* in presence of TTX 0.5 μmol·L^{-1} or induced by NE 10 μmol·L^{-1}. $n=6$. $\bar{x}\pm s$. $^*P>0.05$ *vs* corresponding control.

Group	Contractile tension/mN	
	FS	NE
Control	15.6±0.8	18.9±7.1
α-MeHA	14.9±3.7*	18.9±6.8*
Pyr	15.9±1.0*	18.9±3.5*

DISCUSSION

The contractile responses of guinea pig *vas deferens* elicited by electric field pulses with short duration could be abolished by TTX (0.5 μmol·L^{-1}), suggesting a neurogenic origin. The phase II of the contractile response is always induced by the release of NE from sympathetic terminals which has been taken as a reliable index for testing effects of drugs, which would interrupt the sympathetic neurotransmission[5-9].

Under normal conditions, contractile amplitudes were not significantly changed when the preparation was excited by field pulses every 7 min for 6 times.

The action of α-MeHA on the contractile responses induced by field pulses consisted of 2 components, the "inhibition" (at 0.1—100 nmol·L^{-1}) and the "enhancement" (at 1—10 μmol·L^{-1}). Chlorpheniramine (1 μmol·L^{-1}) could reverse the "enhancement," but could not influence the "inhibition." In contrast, the sympathetic responses of *vas deferens* could be facilitated by pyridelethylamine in a concentration-dependent manner, but not modified by dimaprit. α-MeHA, although highly specific for histamine H_3-receptors, would interact with H_1- and H_2-receptors when the concentration reached 10 μmol·L^{-1}[10,11]. Our results indicated that the 2 components of sympathetic responses to α-MeHA might be mediated by 2 different subclasses of histamine receptors, H_1- and H_3-recptors, respectively.

Thioperamide reversed the inhibitory effects of α-MeHA on responses induced by field pulses concentration-dependently; while chlorpheniramine only antagonized the effect of pyridelethylamine. Since α-MeHA and pyridelethylamine did not modify the contractile response elicited either by direct electric stimulation of *vas deferens* or by application of exogenous NE, they may possibly consist of reduction or facilitation of NE release due to prejunctional effect. It was strongly suggested that histamine H_3- and H_1-recptors were present in the sympathetic terminals, but were not distributed on the postsynaptic membrane of guinea pig *vas deferens*.

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突触前组胺 H_1和 H_3受体对豚鼠输精管交感神经冲动传递的影响

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A **摘要** (R)-α-甲基组胺(α-MeHA)低浓度抑制，高浓度增强电场刺激诱发的离体输精管收缩. 上述效应可分别被 thioperamide 和氯苯那敏拮抗. Pyridelethylamine (Pyr)能增强电场刺激诱发的输精管收缩. α-MeHA 和 Pyr 对于直接电刺激或去甲肾上腺素(NE)诱发的输精管收缩均无影响. 以上表明，豚鼠输精管交感神经末稍分布有组胺 H_3和 H_1两种受体，它们分别介导抑制和促进 NE 的释放.

关键词 组胺受体；甲基组胺；thioperamide；pyridelethylamine；dimaprit；雷尼替丁；氯苯那敏；输精管；组胺 H_1受体拮抗剂；组胺 H_2受体拮抗剂

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