Dual effects of 5-hydroxytryptamine on the release of γ -aminobutyric acid from myenteric neurones of the guinea-pig ileum

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The effects of 5-hydroxytryptamine (5-HT) on the release of γ -aminobutyric acid (GABA) were examined in the longitudinal muscle-myenteric plexus (LM-MP) preparation of guinea-pig ileum. 5-HT increased the spontaneous release and inhibited the electrically-evoked release of [³H]-GABA. The 5-HT-evoked release was Ca^{2+} -dependent and tetrodotoxin-sensitive, and was antagonized by (3α -tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930), but not by methysergide and ketanserin. The inhibitory effect of 5-HT was antagonized by methysergide, but not by ketanserin and ICS 205-930. 8-Hydroxy-2-(di-*n*-propylamino)tetralin mimicked the inhibitory effect of 5-HT. Thus, 5-HT may exert an excitatory effect on the enteric GABAergic neurone via the 5-HT₃ receptor and an inhibitory effect via the 5-HT_{1A} receptor.

Introduction 5-Hydroxytryptamine (5-HT) has been shown to exert stimulatory and inhibitory effects on acetylcholine (ACh) release from the guinea-pig ileum (Kilbinger & Pfeuffer-Friederich, 1985). γ -Aminobutyric acid (GABA) has similar dual effects on ACh release (Kleinrok & Kilbinger, 1983; Erdö, 1985; Tanaka, 1985; Jessen *et al.*, 1987). We proposed that excitatory peptides present in the ileum, including substance P, neurotensin and cholecystokinin may modulate the enteric GABAergic neurone (Tanaka & Taniyama, 1987). To assess 5-HT as a possible modulator of GABAergic activity in the guinea-pig ileum, we have now examined the effect of 5-HT on the release of GABA from longitudinal muscle-myenteric plexus preparations.

Methods Adult guinea-pigs of either sex, weighing between 300 and 350 g, were killed by cervical dislocation. Strips of ileum were excised 10 cm proximal to the ileocaecal sphincter, and longitudinal muscle-myenteric plexus (LM-MP) preparations were dissected. The methods of incubation and perfusion were as described by Hashimoto *et al.* (1986). Strips were incubated at 35°C for 60 min with [³H]-GABA at a final concentration of 5×10^{-8} M in Krebs solution of the following composition (in mM): NaCl 118,

KCl 4.8, CaCl_2 2.5, MgSO_4 1.19, NaHCO_3 25.0, KH_2PO_4 1.18 and glucose 11. After washing in fresh Krebs solution for 30 min, the strips were mounted between two parallel platinum electrodes and superfused at a rate of 1.2 ml min^{-1} with Krebs solution. The Krebs solution used throughout contained 10^{-5} M aminooxyacetic acid (AOAA) to prevent the metabolism of [³H]-GABA and 10^{-3} M β -alanine to prevent the glial uptake of [³H]-GABA, and was gassed with 95% O_2 -5% CO_2 . The superfusate was collected every 1 min, and the radioactivity was measured with a liquid scintillation spectrometer. The proportion of unchanged GABA in the tritium efflux was determined as described by Hashimoto *et al.* (1986). The release of GABA was represented as the fractional rate in which the amount of GABA in the superfusate was divided by the respective GABA contents in the tissue at the start of the respective period. The amount of GABA contained in the tissue at the start of the respective period was calculated by adding cumulatively the amounts of GABA released during the collection period to the amount of GABA in the tissue at the end of the experiment. Data were analyzed by Students' *t* test and a $P \leq 0.05$ was considered statistically significant. All chemicals and drugs were obtained commercially.

Results The experiment was started 60 min after the superfusion, during which time the spontaneous release of [³H]-GABA declined in a multiphasic exponential course and approached a slow phase with a fractional rate of $0.00411 \pm 0.00035 \text{ min}^{-1}$ ($n = 30$) 60 min after superfusion. The effect of 5-HT on the GABA release was examined in the presence of 10^{-6} M imipramine. Superfusion with 5-HT at 10^{-9} to 3×10^{-5} M for 1 min led to an increase in the release of [³H]-GABA from the LM-MP preparations (Figure 1a), which then returned to the basal release levels within 2 min during the continued presence of 5-HT. The EC_{50} value of 5-HT was 6.0×10^{-8} M ($n = 6$). The 5-HT (10^{-6} M)-evoked release of [³H]-GABA was prevented by pretreatment with tetrodotoxin (TTX) at 10^{-6} M for 15 min

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and in Ca^{2+} -free medium containing 1 mM EGTA, thereby indicating that the released $[^3\text{H}]$ -GABA is of neuronal origin. (3α -Tropanyl)-1H-indole-3-carboxylic acid ester (ICS 205-930, 3×10^{-7} M), a 5-HT_3 receptor antagonist, but not methysergide (10^{-6} M) and ketanserin (10^{-5} M) antagonized the facilitatory effect of 5-HT (Figure 1a). Hexamethonium at 3×10^{-4} M, atropine at 10^{-6} M and $[\text{D-Pro}^2, \text{D-Trp}^7, ^9]\text{substance P}$ at 3×10^{-6} M, a substance P antagonist, did not affect the 5-HT (10^{-6} M)-evoked release of $[^3\text{H}]$ -GABA.

Electrical field stimulation (EFS) with pulses of 0.5 ms duration, 15 V intensity, and 5 Hz frequency for 30 s stimulated $[^3\text{H}]$ -GABA release, which was prevented by superfusion with 10^{-6} M TTX or with Ca^{2+} -free medium containing 1 mM EGTA. Imipramine (10^{-6} M) did not affect the EFS-evoked release of $[^3\text{H}]$ -GABA. Pretreatment with 5-HT for 3 min, but not 1 or 2 min, inhibited the release of $[^3\text{H}]$ -GABA evoked by EFS (in the presence of ICS 205-930 (10^{-6} M) to prevent a transient increase in the release of $[^3\text{H}]$ -GABA), in a concentration-dependent manner (Figure 1b). The IC_{50} value for 5-HT was 1.46×10^{-7} M ($n = 6$). 8-Hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) at 10^{-7} M, a 5-HT_{1A} receptor agonist, also inhibited the EFS-evoked release of $[^3\text{H}]$ -GABA to $62.1 \pm 5.3\%$ ($n = 5$) of the release in the absence of 8-OH-DPAT. The inhibitory effects of 5-HT and 8-OH-DPAT on the EFS-evoked release of $[^3\text{H}]$ -GABA were antagonized by methysergide (3×10^{-7} M) (Figure 1b), but not by ketanserin (10^{-5} M) and ICS 205-930 (10^{-6} M). Prazosin (10^{-6} M), an α_1 -adrenoceptor antagonist and yohimbine (10^{-7} M), an α_2 -adrenoceptor antagonist did not affect the inhibition by 5-HT of the EFS-evoked release of $[^3\text{H}]$ -GABA.

Discussion The present study revealed that in guinea-pig ileum, 5-HT exerts a dual effect on the release of GABA: a transient increase in a spontaneous release of GABA and an inhibition of EFS-evoked release of GABA. This finding is similar to certain effects of 5-HT on the release of ACh (Kilbinger & Pfeuffer-Friederich, 1985). The excitatory 5-HT receptor on the GABAergic neurone may be the 5-HT_3 receptor, because the 5-HT-evoked release of GABA was antagonized by ICS 205-930, a 5-HT_3 antagonist, but not by methysergide and ketanserin. However, ICS 205-930 has also been reported to antagonize responses evoked by 5-HT acting at a receptor other than 5-HT_1 , 5-HT_2 or 5-HT_3 (Craig & Clarke, 1989; Dumuis *et al.*, 1989) and it remains a possibility that the 5-HT-evoked release of GABA may be mediated by the stimulation of a non-classical 5-HT receptor. It has been reported that 5-HT induces the release of sub-

Figure 1. Concentration-response curve for the effect of 5-hydroxytryptamine (5-HT) on the release of $[^3\text{H}]$ - γ -aminobutyric acid ($[^3\text{H}]$ -GABA). (a) 5-HT-evoked release of $[^3\text{H}]$ -GABA in the absence (●) and presence (○) of ICS 205-930. ICS 205-930 (3×10^{-7} M) was applied 10 min before and during stimulation with 5-HT for 1 min, in the presence of 10^{-6} M imipramine. (b) Inhibition by 5-HT of electrical field stimulation (EFS, 5 Hz, 15 V, 0.5 ms for 30 s)-evoked release of $[^3\text{H}]$ -GABA in the absence (●) and presence of methysergide (○). Pretreatment with 5-HT was for 3 min. Methysergide (3×10^{-7} M) was applied 10 min before and during stimulation with EFS.

The effect of 5-HT at concentrations of 10^{-9} , 10^{-7} and 10^{-5} M was examined in one preparation and that at concentrations of 10^{-8} , 10^{-6} and 3×10^{-5} M in another preparation from the same animal. Each point represents the mean from 5 or 7 animals with s.e. shown by vertical lines.

stance P (Buchheit *et al.*, 1985), and that substance P induces GABA release (Tanaka & Taniyama, 1987). However the lack of effect of [D-Pro², D-Trp^{7,9}]substance P on the 5-HT-evoked release of GABA does not support an involvement of substance P in the excitatory effect of 5-HT on the GABAergic neurone.

5-HT and 8-OH-DPAT (5-HT_{1A} agonist) inhibited the EFS-evoked release of GABA. These inhibitory effects were antagonized by methysergide, but not by ketanserin or ICS 205-930. The inhibitory effect of 5-HT on the GABA release may, therefore, occur via the 5-HT_{1A} receptor.

The affinity of 5-HT for the putative 5-HT₃ receptor was only twice as high as that for 5-HT_{1A} receptor, calculating from the EC₅₀ value. However, the

time course of these receptor-mediated effects of 5-HT differed. The 5-HT₃ receptor-mediated effect occurred immediately after treatment with 5-HT and returned to the basal level within 2 min, even in the presence of 5-HT. The 5-HT_{1A} receptor-mediated effect occurred 3 min after treatment with 5-HT. It remains to be determined whether the 5-HT_{1A} and 5-HT₃ receptors are present in the same or distinct GABAergic neurones.

We thank M. Ohara for pertinent comments. This work was supported by grants from the Ministry of Education, Science and Culture, Japan. ICS 205-930 was a generous gift from Sandoz Ltd., Basle, Switzerland.

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(Received June 30, 1989
Accepted July 7, 1989)