

## 5-HT<sub>1P</sub> receptor-mediated slow depolarization in neurons of guinea pig inferior mesenteric ganglion<sup>1</sup>

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**KEY WORDS** serotonin; sympathetic ganglia; cyproheptadine; quipazine; serotonin uptake inhibitors; serotonin receptors; serotonin antagonists; serotonin agonists

### ABSTRACT

**AIM:** To study the effects of several 5-hydroxytryptamine (5-HT) receptor subtype antagonists on 5-HT-induced depolarization and the effects of 5-HT<sub>1P</sub> receptor agonist on the membrane potential in the neurons of guinea pig inferior mesenteric ganglion (IMG). **METHODS:** Intracellular recordings were made from neurons of the isolated guinea pig IMG. **RESULTS:** Cyproheptadine (5-HT<sub>1/2</sub> antagonist 10  $\mu\text{mol}\cdot\text{L}^{-1}$ ,  $n = 7$ ) and BRL 24924 (5-HT<sub>1P</sub> antagonist 10  $\mu\text{mol}\cdot\text{L}^{-1}$ ,  $n = 19$ ) reversibly suppressed 5-HT slow response; pressure ejection of MCPP (5-HT<sub>1P</sub> agonist 10  $\text{mmol}\cdot\text{L}^{-1}$ ) induced a slow depolarization in most of 5-HT sensitive neurons (10/14). **CONCLUSION:** 5-HT-induced slow depolarization is mediated by 5-HT<sub>1P</sub> receptor.

### INTRODUCTION

5-Hydroxytryptamine (5-HT) has been shown to evoke both fast depolarization and slow response in most of neurons of prevertebral sympathetic ganglia in guinea pig by us<sup>(1-3)</sup>. It has been discovered by Bradley<sup>(4)</sup> and Fozard<sup>(4,5)</sup> that the fast depolarizing

action of 5-HT was sensitive to 5-HT<sub>3</sub> antagonist (eg, bemesetron, ICS 205-930, and quipazine) in peripheral neurons. This result was confirmed in guinea pig inferior mesenteric ganglion (IMG), celiac ganglion (CG), and rabbit ganglion nodosum<sup>(2,6,7)</sup>. But it has not been proved what kind of 5-HT receptor subtype caused the slow depolarization. Using intracellular recordings *in vitro*, we have investigated the effects of several 5-HT receptor subtype antagonists on 5-HT-induced slow depolarization and the effects of 5-HT<sub>1P</sub> receptor agonist on the membrane potential in the neurons of guinea pig IMG to detect the receptor subtype which mediates 5-HT slow depolarization.

### MATERIALS AND METHODS

Experiments were carried out on the adult guinea pigs (200 - 300 g, Certificate No AH-06) of either sex. The IMG with attached nerve trunks was superfused with a modified Krebs' solution; NaCl 123.0, KCl 4.7, MgCl<sub>2</sub> 1.2, NaH<sub>2</sub>PO<sub>4</sub> 1.8, CaCl<sub>2</sub> 2.5, glucose 11.5, NaHCO<sub>3</sub> 18.0  $\text{mmol}\cdot\text{L}^{-1}$ , pH 7.40  $\pm$  0.05, saturated with 95 % O<sub>2</sub> + 5 % CO<sub>2</sub> at 35  $^{\circ}\text{C} \pm 0.5$   $^{\circ}\text{C}$ . Intracellular recordings were obtained from the isolated ganglion neurons by glass micro-electrode filled with KCl 3  $\text{mol}\cdot\text{L}^{-1}$ , having a tip resistance of 30 - 60 M $\Omega$ . The transmembrane current was past through the recording electrode utilizing a bridge circuit of the preamplifier (WPI-707A). The bioelectrical signals were displayed on an Iwatsu oscilloscope (SS-5702) and recorded on a pen recorder (Chengdu Instrument Plant, LMS-2B) and an addscope (Nihon Kohden, ATAC-350). In partial neurons, downward deflexions represented electrotonic potentials induced by hyperpolarizing current pulses (0.05 - 0.4 nA, 100 ms) and the  $R_{in}$  could be calculated by Ohm's Law.

5-Hydroxytryptamine creatinine sulfate, cypro-

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heptadine hydrochloride (5-HT<sub>1/2</sub> antagonist), mianserin hydrochloride (5-HT<sub>2</sub> antagonist), ketanserin (5-HT<sub>2</sub> antagonist), spiperone hydrochloride (5-HT<sub>1A</sub> antagonist), and MCPP (5-HT<sub>1P</sub> agonist) were purchased from Sigma. Bemesebron (5-HT<sub>3</sub> antagonist) was supplied by Res Biochemicals Inc. BRL 24924 (5-HT<sub>1P</sub> antagonist) was supplied by Beecham Pharmaceuticals Inc. 5-HT antagonists were dissolved in Krebs' solution and applied to the ganglia by superfusion. The 5-HT agonist was dissolved in Krebs' solution, filled in glass micropipettes and applied by pressure ejection in close proximity to the impaled neuron using a picospritzer (General Valves Co). 5-HT was applied by superfusion and pressure ejection.

Data were expressed as  $\bar{x} \pm s$  and compared using Student's *t*-test and  $\chi^2$  test.

## RESULTS

**5-HT depolarization** Applied by superfusion, 5-HT (100  $\mu\text{mol} \cdot \text{L}^{-1}$ , 30 s) induced a depolarization in 62 % (77/124) of the neurons sampled, and 75 % (58/77) of them showed slow depolarization with mean amplitude of  $5.7 \text{ mV} \pm 2.3 \text{ mV}$  and average duration  $130 \text{ s} \pm 79 \text{ s}$ , respectively; 25 % (19/77) of them showed a biphasic response which consisted of an initial fast depolarization followed by a slow one. The amplitude and duration were  $7 \text{ mV} \pm 5 \text{ mV}$  and  $24 \text{ s} \pm 19 \text{ s}$ , respectively for fast response and  $6 \text{ mV} \pm 3 \text{ mV}$  and  $140 \text{ s} \pm 64 \text{ s}$  for slow one. Applied by pressure ejection, 5-HT (10  $\text{mmol} \cdot \text{L}^{-1}$ ) induced membrane depolarization in 70 % (53/76) of neurons sampled. There were 17 % (9/53) and 40 % (21/53) of them showing only a fast depolarization and only a slow one respectively, and the remaining neurons (23/53) showed a biphasic response (Fig 1).

In partial neurons, downward deflexions represented electrotonic potentials induced by hyperpolarizing current pulses (0.05 – 0.4 nA, 100 ms). The slow depolarization was accompanied by a small increase in membrane resistance by  $(19 \pm 13) \%$  ( $n = 18$ ,  $P < 0.01$ ), but the fast depolarization was accompanied by a small decrease in membrane resistance by  $(27 \pm 11) \%$  ( $n = 12$ ,  $P < 0.01$ , Fig 1). It indicated that the ionic mechanisms underlying fast and slow depolarization were different<sup>(6)</sup>.

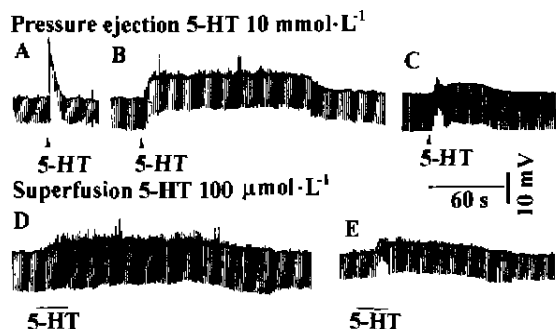


Fig 1. 5-HT-induced depolarization and the membrane resistance change associated with 5-HT fast and slow depolarization.

**Effect of 5-HT receptor subtype antagonists** Superfusion of cyproheptadine<sup>(8)</sup> (10  $\mu\text{mol} \cdot \text{L}^{-1}$ , 10 min) markedly suppressed or completely blocked 5-HT slow response (Tab 1, Fig 2A). It suggested that 5-HT slow response was mediated by 5-HT<sub>1</sub> or 5-HT<sub>2</sub> receptor.

Tab 1. Effects of different 5-HT antagonists on amplitude of 5-HT slow depolarization.  $\bar{x} \pm s$ .  $^{\circ}P < 0.01$  vs control.

	Cell number	Before treatment	After treatment
Cyproheptadine (5-HT <sub>1/2</sub> )	7	7 ± 3	4 ± 4 <sup>°</sup>
Mianserin (5-HT <sub>2</sub> )	9	5 ± 3	5 ± 4
Ketanserin (5-HT <sub>2</sub> )	6	3.1 ± 1.7	4 ± 3
Bemesebron (5-HT <sub>3</sub> )	10	4.6 ± 2.9	2.9 ± 1.3
Spiperone (5-HT <sub>1A</sub> )	9	3.6 ± 1.2	4.2 ± 1.8
BRL 24924 (5-HT <sub>1P</sub> )	19	5.1 ± 2.6	2 ± 3 <sup>°</sup>

Superfusion of mianserin and ketanserin<sup>(9)</sup> (10  $\mu\text{mol} \cdot \text{L}^{-1}$ , 10 min) had no significant effect on 5-HT slow depolarization (Tab 1). It indicated that 5-HT slow depolarization was not mediated by 5-HT<sub>2</sub> receptor.

Bemesebron was a selective 5-HT<sub>3</sub> antagonist<sup>(9)</sup>. Superfusion of bemesebron (10  $\mu\text{mol} \cdot \text{L}^{-1}$ , 10 min) had no significant effect on 5-HT slow response (Tab 1). It indicated that 5-HT slow depolarization was not interrelated with 5-HT<sub>3</sub> receptor.

Superfusion of spiperone<sup>(9)</sup> (10  $\mu\text{mol} \cdot \text{L}^{-1}$ , 10

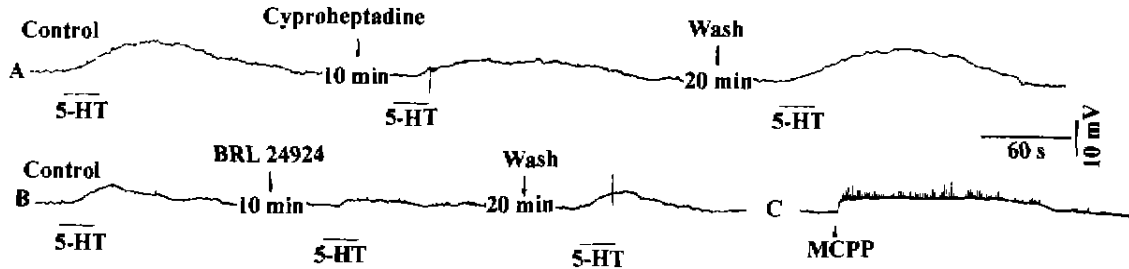


Fig 2. Effect of cyproheptadine and BRL 24924 on 5-HT depolarization and the effect of MCPP on 5-HT-sensitive neuron membrane potentials.

min) had no significant effect on 5-HT slow depolarization, but BRL 24924<sup>(10)</sup> ( $10 \mu\text{mol} \cdot \text{L}^{-1}$ , 10 min) attenuated 5-HT slow depolarization (Fig 2B, Tab 1). It indicated that 5-HT slow depolarization was mediated by 5-HT<sub>1P</sub> receptor and not mediated by 5-HT<sub>1A</sub> receptor.

**Effect of 5-HT<sub>1P</sub> agonist** First, superfusion of IMG with 5-HT ( $100 \mu\text{mol} \cdot \text{L}^{-1}$ , 30 s) was performed to observe whether the cell was sensitive to 5-HT, and then pressure ejection of MCPP<sup>(11)</sup> (5-HT<sub>1P</sub> agonist,  $10 \text{mmol} \cdot \text{L}^{-1}$ ) to observe the reaction of the cells to the agonists (Fig 2C, Tab 2).

Tab 2. Reaction of MCPP on 5-HT-sensitive neurons and 5-HT-insensitive neurons.  $\bar{x} \pm s$ .

\* $P < 0.01$  vs control.

	n	Fast response	Slow response	Biphasic response	No response
5-HT-sensitive neurons	14	0	10	4	0
5-HT-insensitive neurons	7	0	0	0	7 <sup>c</sup>

MCPP had no significant effect on 5-HT-insensitive cells either (7/7), but it induced slow response in most of 5-HT-sensitive cells by activating 5-HT<sub>1P</sub> receptor (10/14). (Tab 2)

## DISCUSSION

The superfusion method and pressure ejection method have their respective strong and weak points.

For the superfusion method, the strong point is that the accurate concentration of agents can be made, so as to gain a good dose-reaction relationship. The weak point was that there was a longer latent period from superfusion to appearance of reaction because of the dead space of superfusion system, and because the fast reaction to agents was easily submerged. In the present study, the occurrence rate of 5-HT-induced fast reaction during superfusion was really less than that using pressure ejection. Moreover, the superfused solution of agents acts on the whole ganglion, but not only on the impaled neuron. Oppositely, the agent applied by pressure ejection acted on the proximity to the impaled neuron and evoked a reaction with very short latent period. However, the agent solution applied to Krebs' solution is fast diluted, so the accurate concentration of the agent acted on the impaled neuron is unclear. In the present work, both superfusion and pressure ejection were used for administration of 5-HT in order to obtain more reliable results.

Cyproheptadine and methysergide markedly suppressed slow depolarization in guinea pig IMG and CG neurons<sup>(1,2)</sup>, but both cyproheptadine and methysergide are not selective 5-HT antagonist. Those two drugs antagonized both 5-HT<sub>1</sub> and 5-HT<sub>2</sub><sup>(8)</sup>. 5-HT slow depolarization was reversibly attenuated by 5-HT<sub>1P</sub> antagonist (BRL24924) and imitated by 5-HT<sub>1P</sub> agonist (MCPP). It indicates that 5-HT-induced slow response is mediated by 5-HT<sub>1P</sub> receptor.

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### 豚鼠肠系膜下神经节细胞的 5-HT<sub>1P</sub>受体介导的慢去极化反应<sup>1</sup>

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关键词 血清素; 交感神经节; 赛庚啉; 唑派啉; 血清素摄取抑制剂; 血清素受体; 血清素拮抗剂; 血清素激动剂

目的: 观察不同 5-羟色胺(5-HT)受体亚型拮抗剂对 5-HT 去极化反应的作用, 并观察 5-HT<sub>1P</sub>受体激动剂对肠系膜下神经节(IMG)细胞膜电位的作用。  
方法: 离体豚鼠 IMG 细胞内生物电记录。结果: 赛庚啉(5-HT<sub>1,2</sub>受体拮抗剂 10 μmol·L<sup>-1</sup>, n=7)和 BRL 24924 (5-HT<sub>1P</sub>受体拮抗剂 10 μmol·L<sup>-1</sup>, n=19)可逆地阻抑 5-HT 慢去极化反应; 压力注射 MCPP (5-HT<sub>1P</sub>受体激动剂 10 mmol·L<sup>-1</sup>)则使大部分 5-HT 敏感细胞出现慢去极化反应(10/14)。结论: 5-HT 慢去极化反应是由 5-HT<sub>1P</sub>受体介导。

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