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Release of endogenous 5-hydroxytryptamine from the myenteric plexus of the guinea-pig isolated small intestine

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1 The presence of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in, and the release of these substances from, the myenteric plexus-longitudinal muscle (MPLM) layer of the guinea-pig isolated small intestine were investigated. 5-HT and 5-HIAA were measured by high performance liquid chromatography and electrochemical detection.

2 Freshly prepared MPLM contained measurable amounts of 5-HT and 5-HIAA. For the release experiments, the MPLM was incubated in a medium containing the 5-HT uptake inhibitor fluoxetine and the monoamine oxidase inhibitor nialamide; this led to a decrease in the 5-HIAA content of the MPLM whereas the 5-HT content remained unchanged.

3 There was a spontaneous release of 5-HT and 5-HIAA from the MPLM. The release of 5-HT was so small that it was just detectable; it seemed equivalent to about 0.8% of the tissue stores released per min.

4 Depolarization of the tissue by increasing the $[K^+]$ or by exposing it to veratridine enhanced the release of 5-HT in a Ca^{2+}-dependent manner whereas the release of 5-HIAA was not increased. Tetrodotoxin inhibited the veratridine-evoked release of 5-HT but did not affect the K^+-evoked release of 5-HT.

5 The presence of 5-HT in myenteric neurones and the characteristics of the release of 5-HT from these neurones strongly support the hypothesis that 5-HT is an enteric neurotransmitter.

Introduction

Within the gastrointestinal tract there is a population of intrinsic neurones which can take up and retain 5-hydroxytryptamine (5-HT) and can synthesize and inactivate this amine (for reviews see Gershon & Tamir, 1981; Furness & Costa, 1982a). Recent immunohistochemical studies have revealed that a 5-HT like substance is indeed present in nerve cell bodies in the myenteric plexus and in varicose nerve fibres in the ganglia of the myenteric and submucous plexus (Costa *et al.*, 1982; Furness & Costa, 1982b; Dahlström & Ahlman, 1983) and there is increasing evidence that 5-HT is an enteric neurotransmitter. For a substance to be accepted as a neurotransmitter it is commonly held that several criteria should be fulfilled (see Furness & Costa, 1982a). The two major criteria are that the substance is released under the appropriate conditions and that the actions of the endogenously released and exogenously added substance are identical. In the case of 5-HT both these criteria proved difficult to meet. The problems are due to the multiplicity of the actions of 5-HT in the gut (Johnson *et al.*, 1980; Furness & Costa, 1982a) and the difficulties in providing a convincing demonstration of the release of endogenous 5-HT from enteric neurones.

There is pharmacological evidence indicating a release of 5-HT from enteric nerves (see Wood & Mayer, 1979; Furness & Costa, 1982a) and there have been numerous attempts to demonstrate a release of 5-HT directly. Myenteric neurones have been shown to take up $[^3H]$-5-HT and to release it in response to stimulation with electrical pulses or high $[K^+]$ (Schulz & Cartwright, 1974; Jonakait *et al.*, 1979). The release of newly taken up $[^3H]$-5-HT induced by electrical stimulation is Ca^{2+}-dependent and therefore likely to represent a synaptic release mechanism whereas the release induced by high $[K^+]$ is not Ca^{2+}-dependent (Jonakait *et al.*, 1979).

Recently Gershon & Tamir (1981), using a radioenzymatic assay for the detection of 5-HT, were able to show that electrical stimulation increases the release of 5-HT into the solution bathing the serosal surface of everted segments of the guinea-pig small intestine in a Ca^{2+}-dependent manner. Examination of the diffusion of [^{3}H]-5-HT through the intestinal wall suggested that the source of 5-HT could not have been the mucosa which, in the enterochromaffin cells, contains large amounts of 5-HT (Erspamer, 1966).

The present study aimed at investigating the release of endogenous 5-HT from the isolated longitudinal muscle-myenteric plexus layer of the guinea-pig small intestine so as to avoid any possible contamination with mucosal 5-HT. 5-HT was measured by a novel, sensitive method, using high performance liquid chromatography and electrochemical detection (Sperk, 1982). This assay for 5-HT is far less laborious than the radio-enzymatic assay and the specificity of the method is based on both the chromatographic separation and electrochemical reaction of 5-HT.

Methods

Experimental protocol

Adult guinea-pigs of either sex (300–500 g) were used. Some guinea-pigs (see Results) were treated with the monoamine oxidase (EC 1.4.3.4) inhibitor pargyline (50 mg kg^{-1} i.p.) 1 h before sacrifice. The animals were killed by decapitation, and the small intestine was rapidly excised and immersed in oxygenated Tyrode solution at room temperature. The myenteric plexus-longitudinal muscle (MPLM) layer was isolated from the small intestine (jejunum and ileum) according to the method of Bolton (1972). By this procedure 4 strips of MPLM of about 30 cm length were obtained from each animal. These strips were halved to yield two portions of tissue weighing 0.5–0.7 g. Each portion of MPLM was transferred to an incubation bath containing 1 ml of oxygenated Tyrode solution at 37°C. The baths were of polyethylene (inner diameter 1.3 cm) with a 15 μm mesh filter base. The incubation medium could be collected by suction, the arrangement being similar to that used by Sharman *et al.* (1982).

After equilibration for 30 min, the experiments were carried out by collecting the incubation medium every 10 min. The medium was exchanged for fresh oxygenated and pre-warmed medium within 10 s. The collected bath fluid was immediately acidified with concentrated perchloric acid to give a final concentration of 0.2 mol l^{-1} perchloric acid and kept at −20°C for assay.

After collection of two 10 min incubation samples to observe the resting release of 5-HT, the tissues were stimulated by increasing the K^+ concentration of the medium or by addition of veratridine. Stimulation was performed only during one incubation period of 10 min; thereafter the resting release of 5-HT was usually observed for a further period of 10 min. At the end of the experiments the tissues were blotted on tissue paper and weighed; in some experiments their 5-HT content was also determined. In this case, the tissues were quickly frozen in liquid nitrogen and kept at −20°C for assay.

The incubation medium was Tyrode solution (composition in mmol l^{-1}: NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, and glucose 5.6, gassed with 95% O_2 plus 5% CO_2). The medium also contained 5 μmol l^{-1} of the 5-HT uptake inhibitor fluoxetine (Gershon & Jonakait, 1979) and 20 μmol l^{-1} of the monoamine oxidase inhibitor nialamide. Media with altered K^+ or Ca^{2+} concentrations were kept isosmotic by appropriate changes in the Na^+ concentration. Ca^{2+}-free Tyrode solution also contained 2 mmol l^{-1} EGTA (ethyleneglycol-bis-(β-aminoethylether)-N, N′-tetraacetic acid).

Determination of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA)

5-HT and 5-HIAA were determined by h.p.l.c. with electrochemical detection according to the method of Sperk (1982) with modifications. Immediately before assay, the frozen incubation samples were thawed and centrifuged (1000 *g*, 15 min) to give a clear supernatant. Aliquots (0.5 ml) of the supernatants were directly applied to the h.p.l.c. system. To extract 5-HT from the MPLM, the frozen tissues were dropped into 5 volumes (w/v) of 0.2 mol l^{-1} perchloric acid and homogenized by ultrasonication. The homogenates were centrifuged (1000 *g*, 15 min) and 0.5 ml aliquots of the clear supernatants applied to the h.p.l.c. system.

The h.p.l.c. system consisted of a Kontron LC410 pump with pulse dampener, a Kontron MSI660 autosampler, a Rheodyne injection valve with a 500 μl sample loop, a RP8 precolumn (Kontron, 3.9 × 30 mm, particle size: 10 μm), a reverse-phase column (Waters, μBondapak C-18, 3.9 × 300 mm, particle size: 10 μm), and a LC-3 amperometric detector equipped with a TL-3 carbon paste electrode (both from Bioanalytical Systems). The voltage setting of the detector was + 0.8 V versus an Ag/AgCl reference electrode. Recordings and calculations were performed with a Shimadzu C-R1A integrator.

The mobile phase was 0.1 mol l^{-1} sodium acetate adjusted to pH 4.5 with acetic acid, degassed by ultrasonication, and containing 1 mmol l^{-1} EDTA

(ethylenediaminetetraacetic acid). The solution was stirred with a magnetic stirrer, thermostatically kept at 10°C above room temperature, and delivered at a constant flow rate of 2.5 ml min^{-1} at a pressure of about 200 bar. The system allowed the simultaneous measurement of dopamine, 3,4-dihydroxyphenylacetic acid, 5-HT, 5-HIAA, and homovanillic acid (Sperk, 1982) as is shown in Figure 1. The sensitivity of the procedure was 0.1 ng 5-HT, the detection limit being defined as a response peak twice the background noise, and the dose-detector response relation was linear for 0.1–20 ng 5-HT (see also Sperk, 1982). The background noise was determined by a blank run, i.e. by injecting blank incubation medium containing 0.2 mol l^{-1} perchloric acid, and then the integrator was programmed in such a way that only peaks exceeding the largest background peak by a factor of 2 were calculated and plotted out. To account for possible changes in the retention times or in the detector responses to the standards which might occur with time, a run of standards was included after every 10 runs of samples and the integrator reprogrammed, if necessary.

Presentation of data

The release of 5-HT is given in ng g^{-1} min^{-1}. In those experiments in which the 5-HT content of the tissues at the end of the experiments was measured, the release rate constant could also be calculated, i.e. $\Delta C/(\Delta t\, C_t)$. ΔC is the amount of 5-HT released in the time interval Δt, and C_t is the 5-HT content of the tissue at the midpoint of the interval Δt. The results are presented as means ± s.e. mean or as medians with range. For statistical evaluation, the one side *U* test of Wilcoxon, Mann & Whitney (Sachs, 1982) was used, *P* values <0.05 being regarded as significant.

Drugs

The following drugs were used: EDTA, EGTA (both from Merck), fluoxetine (Lilly), 5-HIAA (Sigma), 5-HT creatinine sulphate (Calbiochem), nialamide (Pfizer), pargyline hydrochloride, tetrodotoxin and veratridine (all from Sigma).

Results

5-HT and 5-HIAA content of MPLM

The concentrations of 5-HT and 5-HIAA in the MPLM taken from untreated guinea-pigs and from guinea-pigs treated with pargyline and their statistical evaluation by means of the *U* test are presented in Table 1. The concentrations of 5-HT and 5-HIAA in freshly prepared MPLM from untreated animals varied very much as did the molar ratio 5-HIAA/5-HT, which is a measure of 5-HT metabolism. During the release experiments, i.e. incubation of the MPLM in a medium containing nialamide and fluoxetine for 70 min, the content of 5-HIAA but not of 5-HT, and consequently the ratio 5-HIAA/5-HT, fell significantly. Pretreatment of the guinea-pigs with pargyline (50 mg kg^{-1}) inhibited the metabolism of 5-HT in freshly prepared MPLM as indicated by a significant decrease in the ratio 5-HIAA/5-HT and a tendency towards an increase in the 5-HT content. Again, both 5-HIAA content and ratio 5-HIAA/5-HT fell considerably during the release experiments, the decreases being statistically significant.

Table 1 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) content of the myenteric plexus-longitudinal muscle (MPLM) (ng g^{-1} wet weight).

	5-HT	*5-HIAA*	*Molar ratio 5-HIAA/5-HT*
Untreated guinea-pigs			
Fresh MPLM	3.8	14.0	1.48
	1.5–60.6 (7)	6.1–67.6 (7)	0.8–8.8 (7)
Incubated MPLM†	17.4	5.2**	0.27**
	12.6–26.1 (6)	3.1–7.8 (6)	0.18–0.37 (6)
Guinea-pigs treated with pargyline			
Fresh MPLM	38.1	14.4	0.35*
	30.5–74.7 (5)	11.3–29.4 (5)	0.23–0.49 (5)
Incubated MPLM†	20.4**	1.9**	0.12**
	12.4–48.2 (8)	1.0–6.3 (8)	0.02–0.28 (8)

Results are given as medians and range, *n* in parentheses.
*Significant differences ($P<0.01$) between pargyline treated and untreated guinea-pigs.
**Significant differences ($P<0.05$) between fresh and incubated MPLM (*U* test).
†MPLM used for release experiments, i.e. incubation in medium with 20 μmol l^{-1} nialamide and 5 μmol l^{-1} fluoxetine for 70 min. Pargyline (50 mg kg^{-1}) was given as an intraperitoneal injection 1 h before killing the animal.

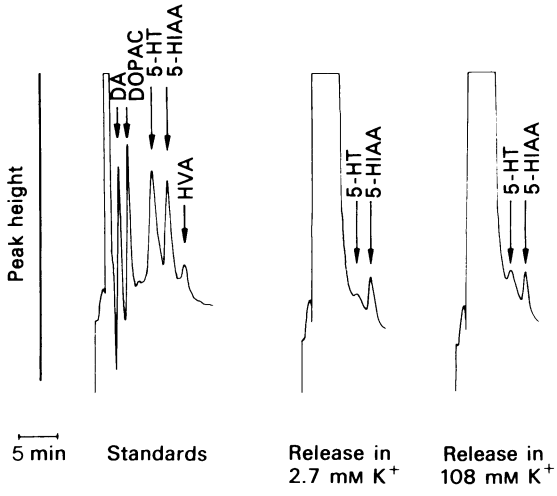

Figure 1 High performance liquid chromatography with electrochemical detection. Chromatograms of authentic standards and of myenteric plexus-longitudinal muscle (MPLM) incubation samples showing the resting (2.7 mM K^+) and stimulated (108 mM K^+) release of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA). Standards: dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-HIAA, and homovanillic acid (HVA) (10 ng of each).

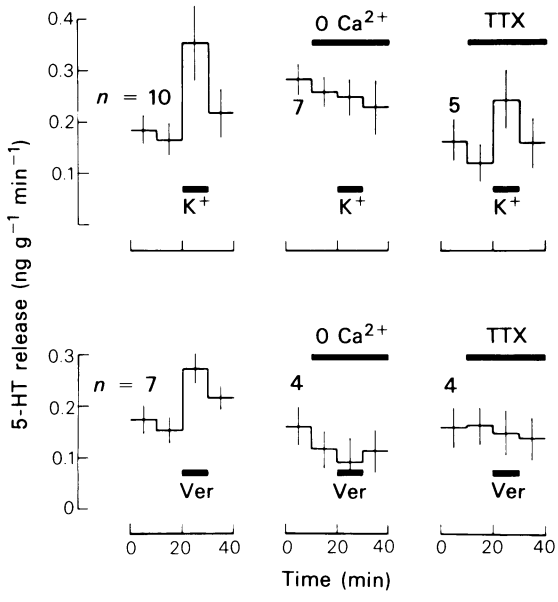

Figure 2 Effect of a 40 fold increase in the K^+ concentration or of veratridine (Ver, 0.1 mmol l^{-1} on the release of 5-hydroxytryptamine (5-HT) from the myenteric plexus-longitudinal muscle (MPLM). O Ca^{2+}: omission of Ca^{2+} from and addition of 2 mmol l^{-1} EGTA to the incubation medium. TTX: presence of 0.3 μmol l^{-1} tetrodotoxin. The results are shown as means ± s.e. mean, n as indicated.

Resting release of 5-HT and 5-HIAA

The release experiments were carried out with MPLM taken from guinea-pigs not pretreated with pargyline unless stated otherwise. The resting release of 5-HT from the MPLM was small but measurable (Figure 1) and quite variable from one preparation to another (0.03–0.32 ng 5-HT g^{-1} min^{-1}; Figure 2). The mean release rate constant in the first release sample collected after equilibration was 0.0078 ± 0.0012 min^{-1} ($n = 6$). Pretreatment of the guinea-pigs with pargyline did not enhance the resting release of 5-HT ($n = 8$). Of the 6 ng 5-HT added to the bath, 68 ± 8% ($n = 4$) was recovered in the incubation sample after 10 min and 31 ± 7% ($n = 4$) in the following sample, the values being corrected for the resting release of 5-HT as measured in the preceding sample. The total recovery of added 5-HT thus appeared to be quantitative (99 ± 9%, $n = 4$). 5-HIAA was easily detectable in all release samples (Figure 1), and the mean molar ratio 5-HIAA/5-HT in the first sample collected after equilibration amounted to 2.84 ± 0.39 ($n = 5$).

Stimulation of 5-HT release

Increasing the K^+ concentration of the medium by a factor of 40 increased the release of 5-HT by a factor of approximately 2 (Figures 1 and 2). In contrast, the release of 5-HIAA remained unaltered or even decreased slightly (Figure 1) and consequently the molar ratio 5-HIAA/5-HT fell from 2.84 ± 0.39 to 1.28 ± 0.21 ($n = 5$; $P < 0.01$, U test). Omission of Ca^{2+} from the medium together with addition of 2 mmol l^{-1} EGTA completely prevented the effect of K^+ on the 5-HT release whereas 0.3 μmol l^{-1} tetrodotoxin had no significant effect (Figure 2).

Veratridine (0.1 mmol l^{-1}) also stimulated the release of 5-HT from the MPLM (Figure 2). Veratridine had no effect when Ca^{2+} had been omitted from, or tetrodotoxin (0.3 μmol l^{-1}) added to, the incubation medium.

Discussion

The present study has confirmed that endogenous 5-HT is contained in and released from the isolated MPLM layer of the guinea-pig small intestine. The concentrations found in the MPLM were smaller than, but of the same order of magnitude as, the concentrations reported in the literature

(81–110 ng g^{-1} wet weight; Robinson & Gershon, 1971; Juorio & Gabella, 1974; Gershon & Tamir, 1981). Since the concentrations of 5-HT in the mucosa seem to be more than 100 times higher than in the MPLM layer (Juorio & Gabella, 1974), it is possible that the 5-HT content of the MPLM is entirely due to contamination with mucosal 5-HT, which in the course of the tissue preparation is absorbed by the MPLM (Furness & Costa, 1982a). However, there are two findings which indicate that at least part of the 5-HT found in the MPLM is in fact of neural origin. (i) Immunohistochemistry has shown the presence of a 5-HT-like material in neurones of the myenteric plexus (Costa *et al.*, 1982; Furness & Costa, 1982b; Dahlström & Ahlman, 1983). (ii) If the myenteric 5-HT was due to contamination with mucosal 5-HT, one would expect that most of the 5-HT contained in the MPLM would be lost during incubation in a medium containing the uptake inhibitor fluoxetine. However, the 5-HT content of the MPLM did not fall significantly during the release experiment.

There was a spontaneous, or resting, release of 5-HT from the MPLM which did not depend on the presence of Ca^{2+}. It should be pointed out that the amounts of 5-HT released spontaneously were very close to the limit of detectability. The absolute identification of these trace amounts as being 5-HT is difficult to prove but there are some arguments which suggest that the released material was indeed 5-HT rather than some unidentified substance. (i) The standard-detector response curve was linear also in the very low dose range. (ii) The detector responses were at least twice as high as the largest background peaks. (iii) The material released spontaneously was consistently eluted at the position of the 5-HT standard. The spontaneous efflux of 5-HT amounted to about 0.8% of the tissue stores released per min. This is quite a high rate of release but it should be borne in mind that inactivation and reuptake of the released 5-HT were blocked by nialamide and fluoxetine, respectively, and thus the rate of release measured in this study may substantially differ from that in the intact animal. That the concentrations of fluoxetine and nialamide used in this study were maximally effective is shown by the finding that exogenously added 5-HT was recovered quantitatively. When expressed in absolute amounts, the resting release of 5-HT was small, but still measurable, and varied from tissue to tissue. A similar variability has been observed with the resting release of substance P from the myenteric plexus of the guinea-pig small intestine and evidence was presented showing that this resulted from variable degrees of damage caused to the myenteric neurones in the course of the preparation of the MPLM (Holzer, 1983).

Depolarization of the MPLM by increasing the $[K^+]$ enhanced the release of 5-HT, but not of its metabolite 5-HIAA, in a Ca^{2+}-dependent manner. Also depolarization with veratridine, which acts by opening Na^+ channels in excitable cells (Ulbricht, 1969), increased the release of 5-HT but only when Ca^{2+} was present. This ionic dependence of the evoked release of 5-HT is consistent with the characteristics of release mechanisms for other established neurotransmitters (see Kelly *et al.*, 1979). Tetrodotoxin inhibited the effect of veratridine but not that of K^+, which indicates that the effect of K^+ does not involve action potential conduction via Na^+ channels.

The present results are in agreement with the findings of Gershon & Tamir (1981), that the release of endogenous 5-HT into the solution bathing the serosal surface of the guinea-pig small intestine is enhanced by electrical stimulation in a Ca^{2+}-dependent fashion and that the increase in the release of newly taken up [^{3}H]-5-HT is greater than that of labelled metabolites of 5-HT. However, they are at variance with those of Jonakait *et al.* (1979) who found that the K^+-evoked release of newly taken up [^{3}H]-5-HT from enteric neurones is not Ca^{2+}-dependent. This discrepancy may be explained by assuming that the pool of newly taken-up [^{3}H]-5-HT is different from the pool of endogenous 5-HT, a possibility that is not without experimental support (Gershon & Tamir, 1981).

In conclusion, these experiments have shown that authentic 5-HT is present in myenteric neurones and that depolarization of these neurones increases the release of 5-HT in preference to its metabolite 5-HIAA. The stimulated release of 5-HT is Ca^{2+}-dependent and thus likely to reflect a synaptic release mechanism. These results strongly support the hypothesis that 5-HT is an enteric neurotransmitter (Gershon & Tamir, 1981). However, the physiological roles of the enteric neurones containing 5-HT remain to be elucidated.

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References

BOLTON, T.B. (1972). The depolarizing action of acetycholine or carbachol in intestinal smooth muscle. *J. Physiol.*, **220**, 647–671.

COSTA, M., FURNESS, J.B., CUELLO, A.C., VERHOFSTAD, A.A.J., STEINBUSCH, H.W.J. & ELDE, R.P. (1982). Neurons with 5-hydroxytryptamine-like immunoreac-

tivity in the enteric nervous system: their visualization and reactions to drug treatment. *Neuroscience*, **7**, 351–363.

DAHLSTRÖM, A. & AHLMAN, H. (1983). Immunocytochemical evidence for the presence of tryptaminergic nerves of blood vessels, smooth muscle and myenteric plexus in the rat small intestine. *Acta physiol. scand.*, **117**, 589–591.

ERSPAMER, V. (1966). Occurrence of indolealkylamines in nature. In *5-Hydroxytryptamine and Related Indolealkylamines, Handb. exp. Pharmac.* Vol. 19, ed. Erspamer, V., pp. 132–181. Berlin, Heidelberg and New York: Springer-Verlag.

FURNESS, J.B. & COSTA, M. (1982a). Identification of gastrointestinal neurotransmitters. In *Mediators and Drugs in Gastrointestinal Motility I, Handb. exp. Pharmac.* Vol. 59/I, ed. Bertaccini, G., pp. 383–462. Berlin, Heidelberg and New York: Springer-Verlag.

FURNESS, J.B. & COSTA, M. (1982b). Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system: their projections in the guinea-pig small intestine. *Neuroscience*, **7**, 341–349.

GERSHON, M.D. & JONAKAIT, G.M. (1979). Uptake and release of 5-hydroxytryptamine by enteric 5-hydroxytryptaminergic neurones: effects of fluoxetine (Lilly 110140) and chlorimipramine. *Br. J. Pharmac.*, **66**, 7–9.

GERSHON, M.D. & TAMIR, H. (1981). Release of endogenous 5-hydroxytryptamine from resting and stimulated enteric neurons. *Neuroscience*, **6**, 2277–2286.

HOLZER, P. (1983). Characterization of the stimulus-induced release of immunoreactive substance P from the myenteric plexus of the guinea-pig small intestine. *Brain Res.*, (in press.)

JOHNSON, S.M., KATAYAMA, Y. & NORTH, R.A. (1980). Multiple actions of 5-hydroxytryptamine on myenteric neurones of the guinea-pig ileum. *J. Physiol.*, **304**, 459–470.

JONAKAIT, G.M., TAMIR, H., GINTZLER, A.R. & GERSHON, M.D. (1979). Release of [^{3}H] serotonin and its binding protein from enteric neurons. *Brain Res.*, **174**, 55–69.

JUORIO, A.V. & GABELLA, G. (1974). Noradrenaline in the guinea-pig alimentary canal: regional distribution and sensitivity to denervation and reserpine. *J. Neurochem.*, **22**, 851–858.

KELLY, R.B., DEUTSCH, J.W., CARLSON, S.S. & WAGNER, J.A. (1979). Biochemistry of neurotransmitter release. *Ann. Rev. Neurosci.*, **2**, 399–446.

ROBINSON, R.G. & GERSHON, M.D. (1971). Synthesis and uptake of 5-hydroxytryptamine by the myenteric plexus of the guinea-pig ileum: a histochemical study. *J. Pharmac. exp. Ther.*, **178**, 311–324.

SACHS, L. (1982). *Applied Statistics.* New York, Heidelberg and Berlin: Springer-Verlag.

SCHULZ, R. & CARTWRIGHT, C. (1974). Effect of morphine on serotonin release from myenteric plexus of the guinea-pig. *J. Pharmac. exp. Ther.*, **190**, 420–430.

SHARMAN, D.F., HOLZER, P. & HOLZBAUER, M. (1982). *In vitro* release of endogenous catecholamines from the neural and intermediate lobe of the hypophysis. *Neuroendocrinology*, **34**, 175–179.

SPERK, G. (1982). Simultaneous determination of serotonin, 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid and homovanillic acid by high performance liquid chromatography with electrochemical detection. *J. Neurochem.*, **38**, 840–843.

ULBRICHT, W. (1969). The effect of veratridine on excitable membranes of nerve and muscle. *Ergebn. Physiol.*, **61**, 19–61.

WOOD, J.D. & MAYER, C.J. (1979). Serotoninergic activation of tonic-type enteric neurons in the guinea-pig small bowel. *J. Neurophysiol.*, **42**, 582–593.

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