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Tetraethylammonium and 4-aminopyridine enhancement of 5-HT_3 receptor-mediated contraction of guinea pig ileum *in vitro*[1]

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KEY WORDS serotonin receptors; tetraethylammonium compounds; 4-aminopyridine; ileum; myenteric plexus; carbachol; serotonin agonists; serotonin antagonists; radioligand assay

AIM: To study the effects of tetraethylammonium (TEA) and 4-aminopyridine (4-AP) on 5-HT_3 receptor-mediated contractions of the isolated guinea pig ileum longitudinal muscle-myenteric plexus strip preparations (GPI). **METHODS:** GPI contractions were recorded with a chart recorder through isometric transducers. The effect of TEA and 4-AP on binding properties of 5-HT_3 receptors was assessed using [^{3}H]GR65630 binding assay in membrane preparation of rat entorhinal cortex. **RESULTS:** (1) Both TEA 0.5 mmol · L^{-1} and 4-AP 5 μmol · L^{-1} increased the spontaneous activity, and elicited contractions of GPI; atropine 10 μmol · L^{-1} or the selective 5-HT_3 receptor antagonist MDL72222 100 μmol · L^{-1} prevented these effects. (2) Both TEA 0.05 - 0.5 mmol · L^{-1} and 4-AP 1 - 10 μmol · L^{-1} enhanced GPI contractions induced by the selective 5-HT_3 receptor agonist 2-methyl-5-HT in concentration-dependent manners. (3) Both TEA 0.5 mmol · L^{-1} and 4-AP 5 μmol · L^{-1} attenuated the inhibitory effects of the selective 5-HT_3 receptor antagonists tropisetron 0.1 μmol · L^{-1} and benesetron 1 μmol · L^{-1} on 5-HT_3 receptor-mediated GPI contractions. (4) Neither TEA 0.1 - 0.5 mmol · L^{-1} nor 4-AP 5 - 10 μmol · L^{-1} affected GPI contractions evoked by the selective M-ACh receptor agonist carbachol 1 μmol · L^{-1}. (5) TEA 0.5 mmol · L^{-1} and 4-AP 10 μmol · L^{-1} had no effect on the properties of binding of the selective 5-HT_3 receptor radioligand [^{3}H]GR65630 to 5-HT_3 receptors. **CONCLUSION:** The enhancement by TEA and 4-AP of 5-HT_3 receptor-mediated GPI contractile responses was due to blocking K^+ channels in prejunctional myenteric neurons.

5-HT_3 receptors are cation-selective channels with essentially equal permeability to Na^+ and K^+. Activation of the receptors by 5-HT elicits a rapidly activating and desensitizing inward current that leads to fast depolarization of the neuronal membrane. The main function of 5-HT_3 receptors in the peripheral and central nervous systems is modulation of the release of neuro ' transmitters, including ACh, GABA, dopamine, norepinephrine, cholecystokinin, *etc*[1,2].

The isolated guinea pig ileum longitudinal muscle-myenteric plexus strip (GPI) provides the simplest model for studying the physiological and pharmacological properties of 5-HT_3 receptors. 5-HT, depending upon the concentrations used, interacted with three types of 5-HT receptors in this preparation, *ie*, neuronal 5-HT_3 and 5-HT_4 receptors, 5-HT_2 receptors located on the smooth muscle[3,4]. The main component of contractions induced by 5-HT 0.1 - 10 μmol · L^{-1} was inhibited by low concentration of Na^+ channel blocker tetrodotoxin, or the M-ACh receptor antagonist atropine, thus was proposed to be mediated via activation of neuronal 5-HT_3 receptors that led to the release of ACh from the myenteric plexus neurons[5,6].

Tetraethylammonium (TEA) and 4-aminopyridine (4-AP) enhanced neurotransmitter release in various preparations via blockade of K^+ channels in the presynaptic terminals[7-9]. If 5-HT_3 receptors in the myenteric plexus neurons do mediate the main component of GPI contractile responses, the responses should also be affected by the K^+ channel blockers. For address this issue, the interaction between the

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K^+ channel blockers and 5-HT_3 receptor-mediated GPI contractile responses were examined in the present study.

MATERIALS AND METHODS

Chemicals 2-Methyl-5-HT, benesetron, and TEA (Research Biochemicals International, USA); 5-HT, carbachol, 4-AP, and metoclopromide (Sigma); [^{3}H]GR65630 (DuPont NEN, USA); tropisetron (synthesized and gifted by Prof ZHU You-Cheng, Shanghai Institute of Materia Medica, Chinese Academy of Sciences).

Measurement of contractions of GPI Experiments were performed on strips of longitudinal muscle with adherent myenteric plexus from guinea pig (♂, 300 – 400 g, Certificate № 117) ileum mounted in organ baths (5 mL) containing Tyrode solution, which consisted of: NaCl 137.0, $CaCl_2$ 1.8, KCl 2.7, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.6 mmol·L^{-1}, and gassed with 95 % O_2 + 5 % CO_2 at 37 ℃[10]. Contractions were recorded with a chart recorder via isometric transducers. Non-cumulative concentration-response curves were established with and without TEA or 4-AP applied 5 min before each addition of the agonist. Experiments with the same protocol were repeated with tropisetron or benesetron added 10 min before agonist application. The peak amplitude of the response to each concentration of agonist was expressed as a % of that of the response to carbachol 10 μmol·L^{-1}.

Radioligand binding assay Crude membrane preparation was made from the entorhinal cortex of Sprague-Dawley rats (♂, 200 – 250 g)[11]. For binding assay, [^{3}H]GR65630 100 μL (2286.6 TBq·mol^{-1}, final concentration of 0.3 nmol·L^{-1}), membrane preparation 100 μL (0.3 – 0.4 mg protein), and 5-HT_3 receptor agonists or antagonists 100 μL (different concentrations) were mixed at 4 ℃, and added with ice-cold HEPES buffer (50 mmol·L^{-1}, pH 7.4) to a volume of 1 mL. The mixture was incubated at 37 ℃ for 30 min and then subjected to a rapid vacuum filtration through Whatman GF/B filters using a Brandel Cell Harvester. The filters were washed immediately with 3 mL ice-cold Tris·HCl buffer (50 mmol·L^{-1}, pH 7.4) for three times. Radioactivity was assayed with a Beckman LS6000LL scintillometer (efficiency = 47 %). Nonspecific binding was determined by the inclusion of 5-HT_3 receptor antagonist metoclopramide 100 μmol·L^{-1}, which inhibited 50 % – 60 % of total binding of [^{3}H]GR65630 0.3 nmol·L^{-1} in the washed crude membrane preparation. All individual assays were carried out in replicates of three.

Data analysis IC_{50} values were calculated using computer software 'GraphPad InPlot.' The data were presented as $\bar{x} \pm s$ and compared with Student's t-test.

RESULTS

GPI contractions elicited by TEA and 4-AP Addition of TEA 0.5 mmol·L^{-1} into the organ bath caused a substantial increase in the spontaneous activity, and elicited contractions of GPI. Pretreatment of GPI with atropine 10 μmol·L^{-1} prevented TEA-induced GPI contractions. Pretreatment of GPI with the selective 5-HT_3 receptor antagonist MDL72222 100 μmol·L^{-1} not only blocked TEA-induced GPI contractions, but also suppressed the spontaneous activity of GPI (Fig 1). Similar effects were observed when 4-AP 5 μmol·L^{-1} was tested.

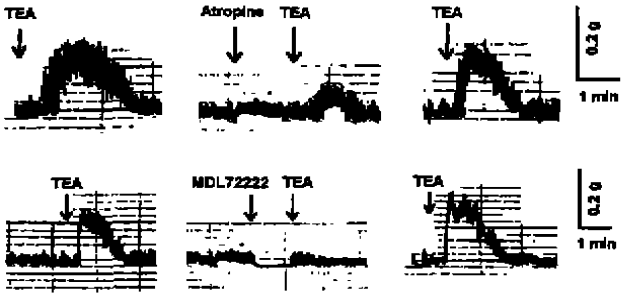

Fig 1. Atropine and MDL72222 blocked TEA-induced GPI contraction. The left: TEA 0.5 mmol·L^{-1} induced GPI contraction. The middle: the contraction was blocked by atropine 10 μmol·L^{-1} or MDL72222 100 μmol·L^{-1} applied 10 min before adding TEA. To obtain comparable chart lengths, the recorder was slowed down between the drug applications. The right: the contraction recovered after washout of atropine or MDL72222, n = 8 strips from 8 guinea pigs.

Effects of TEA and 4-AP on 5-HT_3 receptor-mediated GPI contraction TEA

0.05 – 0.5 mmol·L^{-1} and 4-AP 1 – 10 μmol·L^{-1} enhanced 5-HT_3 receptor-mediated GPI contractions in concentration-dependent manners. Increasing concentrations of TEA or 4-AP produced progressive enhancement of GPI contraction induced by the selective 5-HT_3 receptor agonist 2-methyl-5-HT (Fig 2).

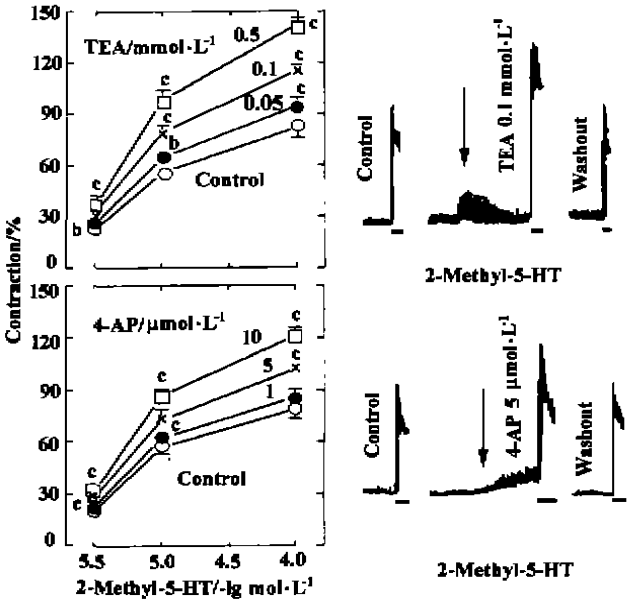

Fig 2. TEA and 4-AP enhanced 2-methyl-5-HT-induced GPI contraction. $n = 5$ strips. $\bar{x} \pm s$. Concurrent contractions are shown for 2-methyl-5-HT 10 μmol·L^{-1} with and without TEA or 4-AP. $^bP < 0.05$, $^cP < 0.01$ vs control.

With TEA 1 mmol·L^{-1} or 4-AP 20 μmol·L^{-1} in the organ baths, the spontaneous contractions became extremely intense, so that the effect of 2-methyl-5-HT could not be tested. Similar results were obtained when 5-HT 0.1 – 10 μmol·L^{-1} was used as agonist.

TEA and 4-AP attenuated the inhibitory effects of tropisetron and benesetron on 5-HT-evoked GPI contraction GPI contractions induced by high concentrations of 5-HT (≥0.1 μmol·L^{-1}) have been shown to be mediated mainly via 5-HT_3 receptors[12]. These contractile responses were competitively inhibited by the selective 5-HT_3 receptor antagonists tropisetron and benesetron. When TEA 0.5 mmol·L^{-1} or 4-AP 5 μmol·L^{-1} was added, the inhibitory effects of tropisetron and benesetron on GPI contractions induced by 5-HT 0.1 – 5 μmol·L^{-1} were reversed (Fig 3).

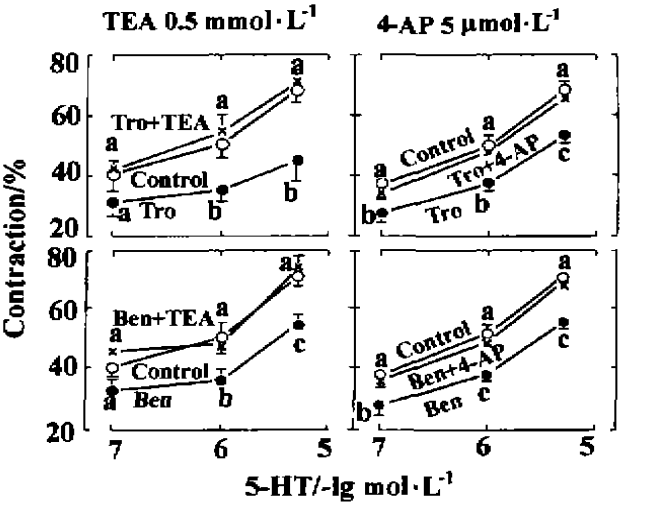

Fig 3. TEA and 4-AP attenuated the inhibitory effects of tropisetron (Tro) 0.1 μmol·L^{-1} or benesetron (Ben) 1 μmol·L^{-1} on the 5-HT-induced GPI contraction. $n = 3$ strips, $\bar{x} \pm s$, $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

Effect of TEA and 4-AP on carbachol-evoked GPI contraction TEA (0.1 and 0.5 mmol·L^{-1}) or 4-AP (5 and 10 μmol·L^{-1}) which enhanced 5-HT_3 receptor-mediated GPI contractions (Fig 2), had no detectable effects on GPI contractions induced by the selective M-ACh receptor agonist carbachol 1 μmol·L^{-1} (n = 5 strips). The carbachol-induced contractions in presence of TEA 0.1 and 0.5 mmol·L^{-1} [(74 ± 14) %, (73 ± 8) %] or 4-AP 5 and 10 μmol·L^{-1} [(72 ± 9) %, (67 ± 17) %] had no significant difference with those induced by carbachol alone [(71 ± 11) %].

Effect of TEA and 4-AP on 5-HT_3 receptor binding TEA 0.5 mmol·L^{-1} and 4-AP 10 μmol·L^{-1} neither inhibited the total binding of the selective 5-HT_3 receptor radioligand [^{3}H]GR65630 0.3 nmol·L^{-1} to 5-HT_3 receptors in rat entorhinal cortex nor affected the IC_{50} of 5-HT, 2-methyl-5-HT, tropisetron, and benesetron in [^{3}H]GR65630 0.3 nmol·L^{-1} binding (Tab 1). These results suggested that TEA and 4-AP had no direct effect on the properties of binding of [^{3}H]GR65630 to 5-HT_3 receptors.

Tab 1. Effects of TEA and 4-AP on binding of [^{3}H]GR65630 0.3 nmol·L^{-1} to 5-HT$_3$ receptors. n = 3 membrane homogenates of 5 rat entorhinal cortices. $\bar{x} \pm s$. $^aP > 0.05$ vs control.

	Control	TEA/ 0.5 mmol·L^{-1}	4-AP/ 10 μmol·L^{-1}
Total binding/Bq	71 ± 6	67 ± 7^a	74 ± 9^a
IC_{50}/nmol·L^{-1}			
5-HT	208 ± 45	195 ± 39^a	234 ± 49^a
2-Methyl-5-HT	156 ± 37	178 ± 41^a	140 ± 29^a
Tropisetron	18 ± 4	15 ± 3^a	21 ± 6^a
Benesetron	99 ± 21	106 ± 31^a	98 ± 26^a

DISCUSSION

The present study demonstrated that low concentrations of TEA (< 0.5 mmol·L^{-1}) or 4-AP (< 10 μmol·L^{-1}) exerted two effects on GPI preparations: (1) increased the spontaneous activity and elicited contractions; (2) enhanced the 5-HT$_3$ receptor-mediated GPI contractile responses. The TEA- and 4-AP-induced GPI contractions were blocked by atropine. Neither TEA nor 4-AP affected the carbachol-induced GPI contractile responses. Furthermore, the concentrations of TEA and 4-AP needed to exert direct excitatory effects on smooth muscle cells were at least one magnitude higher[7,13] than those used in the present study. Therefore, the action of the low concentrations of TEA and 4-AP should be confined to the prejunctional myenteric plexus neurons.

The effects of TEA or 4-AP seemed not to be due to changes in the 5-HT$_3$ receptors *per se*, at least in their property of binding to the specific ligand. Therefore, the reverse by the K$^+$ channel blockers of the inhibitory effect of tropisetron or benesetron on the 5-HT$_3$ receptor-mediated GPI contractile responses should be attributed to a certain type of functional antagonism. Furthermore, the fact that the selective 5-HT$_3$ receptor antagonist MDL72222 suppressed the spontaneous activity of GPI (Fig 1) suggested the existence of tonic activation of 5-HT$_3$ receptors in myenteric plexus neurons.

In guinea-pig cerebral cortical synaptosome preparation, 4-AP has been demonstrated to elevate the cytosolic free Ca^{2+} concentration and to evoke glutamate release by blocking the presynaptic K$^+$ channels responsible for stabilizing the membrane potential, and eliciting repetitive firing[8]. In cultured bovine adrenal chromaffin cells, TEA stimulated catecholamine secretion through similar mechanisms[9]. The same would occur in myenteric plexus neurons where TEA and 4-AP increased the release of ACh evoked by activation of 5-HT$_3$ receptors. Our results demonstrated that some functional interactions existed between 5-HT$_3$ receptors and several types of K$^+$ channels, including ATP-sensitive K$^+$ channels[12], in prejunctional myenteric plexus neurons of GPI. In electrophysiological studies 5-HT$_3$ receptor-mediated current responses have been shown a rapid desensitization[1,2]. Since the activity of K$^+$ channels *in vivo* is modulated by a variety of endogenous substances through protein phosphorylation and dephosphorylation, or direct interaction with activated G proteins[14,15], the interaction between 5-HT$_3$ receptors and K$^+$ channels may underlie a mechanism that could compensate for desensitization of 5-HT$_3$ receptors.

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四乙铵和 4-氨基吡啶增强 5-HT_3 受体介导的离体豚鼠回肠收缩[1]

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关键词 血清素受体；四乙铵化合物；4-氨基吡啶；回肠；肠肌丛；卡巴胆碱；血清素激动药；血清素拮抗药；放射配体试验

目的：研究四乙铵（TEA）、4-氨基吡啶（4-AP）对 5-HT_3受体介导的豚鼠回肠收缩的影响. **方法**：等长换能器记录回肠收缩；[^{3}H]GR65630 结合试验测定 5-HT_3 受体结合特性. **结果**：TEA、4-AP 引起回肠收缩并增强自发活动，被阿托品或 MDL72222 阻断. TEA、4-AP 增强 2-甲基-5-HT 和 5-HT 引起的收缩；逆转托烷司琼或 Benesetron 的抑制作用；不影响卡巴胆碱引起的收缩. TEA、4-AP不影响 5-HT_3 受体结合配体. **结论**：TEA、4-AP可能通过阻断突触前神经元钾通道增强 5-HT_3 受体介导的回肠收缩.

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L-type calcium channel blockade mechanisms of panaxadiol saponins against anoxic damage of cerebral cortical neurons isolated from rats

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KEY WORDS patch-clamp techniques; neurons; ion channels; calcium; anoxia; ginseng; saponins; cerebral cortex

AIM: To identify the changes of L-type Ca^{2+} channel on cerebral cortical neurons of rats during anoxia and the protective mechanisms of panaxadiol saponins (PDS) against anoxic injury. **METHODS**: Patch-clamp technique of cell-attached configuration and *in vitro* cerebral anoxic modle built with actuely isolated cortical cells of Wistar rats. **RESULTS**: The open time of L-type Ca^{2+} channel of cortical neurons increased significantly from (2.85 ± 0.21) ms to (9.1 ± 1.0) ms ($P < 0.01$) under anoxia. The particular change was a long-lasting open, which was more than 20 ms in some cases. At the same time, the close time decreased from (38 ± 8) ms to (10 ± 3) ms ($P < 0.01$) and the open-state probability raised from (0.047 ± 0.008) to (0.165 ± 0.025) ($P < 0.01$). PDS (1.5 g·L^{-1}) inhibited the activity of L-type Ca^{2+} channel both in normal and anoxic condition [open time from (2.23 ± 0.47) ms and (9.1 ± 1.0) ms to (1.03 ± 0.25) ms and (2.1 ± 0.4) ms; close time from (38 ± 10) ms and (10 ± 3) ms to (74 ± 16) ms and (46 ± 10 ms); open-state probability from (0.043 ± 0.006) and (0.165 ± 0.025) to (0.012 ± 0.004) and (0.021 ± 0.009), respectively, P all < 0.01]. The results of PDS were similar to those of

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