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Abstract

In the present study, tryptamine produced a slow hyperpolarization in a few neurons other than a slow depolarization in myenteric neurons of the isolated guinea-pig ileum. Neither the adrenergic neuron blocker, guanethidine nor the 5-hydroxytryptamine uptake inhibitor, zimelidine, which can inhibit the release of 5-hydroxytryptamine from enteric neurites induced by tryptamine (M. Takaki et al. (1985) Neuroscience 16, 223-240), affected this slow hyperpolarization. Therefore, it was concluded that the slow hyperpolarization induced by tryptamine in myenteric neurons was not mediated via the release of 5-hydroxytryptamine or noradrenaline. It might be possible that the hyperpolarization was induced by a direct action of tryptamine on myenteric neurons per se.

KEYWORDS: intracelluar recording, tryptamine, hyperpolarization, myenteric neurons

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Slow Hyperpolarizing Action of Tryptamine on Myenteric Neurons of the Isolated Guinea-Pig Ileum

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In the present study, tryptamine produced a slow hyperpolarization in a few neurons other than a slow depolarization in myenteric neurons of the isolated guinea-pig ileum. Neither the adrenergic neuron blocker, guanethidine nor the 5-hydroxytryptamine uptake inhibitor, zimelidine, which can inhibit the release of 5-hydroxytryptamine from enteric neurites induced by tryptamine (M. Takaki *et al.* (1985) Neuroscience 16, 223-240), affected this slow hyperpolarization. Therefore, it was concluded that the slow hyperpolarization induced by tryptamine in myenteric neurons was not mediated via the release of 5-hydroxytryptamine or noradrenaline. It might be possible that the hyperpolarization was induced by a direct action of tryptamine on myenteric neurons *per se*.

Key words : intracelluar recording, tryptamine, hyperpolarization, myenteric neurons

Recently, Takaki *et al.* (1) found that tryptamine (2) evokes a 5-hydroxytryptamine (5-HT)-like depolarizing action on myenteric neurons in the isolated guinea-pig ileum. This action was not a direct action of tryptamine on the neural 5-HT receptor (3), but an indirect effect mediated via the release of endogenous neural 5-HT. This finding was further supported by our recent paper (4) on the cholinergic contractile response induced by tryptamine mediated via the release of 5-HT in the isolated guinea-pig ileum. The present study was performed to find a new effect of tryptamine, namely, a slow hyperpolarization which seems to be due to a direct effect on myenteric neurons *per se*.

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Materials and Methods

Segments of intestine were removed from the ileum of adult guinea pigs (200-350 g) that had been stunned by a blow to the head and exsanguinated. Flat sheet preparations of longitudinal muscle with the myenteric plexus attached were prepared, mounted in a superfusion chamber and viewed with a binocular stereomicroscope, lighted by a microelectrode illumination system (Narishige, Tokyo, Japan). The preparations were immobilized for intracellular recording by pressing two L-shaped wires onto the surface of the muscle on either side of an identified myenteric ganglion. Intracellular recordings were made with glass microelectrodes (resistances of 40-80 MΩ) that were filled with 3 M KCl. A high-impedance preamplifier (Nihon-Kohden, Tokyo, Japan), that was used for recording membrane potentials, had negative-capacity compensation and bridge circuitry for injecting current (200 msec in duration) through the recording micropipette. Some of the records were played back on a Recticorder (Nihon-Kohden, Tokyo, Japan)

from data previously recorded on magnetic tape. The tissues were maintained in Krebs solution (1, 5) at 37°C and gassed with 95 % O_2–5 % CO_2. The tissue chamber was perfused at a rate of 11–12 ml/min. The perfusate completely reached the tissue chamber 3–4 min after onset of the perfusion. Tryptamine (1 mM) and 5-HT (1 mM) were applied by microejection from fine-tipped pipettes (tip diameter 10–20 μm) with nitrogen pulses of controlled pressure amplitude (0.71–1.41 kg/cm^2) and duration (300–999 msec). By adding 0.01 % fast green, exposure of the impaled neurons to the microejected solution could be confirmed by direct observation through the microscope. Fast green in concentrations up to 10 times that used in these experiments had no effect on myenteric neurons (1, 5). Drugs used were: guanethidine sulfate (Tokyo Kasei Kogyo, Tokyo, Japan), 5-hydroxytryptamine creatinine sulfate (5-HT), tryptamine hydrochloride (Sigma Chemical Company, St. Louis, MO, USA) and zimelidine dihydrochloride, which was donated by Astra Läkemedel (Sodertalje, Sweden).

Results

Tryptamine evoked a slow depolarizing response in 12 out of 35 myenteric neurons studied. This depolarization was due to the release of 5-HT from enteric neurites as previously reported (1). In 6 of the other 23 cells, however, tryptamine produced a slow hyperpolarization (Table 1). The input resistance was monitored throughout the tryptamine-induced slow depolarization in the 12 neurons and the hyperpolarization in the 6 neurons by intracellularly injecting hyperpolarizing current pulses (200 msec in duration). During the slow depolariza-

Table 1 Myenteric neural responses to tryptamine and 5-hydroxytryptamine[a]

Response	Tryptamine	5-hydroxy-tryptamine
Depolarization (slow and/or fast)	12	10
Slow hyperpolarization	6	5
No response	17	7
Total	35	22

a : Numerals indicate the number of neurons. Some of the neurons responsive to tryptamine were also affected by 5-hydroxytryptamine.

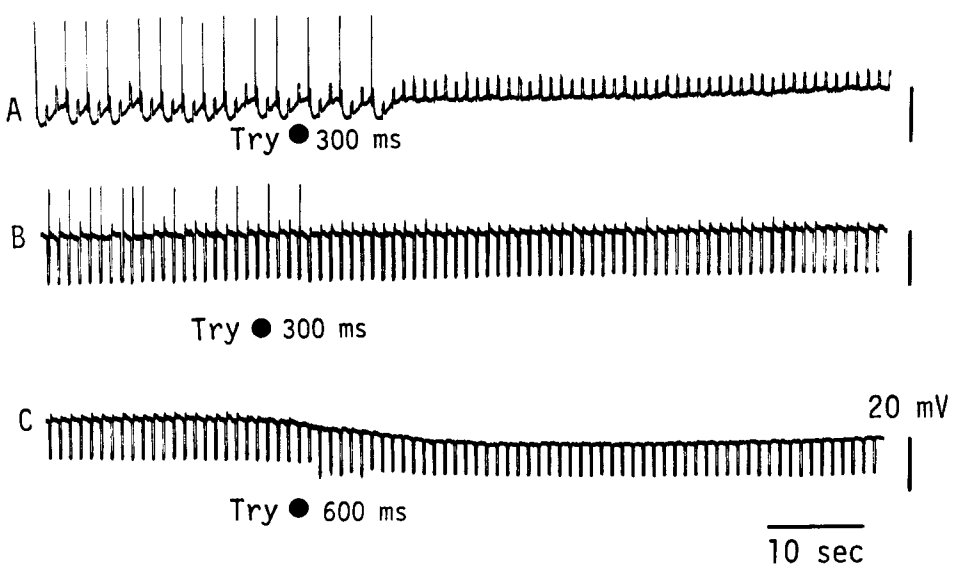

Fig. 1 Effect of tryptamine (Try) on a myenteric neuron. The cell was injected with constant depolarizing (A) or hyperpolarizing (B, C) current pulses through the recording micropipette. A, A microejection of tryptamine (dot; 300-msec pulse) abolished the generation of action potentials. B, A microejection of tryptamine (dot; 300-msec pulse) abolished anodal-break excitation. C, A longer pulse (600 msec) of tryptamine at the dot produced a slow hyperpolarization associated with a transient increase and following decrease in input resistance (cf. amplitude of electrotonic deflections). The resting membrane potential was −70 mV.

tion, input resistance was consistently increased. In contrast, during the slow hyperpolarization, the magnitude and direction of the change in membrane resistance varied in different neurons. Resistance increased in 2 (Fig. 2), decreased in 2 (Fig. 1) and was unaffected in 2 neurons (Fig. 3). A typical response of a myenteric neuron is shown in Fig. 1. Tryptamine (300 msec, 1 pulse) increased the duration of after-hyperpolarization (140 % of the pre-drug duration) and inhibited the spike generation when the depolarizing current pulses were injected (Fig. 1, A). Tryptamine (300 msec, 1 pulse) also inhibited the spike generation at the termination of the hyperpolarizing current pulses (Fig. 1, B). A longer pulse of tryptamine (600 msec) induced a slow hyperpolarizing response associated with decreased input resistance (81.2 % of the pre-drug amplitude) in the

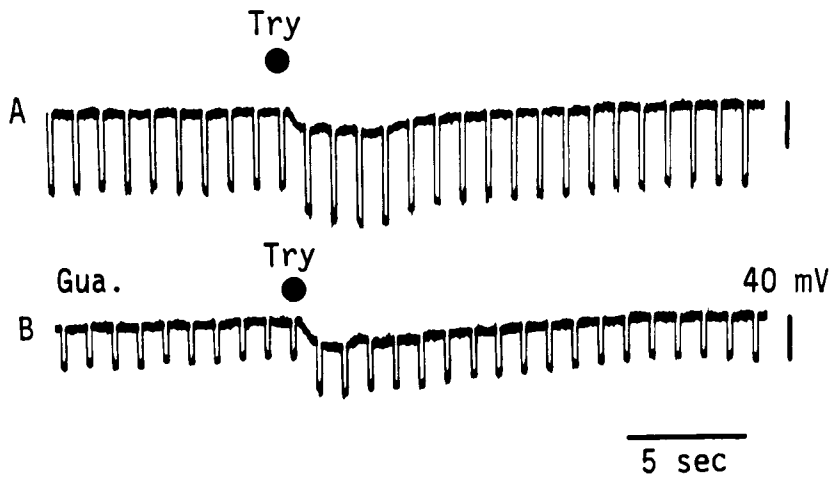

Fig. 2 No effect of superfusion with guanethidine (Gua; 10 μM) on the tryptamine (Try)-induced slow hyperpolarization. The cell was injected with constant hyperpolarizing current pulses. A, Microejection of tryptamine (dot; three 900-msec pulses) evoked a slow hyperpolarization associated with a slight increase in input resistance. B, The slow hyperpolarizing response to tryptamine was not affected 19 min after beginning superfusion with guanethidine. Between A and B, the current injected was decreased. The resting membrane potential was −50 mV.

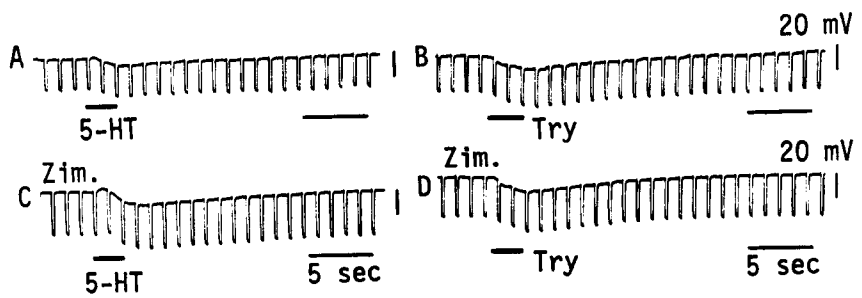

Fig. 3 No effect of superfusion with zimelidine (Zim; 10 μM) on the tryptamine (Try)-induced slow hyperpolarization in comparison with that on the 5-hydroxytryptamine (5-HT)-induced slow hyperpolarization in the same cell. The cell was injected with constant hyperpolarizing current pulses. A, Microejection of 5-HT (short horizontal bar; two 999-msec pulses) evoked a slow hyperpolarization not associated with changes in input resistance. B, Microejection of tryptamine (short horizontal bar, two 999-msec pulses) evoked a slow hyperpolarization without changes of input resistance. C, The slow hyperpolarizing response to 5-HT was not attenuated 7 min after beginning superfusion with zimelidine. D, The slow hyperpolarizing response to tryptamine was not attenuated 4 min after beginning superfusion with zimelidine. In C and D, zimelidine increased the amplitude of the electrotonic potentials. The resting membrane potential was −50 mV.

same cell, though transient increase in input resistance also could be seen (Fig. 1, C). The mean amplitude of the hyperpolarization was 11.8 ± 0.8 mV ($n = 6$).

To check whether or not 5-HT released by tryptamine evokes the slow hyperpolarization, effects of exogenous 5-HT were studied. 5-HT caused a slow depolarization associated with increased input resistance and/or fast depolarization associated with decreased input resistance in 10, and hyperpolarization associated with increased, decreased or unaffected input resistance in 5 out of 22 myenteric neurons. Effects of tryptamine and 5-HT on similar myenteric neurons are summarized in Table 1.

Superfusion with 10 μM guanethidine did not affect the slow hyperpolarization induced by tryptamine in three neurons. An example is shown in Fig. 2. In this cell, tryptamine caused the slow hyperpolarization associated with a slight increase in input resistance (120 % of the control) (Fig. 2, A).

Superfusion with 10 μM zimelidine did not affect the slow hyperpolarization induced by tryptamine in three neurons. An example is shown in Fig. 3. In this cell, tryptamine caused the slow hyperpolarization without changes in input resistance (Fig. 3, B). In the same cell, 5-HT caused a similar hyperpolarization, though slight, fast depolarization also occurred (Fig. 3, A). Superfusion with 10 μM zimelidine did not attenuate the hyperpolarizing responses caused by tryptamine and by 5-HT (Fig. 3, C and D), though zimelidine slightly increased the amplitude of the electrotonic potentials. However, zimelidine at the same dose largely attenuated the depolarizing response to tryptamine in another cell (data not shown).

Discussion

The dominant response of myenteric plexus neurons responsive to tryptamine applied focally was a slow depolarization, although a slow hyperpolarization was also recorded (Table 1). The slow depolarization has been thought to be mediated via the release of 5-HT from enteric neurites, since zimelidine antagonized the depolarizing response to tryptamine (1). In the present study, this inhibitory effect of zimelidine on the depolarizing response to tryptamine was reconfirmed. The action of release of endogenous 5-HT induced by tryptamine was not Ca^{2+}-dependent (1). Therefore, Ca^{2+}-free medium is ineffective in blocking the release of 5-HT from enteric neurites. Zimelidine is a 5-HT uptake inhibitor (6), but it actually antagonized 5-HT release induced by tryptamine (1). This antagonism was imcomplete. However, it is likely that such small amount of 5-HT released by tryptamine after zimelidine can not show substantial physiological actions. Therefore, zimelidine is a good tool for testing the physiological actions of tryptamine mediated via the release of endogenous 5-HT. Moreover, other 5-HT uptake inhibitors, citalopram, fluoxetine and indalpine, also inhibit the cholinergic contractions of the guinea-pig ileum induced by tryptamine, possibly by inhibiting 5-HT release (4).

On the other hand, tryptamine caused the slow hyperpolarization in a small number of myenteric neurons. Exogenous 5-HT also caused the slow hyperpolarization of a small number of myenteric neurons in the present study, as well as in the previous one (5). However, zimelidine did not attenuate the slow hyperpolarization of myenteric neurons induced by tryptamine. From this result, the possibility that the 5-HT released from enteric neurites might have caused the slow hyperpolarization can be excluded.

Tryptamine can also release noradrenaline in the myenteric plexus (1). Exogenous noradrenaline causes a slow hyperpolarization of myenteric neurons (7). Therefore, it was hypothesized that the released endogenous noradrenaline from extrinsic noradrenergic nerve terminals might cause the slow hyperpolarization. However, an adrenergic neuron blocker, guanethidine (8) did not affect the slow hyperpolarization.

Therefore, it seems likely that this hyperpolarization is not mediated via the release of noradrenaline. It has been reported that cholinergic contractions of guinea-pig ileum induced by tryptamine are not potentiated by guanethidine and cocaine (4). It also has been reported that endogenous noradrenaline released by tryptamine does not inhibit presynaptically fast excitatory postsynaptic potentials (EPSPs) evoked by stimulation of interganglionic fiber tracts in myenteric neurons (1). It is, therefore, likely that the noradrenaline released by tryptamine has no influence on the muscular contraction and on fast EPSPs by fiber tract stimulation in myenteric neurons. The present results are consistent with these previous findings.

Ionic mechanisms for the slow hyperpolarization were not analyzed in the present study. However, the varying input resistance changes during the slow hyperpolarization could be explained by differences in resting membrane potential.

It was concluded that the slow hyperpolarization of myenteric neurons induced by tryptamine was not an indirect action on myenteric neurons via the release of endogenous 5-HT or noradrenaline. The response may be induced by a direct action on myenteric neurons *per se*, although the possibility that the response was mediated via a release of an unknown inhibitory substance can not be excluded.

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