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Direct evidence for histamine H_3 receptor-mediated inhibition of norepinephrine release from sympathetic terminals of guinea pig myocardium

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KEY WORD heart atrium; electric stimulation; methylhistamines; histamine antagonists; norepinephrine; postganglionic sympathetic fibers; histamine H_3 receptors; high pressure liquid chromatography

AIM: To study the histamine H_3 receptors mediated inhibition of norepinephrine (NE) release from cardiac sympathetic terminals of guinea pig isolated atria. **METHODS:** Release of NE induced by electric field stimulation (50 mA, 5 ms) in the bath solution was measured by HPLC-ECD. **RESULTS:** The release of NE caused by field stimulation was attenuated by (R)-α-methylhistamine (α-MeHA, 0.1 nmol·L^{-1} − 10 μmol·L^{-1}) in a concentration-dependent manner. Thioperamide concentration-dependently antagonized the inhibition of α-MeHA. Blockade of H_1, H_2, α_2, β_2-receptors failed to prevent the inhibitory effect of α-MeHA. Thioperamide (1 nmol·L^{-1} − 10 μmol·L^{-1}), when used alone, concentration-dependently facilitated the release of NE evoked by field stimulation. **CONCLUSION:** The presynaptic histamine H_3-receptors inhibited the NE release from cardiac sympathetic terminals.

Since the first description of histamine H_3-receptors[1], evidence has accumulated that histamine H_3-receptors represent a class of inhibitory presynaptic receptors localized on terminals of various central and peripheral nerves, where both release and metabolism of neurotransmitters can be modulated[2-5]. Our previous study showed that histamine (HA) and (R)-α-methylhistamine (α-MeHA), a selective H_3-receptor agonist, inhibited the positive inotropic response of myocardium induced by field stimulation and the effects of HA and α-MeHA were reversed by thioperamide (Thio), a specific H_3-receptor antagonists, but not by H_1, H_2-receptor and α_2-, β_2-adrenoceptor antagonists[6,7]. Thus it was proposed that histamine H_3-receptor might exist on the sympathetic terminals of guinea pig heart and modulated sympathetic neurotransmission. However, the direct evidence for H_3-receptor inhibiting norepinephrine (NE) release from sympathetic nerve study is to investigate whether H_3-receptors are involved in the inhibition of NE release from cardiac sympathetic terminals.

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MATERIALS AND METHODS

Guinea pig atrial preparation and protocol Guinea pig of either sex weighing 0.59 ± *s* 0.09 kg were stunned. The right heart atrium was prepared[6,7]. After 60 min of equilibration, field stimulation was started and trains of pulses (50 mA, 5 ms) were applied at 2-min intervals. A control circuit allowed timing of the field pulses to begin 20 ms after the driving pulse.

When the enhanced contraction induced by field stimulation reached peak, 3 mL of the bath solution was collected for determination of NE and an antioxidant 5 % sodium metabisulfite 50 μL was added to maintain the NE in its reduced form. The mixture was stored at − 20 ℃ and detected within 1 wk. To observe effects of agonists and antagonists on NE release evoked by field stimulation, the respective drug was added into bath solution 5 min before field stimulation.

Sample preparation The samples were thawed at room temperature, and 150 μL of 5 % sodium metabisulfite and 150 μL of disodium edetate were added. Then 50 mg of acid-washed alumina were added and the pH was adjusted to 8.6 with Tris-HCl buffer. The volume of sample was replenished to 6 mL by adding 1.5 mL distilled water. The tubes were shaken and centrifuged at 1500 × *g* for 5 min. The supernatant was discarded and NE was eluted by 200 μL HCl 0.05 mol·L^{-1}. The eluant (50 μL) was injected onto HPLC column.

Chromatography The HPLC system consisted of a Shimazu LC-6A pump, 7215 injector equipped with a 50 μL

loop, Shim-pack CLC-ODS column (150 mm × 6 mm, 5 μm particles), L-ECD-6A electrochemical detector, C-R3A integrator (Shimazu, Tokyo). The mobile phase was 50 mmol·L^{-1} sodium acetate and 1 % methanol. The pH was 3.5. The electrochemical detector was operated at + 750 mV *vs* an Ag/AgCl reference electrode. The sensitivity was 8 nA and flow rate was 1.2 mL·min^{-1}.

Drugs and reagents NE, yohimbine, butoxamine (Sigma); α-MeHA and Thio were generous gifts from Dr J M Arrang of Unité de Neurobiologie Centre Paul Brocade l'INSERM (Paris); ranitidine (№ 1 Southwest Pharmaceutical Factory, Chongqing, China); chlorphenframine (Beijing Pharmaceutical Factory); sodium octylsulfate (Tianjing Chenical Reagent Co, Tianjing, China); analytical grade sodium acetate, acetic acid, methanol, disodium edetate, HCl, trishydroxymethyl aminomethane, and sodium metabisulfite were all purchased from Xi-an Chemical Co (Xi-an, China).

RESULTS

Standard curve of NE was linear in amounts ranging from 125 pg − 2 ng when injected onto the column at a signal-noise ratio of 3∶1 with $r = 0.998$ and the linear equation was $\hat{Y} = 0.009 + 0.783X$. In fact, at range of 10 − 100 pg, a good correlation between amount of NE injected and electrochemical signals was also observed. The minimal detectable limit was 10 pg at 8 nA of detector range.

The application of α-MeHA (0.1 nmol·L^{-1} − 10 μmol·L^{-1}) depressed NE release induced by 16 field pulses in a concentration-dependent manner. The EC_{50} value of this effect was 5.8 nmol·L^{-1} with 1.2 − 10 nmol·L^{-1} of 95 % confidence limits and the maximal inhibitory rate of α-MeHA was 57 % (Fig 1).

The positive inotropic effect evoked by field stimulation was inhibited simultaneously. Thio (1 nmol·L^{-1} − 10 μmol·L^{-1}) concentration-dependently attenuated the above effects of α-MeHA and IC_{50} of Thio was 4.2 nmol·L^{-1} with 2.4 − 8.2 nmol·L^{-1} of 95 % confidence limits (Fig 2).

Thio, when used alone, facilitated the release of NE induced by field pulses. The inhibitory effect of α-MeHA was influenced neither by ranitidine (1 μmol·L^{-1}) and chlorpheniramine (1 μmol·L^{-1}) nor by yohimbine (1 μmol·L^{-1}) and butoxamine (1 μmol·L^{-1}) (Tab 1).

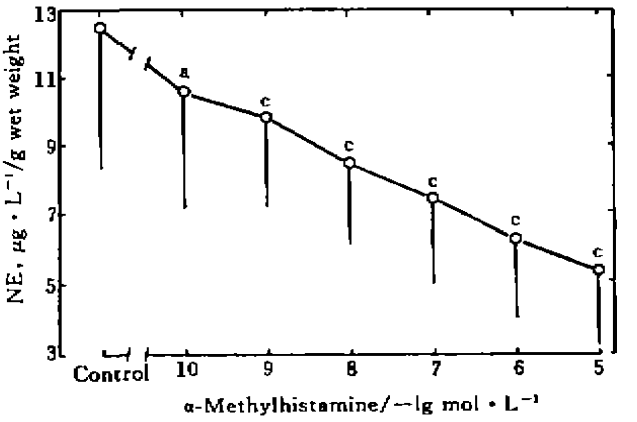

Fig 1. Effect of α-MeHA on release of NE induced by electric field stimulation (16 pulses) in guinea pig isolated atria. $n = 6$ **guinea pigs,** $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* **control.**

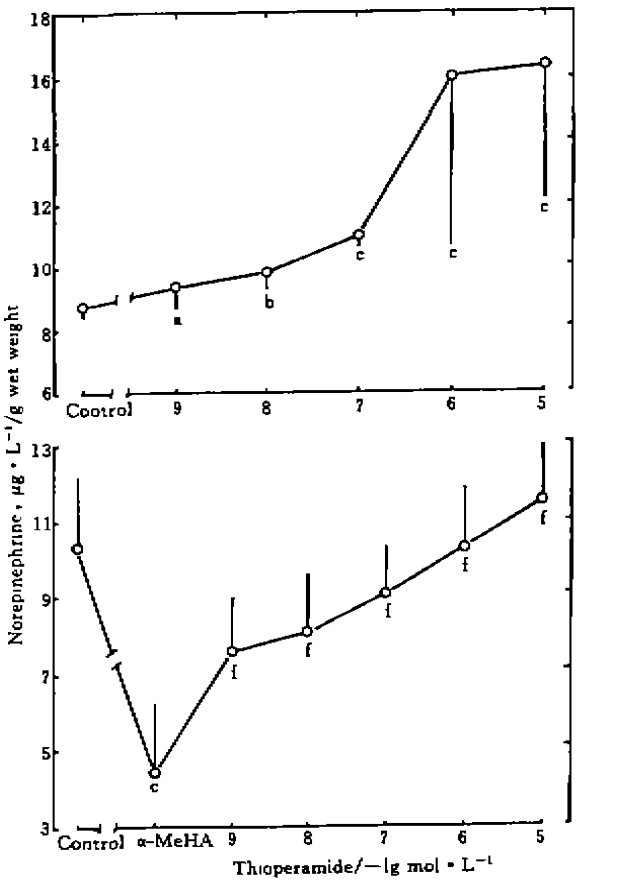

Fig 2. Effect of thioperamide on release of NE from cardiac sympathetic terminals caused by field stimulation (16 pulses) in isolated atria. $n = 4$ **guinea pigs,** $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* **control;** $^fP < 0.01$ *vs* **α-MeHA.**

Tab 1. Effects of ranitidine (1 μmol · L^{-1}), chlorpheniramine (1 μmol·L^{-1}), yohimbine (1 μmol·L^{-1}) and butoxamine (1 μmol·L^{-1}) on the inhibition of NE release induced by electric stimulation (16 pulses) by α-MeHA (1 μmol·L^{-1}) in isolated atria. n = 4 guinea pigs, $\bar{x} \pm s$. $^aP>0.05$ *vs* α-MeHA; $^fP<0.01$ *vs* control.

Group	NE, μg·L^{-1}/g wet weight
Control	10.8 ± 2.1
α-MeHA	6.4 ± 1.8^f
Ranitidine	7.1 ± 1.4^a
Chlorpheniramine	5.3 ± 0.9^a
Yohimbine	7.0 ± 1.1^a
Butoxamine	6.0 ± 0.4^a

DISCUSSION

The field stimulation evoked endogenous NE release from isolated atrium of guinea pig is tetrodotoxin-sensitive[9] and thus probably represents quasi-physiological transmitter release from noradrenergic neurons.

The present experiments revealed that in the isolated guinea pig atrium α-MeHA inhibited field stimulation-evoked NE release from sympathetic neurons in concentration-dependent manner. This inhibition is in accordance with the effects of α-MeHA and histamine in variety of sympathetically innervated tissues isolated from various species[4,5,10,11]. The EC_{50} and maximum inhibitory effect of α-MeHA were nearly similar to those observed in rat brain on inhibition of [^{3}H]histamine release[12]. The selective H_3-receptor antagonist Thio reversed the inhibitory effect of α-MeHA. Thio concentration-dependently facilitated NE release and positive inotropic action induced by field stimulation[6,7]. This suggested that endogenous histamine might be involved in inhibition of NE release by interacting with histamine H_3-receptors. The similar results were also observed when presynaptic α_2-adrenoceptor of myocardium were blocked by yohimbine.

Take together, the present data suggest that the sympathetic nerve terminals of guinea pig atrium are endowed with inhibiting presynaptic histamine H_3-receptors and activation of H_3-receptor could inhibit sympathetic neurotransmission.

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组胺 H_3 受体介导抑制豚鼠心交感神经释放去甲肾上腺素的直接依据

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关键词 心房；电刺激；甲基组胺；组胺拮抗剂；

去甲肾上腺素；节后交感神经纤维；组胺 H_3 受体；高压液相色谱法

目的：观察组胺 H_3 受体介导抑制心交感神经末稍释放去甲肾上腺素(NE)。方法：采用 HPLC-ECD 法检测电场刺激(50 mA，5 ms)诱发交感神经末稍释放 NE。结果：α-甲基组胺浓度依赖性抑制电场刺激诱发的 NE 释放。H_1，H_2，α_2，β_2 受体拮抗剂不能拮抗 α-甲基组胺的作用。Thioperamide 浓度依赖性拮抗 α-甲基组胺的作用　单独应用 thioperamide 可促进 NE 释放　结论：突触前组胺 H_3 受体参与介导抑制心交感神经末稍释放 NE。

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Effects of HI-6 on muscle acetylcholine receptor: analysis on minimal reaction model

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KEY WORDS HI-6; skeletal muscle; cholinergic receptors; messenger RNA; *Xenopus laevis*; oocytes

AIM: To study the action of 1-(2-hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6) on skeletal muscle acetylcholine receptor (N_2-ChR). **METHODS**: N_2-ChR was expressed in *Xenopus laevis* oocyte after injection with mRNA extracted from denervated rat leg muscles. The inward membrane currents induced by various concentrations of carbamylcholine and effects of HI-6 or *d*-tubocurarine on the currents were measured with voltage clamp technique by fast cell flow of agents. The actions of HI-6 and *d*-tubocurarine on N_2-ChR were analyzed by using the minimal reaction model. **RESULTS**: K of 40.05, 156.00, and 334.67 μmol·L^{-1} for HI-6, K of 0.02, 0.10, and 0.18 μmol·L^{-1} for *d*-tubocurarine were obtained by using the competing for single acetylcholine (ACh)-binding site model, the competing for two ACh-binding sites model, and the noncompetitive inhibition model, respectively. **CONCLUSION**: HI-6 and *d*-tubocurarine competed for two ACh-binding sites of N_2-ChR with equal affinity to antagonize the effects of the agonist on N_2-ChR. The N_2-ChR inhibition by HI-6 is much weaker than that by *d*-tubocurarine.

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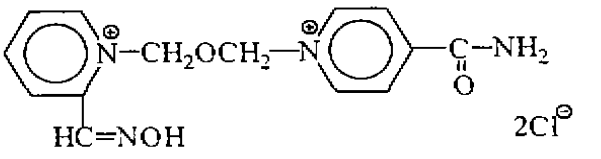
Oximes acetylcholinesterase reactivators such as 1-(2-hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6) and 1,1′-[Oxybis(methylene)]bis-[(4-hydroxyimino)methyl]-pyridinium dichloride (obidoxime chloride) together with certain cholinergic blockers have been used effectively to antagonize organophosphorus agent (OP)-poisoning. However, the acetylcholinesterase reactivation mechanism alone is not sufficient to explain the antidotal effect of oximes against OP-induced toxicity[1]. Some other mechanisms such as antimuscarinic, ganglion-blocking, neuromuscular-blocking properties, and acetylcholinesterase-like activity may also contribute to their antidotal effect. The effects of oximes reactivators on N_2-ChR is quite complicated, including curarine-like inhibition[2], noncompetitive antagonism of N_2-ChR and excitatory actions of N_2-ChR[3]



1-(2-Hydroxyimino-methyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dichloride (HI-6)