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Pinacidil suppression on 5-HT_3 receptor-mediated contraction of guinea pig ileum *in vitro*

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KEY WORDS serotonin receptors; pinacidil; *ileum*; *myenteric plexus*; carbachol; GR65630

AIM: To study the effects of the K^+ channel opener pinacidil on 5-HT_3 receptor-mediated contractions of the isolated guinea pig ileum (GPI) longitudinal muscle-myenteric plexus strip preparations. **METHODS**: GPI contractions were recorded with a chart recorder through isometric transducers. The effect of pinacidil on binding properties of 5-HT_3 receptors was assessed using [^{3}H] GR65630 binding assay in membrane preparations of rat entorhinal cortex. **RESULTS**: (1) A selective 5-HT_3 receptor agonist 2-methyl-5-HT 0.1 – 300 $\mu mol \cdot L^{-1}$ and 5-HT 0.001 – 50 $\mu mol \cdot L^{-1}$ elicited GPI contractile responses in concentration-dependent manners, the EC_{50} values (and 95 % confidence limits) for 2-methyl-5-HT and 5-HT were 10.0 (8.9 – 11.2) $\mu mol \cdot L^{-1}$ and 1.6 (1.3 – 1.9) $\mu mol \cdot L^{-1}$, respectively. Selective 5-HT_3 receptor antagonist tropisetron 0.1 $\mu mol \cdot L^{-1}$ competitively inhibited the responses to 2-methyl-5-HT and 5-HT. (2) Pinacidil 0.5 – 5 $\mu mol \cdot L^{-1}$ inhibited 5-HT_3 receptor-mediated contractions. (3) Pinacidil 1 $\mu mol \cdot L^{-1}$ enhanced the inhibitory effects of tropisetron 0.1 $\mu mol \cdot L^{-1}$ or another selective 5-HT_3 receptor antagonist benesetron 1 $\mu mol \cdot L^{-1}$ on 5-HT-induced GPI contractile responses. (4) Pinacidil 1 – 5 $\mu mol \cdot L^{-1}$ did not affect GPI contractile responses evoked by a selective M-ACh receptor agonist carbachol 1 $\mu mol \cdot L^{-1}$. (5) Pinacidil 1 – 5 $\mu mol \cdot L^{-1}$ had no effect on binding properties of 5-HT_3 receptors with selective 5-HT_3 receptor radioligand [^{3}H] GR65630 in the entorhinal cortex of rat brain. **CONCLUSION**: The inhibition by pinacidil of 5-HT_3 receptor-mediated GPI contractile responses may be mediated through activation of ATP-sensitive K^+ channels located in prejunctional *myenteric neurons*.

The K^+ channel openers (KCO) form a novel class of compounds in clinical applications including the treatment of cardiovascular diseases, bronchial asthma, or irritable bladder syndrome. These KCO acted primarily on a heterogeneous class of K^+ channels, ATP-sensitive K^+ channels, which were found in excitable tissues including heart, skeletal and smooth muscle, neurons, and pancreatic β-cells[1]. Basically, these agents increase potassium conductance by activation of ATP-sensitive K^+ channels and thus lead to a hyperpolarization of the cell membrane and reduced membrane excitability. Consequently, KCO relax smooth muscle preparations precontracted by spasmogens[2].

5-HT_3 receptor was originally described as 'M' type of 5-HT receptor in view of the inhibition by morphine of the contractile response of isolated GPI to 5-HT. In electrophysiologic studies, 5-HT_3 receptors have been characterized as a new member of the ligand-gated ion channel superfamily, similar in many aspects to N-ACh receptor[3]. The function of 5-HT_3 receptors in both the central and peripheral nervous systems is mainly involved in modulation of the release of neurotransmitters, including ACh, dopamine, GABA, noradrenaline, cholecystokinin, *etc*[4]. The main component of GPI contractions induced by 5-HT 0.1 – 10 $\mu mol \cdot L^{-1}$ was sensitive to tetrodotoxin 1 $\mu mol \cdot L^{-1}$, thus mediated via activation of neuronal 5-HT_3 receptors that led to the release of ACh from the myenteric plexus neurons[5,6].

Cromakalim, a K^+ channel opener, reduced cholinergic transmission in airway smooth muscle preparations[7] and inhibited contractions of GPI caused by the release of endogenous ACh via electric stimulation or via activation of 5-HT_3 receptors[8,9]. Pinacidil, a cyanoguandine potassium channel opener, inhibited release of dopamine and norepinephrine in rat

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vas deferens[10]. In the present work, the effects of pinacidil on the 5-HT_3 receptor-mediated contractile responses of GPI were examined.

MATERIALS AND METHODS

Chemicals 2-Methyl-5-HT, benesetron and pinacidil (Research Biochemicals International, USA); 5-HT, carbachol and metoclopromide (Sigma, USA); [^{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 Guinea pigs (♂, 300 – 400 g) were stunned. The ileum was excised approximately 10 cm from the ileo-cecal junction and strips of longitudinal muscle with adherent myenteric plexus were freed from the underlying circular muscle. In each organ bath (5 mL), one longitudinal muscle strip, 2 – 3 cm long, was mounted. The bath contained Tyrode's 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 ℃. After equilibration for 1 h, the strips were placed under a resting tension of 0.5 g. Contractions were recorded with a chart recorder via isometric transducers. Noncumulative concentration-response curves were established by adding increasing concentrations of 5-HT_3 receptor agonist (50 μL) to the organ bath at intervals of at least 20 min, which was found in preliminary experiments to be long enough to avoid tachyphylaxis. Each concentration of the agonist was left in contact with the tissue for about 30 s. The curves were then reconstructed in the presence of pinacidil with increasing concentrations applied 5 min before each application of the agonist. Each strip was used to record only 2 concentration-response curves: one for the agonist alone and the other for the agonist in the presence of one concentration of pinacidil. Experiments with the same protocol were repeated with selective 5-HT_3 receptor antagonists tropisetron or benesetron added 10 min before agonist application. All peak responses to each concentration of the agonist were expressed as a % of the response to carbachol 10 μmol·L^{-1}.

Receptor-binding assay with radioligand Sprague-Dawley rats (♂, 200 – 250 g) were decapitated. Pooled entorhinal cortex tissue was homogenized (Ultra Turrax T25) in 10 vol HEPES buffer (50 mmol·L^{-1}, pH 7.4, 4 ℃) and spun at 4 ℃ and 49 000 × g for 10 min. The supernatant was discarded and the process repeated for the pellet. The final pellet was suspended in 10 vol HEPES buffer. For binding, assay tubes were added with 100 μL selective 5-HT_3 receptor radioligand [^{3}H] GR65630 (2286.6 TBq · mol^{-1}, final concentration of 0.3 nmol·L^{-1}) in HEPES buffer, testing drug 100 μL, and tissue preparation 100 μL (0.3 – 0.4 mg protein). Tubes were incubated at 37 ℃ for 30 min, which was terminated by rapid vacuum filtration through Whatman GF/B filters using a Brandel Cell Harvester. Filters were washed immediately with 3 mL Tris·HCl buffer (50 mmol·L^{-1}, pH 7.4, 4 ℃) for three times. Radioactivity was assayed with a Beckman LS6000LL scintillometer (effeciency = 47 %). All individual assays were carried out in replicates of three. 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 washed crude homogenates of rat entorhinal cortex.

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

RESULTS

5-HT_3 receptor-mediated GPI contraction

2-Methyl-5-HT, a selective 5-HT_3 receptor agonist, evoked GPI contractile responses in a concentration-dependent manner with EC_{50} of 10.0 (8.9 – 11.2) μmol · L^{-1}. Selective 5-HT_3 receptor antagonist tropisetron 0.1 μmol · L^{-1} competitively inhibited 2-methyl-5-HT induced contractile responses. Similar phenomena were seen when 5-HT was used as agonist (Fig 1). The EC_{50} for 5-HT was 1.6 (1.3 – 1.9) μmol·L^{-1}. Note that the responses induced by 5-HT (1 – 10 nmol·L^{-1}) were insensitive to tropisetron. Only the responses induced by 5-HT (≥ 0.1 μmol·L^{-1}) were mediated via 5-HT_3 receptors.

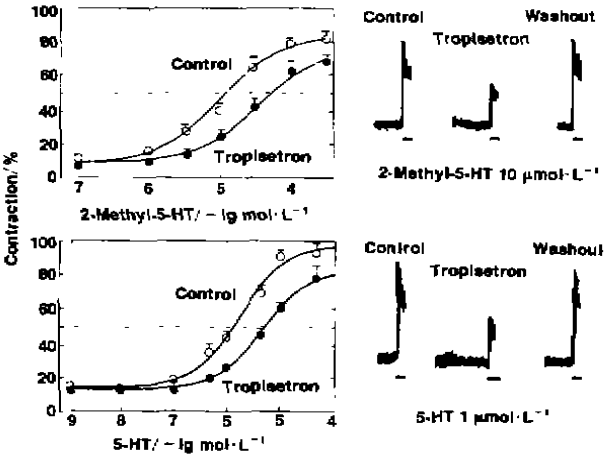

Fig 1. 2-Methyl-5-HT- and 5-HT-induced GPI contractions with and without tropisetron 0.1 μmol·L^{-1}. n = 5 strips, $\bar{x} \pm s$.

Effects of pinacidil on 5-HT_3 receptor-mediated GPI contraction Pinacidil inhibited GPI

contractile responses to 2-methyl-5-HT in a concentration-dependent manner. Increasing concentrations of pinacidil from 0.5 to 5 $\mu mol \cdot L^{-1}$ produced progressive suppression of GPI contraction induced by 2-methyl-5-HT. Addition of pinacidil into the organ bath always caused a substantial decrease of spontaneous activity of GPI preparations (Fig 2). Similar results were obtained when 5-HT 0.1 – 10 $\mu mol \cdot L^{-1}$ was used as agonist.

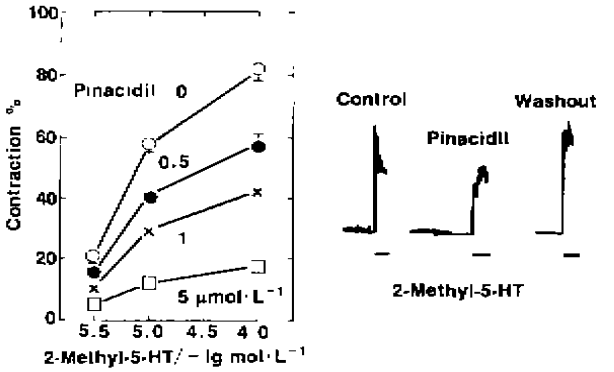

Fig 2. Suppression of 2-methyl-5-HT-induced GPI-contraction by pinacidil. $n = 5$ strips, $x \pm s$. Concurrent contractions are shown for 2-methyl-5-HT 10 $\mu mol \cdot L^{-1}$ with and without pinacidil 1 $\mu mol \cdot L^{-1}$.

Pinacidil enhanced the inhibitory effect of tropisetron and benesetron on 5-HT-evoked GPI contraction GPI contractions induced by 5-HT (0.1 – 10 $\mu mol \cdot L^{-1}$) were competitively suppressed by tropisetron 0.1 $\mu mol \cdot L^{-1}$. When pinacidil 1 $\mu mol \cdot L^{-1}$ was added, the inhibitory effect of tropisetron increased. Similar results were obtained when another selective 5-HT_3 receptor antagonist benesetron 1 $\mu mol \cdot L^{-1}$ was tested (Fig 3).

Effect of pinacidil on carbachol-evoked GPI contraction Pinacidil 1 and 5 $\mu mol \cdot L^{-1}$, which suppressed GPI contractions induced by 2-methyl-5-HT (Fig 2), had no detectable effects on GPI contractile responses induced by a selective M-ACh receptor agonist carbachol 1 $\mu mol \cdot L^{-1}$. The carbachol-induced contractions in presence of pinacidil 1 or 5 $\mu mol \cdot L^{-1}$ (68 % ± 14 %, 71 % ± 12 %) had no significant difference with those induced by carbachol alone (74 % ± 12 %). These observations suggest that the inhibition by pinacidil of 5-HT_3 receptor-mediated GPI contractions may be mediated via a prejunctional mechanism.

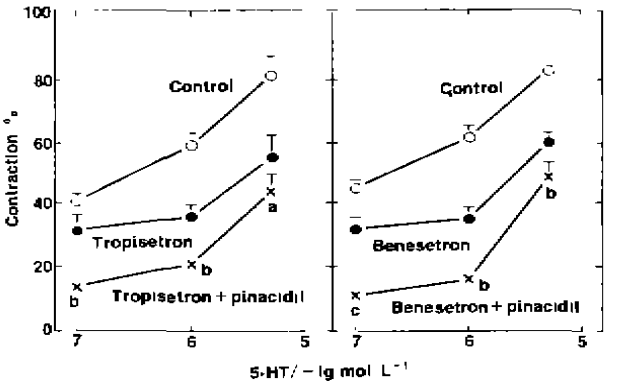

Fig 3. Enhancement of inhibitory effects of tropisetron 0.1 $\mu mol \cdot L^{-1}$ or benesetron 1 $\mu mol \cdot L^{-1}$ on 5-HT-induced contraction of GPI by pinacidil 1 $\mu mol \cdot L^{-1}$. $n = 3$ strips, $x \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* inhibition by tropisetron or benesetron alone.

Effect of pinacidil on binding properties of 5-HT_3 receptors Due to the relatively low density of 5-HT_3 receptors in myenteric plexus neurons of GPI, we tested the effect of pinacidil on the binding properties of 5-HT_3 receptors in rat entorhinal cortex that has been found to have the highest distribution of 5-HT_3 receptors in rat brain[11]. Pinacidil 5 $\mu mol \cdot L^{-1}$ neither inhibited the total binding of 5-HT_3 receptors in the entorhinal cortex with selective 5-HT_3 receptor radioligand [^{3}H]GR65630 0.3 $nmol \cdot L^{-1}$, nor affect the IC_{50} of 5-HT, 2-methyl-5-HT, tropisetron, and benesetron to compete for [^{3}H]GR65630 0.3 $nmol \cdot L^{-1}$ binding (Tab 1). These results suggested that pinacidil had no direct effect on the property of 5-HT_3 receptor binding for [^{3}H]GR65630.

Tab 1. Effects of pinacidil on [^{3}H] GR65630 0.3 $nmol \cdot L^{-1}$ binding with 5-HT_3 receptors. $n = 3$ membrane homogenates of 5 rat entorhinal cortices, $x \pm s$. $^aP > 0.05$ *vs* control.

	Control	Pinacidil (5 $\mu mol \cdot L^{-1}$)
Total binding/Bq	71 ± 6	69 ± 7[a]
IC_{50}/$nmol \cdot L^{-1}$		
5-HT	208 ± 45	228 ± 56[a]
2-Methyl-5-HT	156 ± 37	166 ± 35[a]
Tropisetron	18 ± 4	23 ± 7[a]
Benesetron	99 ± 21	118 ± 29[a]

DISCUSSION

The results of the present study showed that

pinacidil within a certain range of concentrations inhibited the 5-HT_3 receptor-mediated GPI contractile responses. These effects seemed not to be produced via a postjunctional mechanism since the carbachol-induced GPI contractile responses were not affected by the K^+ channel opener. The action site of pinacidil is likely to be in prejunctional myenteric plexus neurons where 5-HT_3 receptors locate as suggested by Zini *et al* that the effect of KCO could occur presynaptically[10]. This notion was also in accord with the previous report that 5-HT-induced GPI contractile responses induced by 5-HT 0.1 – 10 $\mu mol \cdot L^{-1}$ were mediated via neuronally located 5-HT_3 receptors[12]. On the other hand, Sun and Benishin found that pinacidil 1 $\mu mol \cdot L^{-1}$ decreased the basal tension in the plexus-free longitudinal muscle of guinea-pig ileum[13]. This observation has thus been implicated that the mechanisms of action of pinacidil might be involved in ATP-sensitive K^+ channels on postjunctional smooth muscle cells. However, to date there has been no direct ligand-binding and electrophysiological evidence for the presence of ATP-sensitive K^+ channels in the plexus-free longitudinal muscle cells of guinea pig ileum.

Our results showed that the effects of pinacidil on 5-HT_4-mediated contractions of GPI seemed not due to changes in the 5-HT_3 receptors *per se*, at least in their property of binding for the specific ligand. When 5-HT_4 receptors in myenteric plexus neurons were activated by the agonists, Na^+ and K^+ fluxes through the cation-selective channels coupled directly to the receptors caused a rapid depolarization that in turn activated voltage-gated Ca^{2+} channels and caused an increase in the cytosolic free Ca^{2+} concentration[14], then led to neurotransmitter release which caused GPI contractions[16]. Pinacidil may open the ATP-sensitive K^+ channels in the same neurons, thus counteract the 5-HT_3 receptor-mediated effects. The fact that spontaneous activity of GPI greatly decreased after addition of pinacidil supported the above speculation. The enhancement by pinacidil of the inhibitory effect of tropisetron or benesetron on the 5-HT-evoked GPI contractile responses, therefore, could be attributed to a certain type of functional synergism. However, cromakalim, another K^+ channal opener, did not affect the release of [^{3}H]-acetylcholine evoked by electric stimulation or by activation of 5-HT_3 receptors in GPI[9]. In addition, there are recently some debates about the effects of KCO on Ca^{2+} entry which causes contractions of various smooth muscle preparations[12]. The effects of KCO on intestinal muscle are still not fully understood.

Nevertheless, our results suggested that some functional interactions exist between 5-HT_3 receptors and ATP-sensitive K^+ channels in longitudinal muscle-myenteric plexus neurons of GPI. The physiological significance of this finding needs to be addressed at single neuron level using new techniques, such as electrophysiological study, intracellular Ca^{2+} imaging, *etc*.

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吡那地尔对 5-HT$_3$ 受体介导的离体豚鼠回肠收缩反应的抑制

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关键词 血清素受体; 吡那地尔; 回肠; 肠肌丛; 卡巴胆碱; GR65630

目的: 研究钾通道开放剂吡那地尔对 5-HT$_3$ 受体介导的离体豚鼠回肠(GPI)收缩反应的影响. **方法**: 以等长换能器记录 GPI 收缩反应. 以[^{3}H]GR65630 结合试验检测大鼠内嗅皮层 5-HT$_3$ 受体的结合特性. **结果**: (1) 2-甲基-5-HT 及 5-HT 以剂量依赖方式引起 GPI 的收缩; 托品色创竞争性抑制此反应. (2) 吡那地尔以剂量依赖方式抑制 2-甲基-5-HT 和 5-HT 引起的 GPI 收缩, 并增强托品色创或 Benesetron 对 5-HT 诱发 GPI 收缩的抑制作用, 但不影响卡巴胆碱引起的 GPI 收缩; 吡那地尔对大鼠内嗅皮层 5-HT$_3$ 受体与[^{3}H]GR65630 的结合无影响. **结论**: 吡那地尔可能通过激活突触前神经元 ATP 敏感钾通道抑制由 5-HT$_3$ 受体介导的 GPI 收缩反应.

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Effects of long-term application of dopamine HCl on dopamine agonist-induced cAMP production in rat renal cortex[1]

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KEY WORD dopamine receptors; kidney cortex; dopamine; cyclic AMP; dopamine agents; adenyl cyclase; Sch-23390; domperidone

AIM: To study the effects of long-term application of dopamine HCl (DA) on the functional changes of dopamine receptor subtypes coupled to adenyl cyclase in rat renal cortex. **METHODS**: cAMP levels were measured by radioimmunoassay as an index of dopamine receptor function. **RESULTS**: Injection of DA (30 mg · kg^{-1} · d^{-1}, ip, 30 d) reduced the fenoldopam (Fen) (100 μmol · L^{-1})-induced increments of cAMP production from the control group of +1.26 ± 0.04 to the DA-treated group of +0.63 ± 0.22 nmol·min^{-1}/g tissue and the propyl-butyl-dopamine (PBDA) (100 μmol · L^{-1})-induced decrements of cAMP production in the presence of Sch-23390 (Sch) from the control group of −0.38 ± 0.18 to the DA-treated group of −0.11 ± 0.08 nmol·min^{-1}/g tissue with, however, comparable percentile changes for the 2 groups. Sch blocked both Fen- and PBDA-induced increase in cAMP production, while domperidone (Dom) blocked the decreasing effects of PBDA on cAMP accumulation in the presence of Sch. **CONCLUSION**: Long-term application of DA produced a marked "down regulation" of both DA$_1$ and DA$_2$ receptors in rat renal cortex with, however, the responsiveness of the remaining receptors unchanged.

Dopamine HCl (DA) agonists have been extensively used in the treatment of cardiovascular and kidney disease[1,2]. But, long-term application of the drugs may result in the decrease of drug effects in patients and animals, indicating the occurrence of some

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