Br. J. Pharmac. (1985), **84**, 027–034

5-Hydroxytryptamine releases adenosine 5′-triphosphate from nerve varicosities isolated from the myenteric plexus of guinea-pig ileum

Mohammad Al-Humayyd & Thomas D. White*

Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7

1 5-Hydroxytryptamine (5-HT)-evoked release of ATP from nerve varicosities isolated from the myenteric plexus of guinea pig ileum was investigated.

2 5-HT released ATP from myenteric varicosities by a Ca^{2+}-dependent mechanism. The EC_{50} for release of ATP was 7×10^{-7} M 5-HT.

3 5-HT-evoked release of ATP was not blocked by tetrodotoxin (TTX), indicating that release was not initiated by the opening of Na^+-channels in the isolated myenteric varicosities.

4 Release of ATP by 5-HT was diminished to 56% of control values by *in vivo* pretreatment of the guinea-pig with 6-hydroxydopamine (6-OHDA, 250 mg kg^{-1}, i.p.) for 24 h. 6-OHDA pretreatment caused extensive destruction of noradrenergic varicosities as indicated by an 87% loss of noradrenaline content.

5 Quipazine (5×10^{-6} M) and methysergide (10^{-4} M) caused a small release of ATP and blocked subsequent 5-HT-induced release of ATP. Metergoline (2.5×10^{-5} M), (+)-tubocurarine (7×10^{-5} M) and cocaine (10^{-4} M) decreased 5-HT-induced ATP release. 5-Methoxytryptamine (10^{-4} M), picrotoxin (3.5×10^{-6} M), spiroperidol (10^{-6} M), morphine (1.3×10^{-6} M) and phenoxybenzamine (3.7×10^{-7} M) were ineffective.

6 The results demonstrate a 5-HT-receptor-mediated release of ATP from noradrenergic and possibly non-adrenergic varicosities in the myenteric plexus of guinea-pig ileum. The 5-HT-induced release of ATP is consistent with a possible transmitter, cotransmitter or modulatory role for ATP in the myenteric plexus.

Introduction

There is evidence that 5-hydroxytryptamine (5-HT) might function as a neurotransmitter in the myenteric plexus of ileum (Gershon, 1979). Application of 5-HT has been shown either to depolarize or hyperpolarize myenteric neurones (North, 1982), although the overall effect of 5-HT on ileal longitudinal muscle-myenteric plexus preparations is usually an excitatory, contractile response (Huidobro-Toro & Foree, 1980). Burnstock and his colleagues have proposed that adenosine 5′-triphosphate (ATP) might be a non-adrenergic, non-cholinergic inhibitory neurotransmitter in vertebrate gastrointestinal smooth muscle (see Burnstock, 1979, for review). Although recent studies do not support this hypothesis (Westfall *et al.*, 1982; Bauer & Kuriyama, 1982) low concentrations of ATP have been shown to inhibit indirectly the longitudinal muscle of guinea-pig ileum, possibly through adenosine receptors on cholinergic neurones in the myenteric plexus (Sawynok & Jhamandas, 1976; Watt, 1982). Moreover, we have recently described a Ca^{2+}-dependent release of endogenous ATP when varicosities isolated from the myenteric plexus of guinea-pig ileum were depolarized by veratridine or K^+ (White & Leslie, 1982) or exposed to acetylcholine (White, 1982; White & Al-Hymayyd, 1983). Much, but not all, of the acetylcholine-evoked release of ATP appeared to originate from noradrenergic varicosities (White & Al-Humayyd, 1983). These results are consistent with possible transmitter, cotransmitter or modulatory functions for ATP and/or adenosine in the ileum.

In the present study we have investigated the possibility that 5-HT might act as an excitatory neurotransmitter to release ATP from varicosities isolated from the myenteric plexus of guinea-pig ileum. The

* Correspondence

release of ATP by 5-HT was characterized and the possibility that it might arise from noradrenergic varicosities investigated.

Methods

Preparation of myenteric varicosities

A crude preparation of myenteric varicosities (P_2) was isolated from the ileal longitudinal muscles of three or four guinea-pigs (High Oak Ranch, Goodwood, Ontario) of either sex weighing 300–400 g (White & Leslie, 1982). Each muscle was minced thoroughly with scissors before being homogenized in 10 ml of 0.32 M sucrose with seven strokes of a Teflon-glass homogenizer (clearance, 0.25 mm). The homogenates from all the muscles were pooled and centrifuged at 1000 *g* for 10 min and the supernatant decanted and saved. The remaining pellet was resuspended in 0.32 M sucrose and centrifuged at 1000 *g* for 10 min. The resulting supernatant was pooled with the original supernatant and centrifuged at 20,000 *g* for 30 min. The pellet (P_2), containing isolated nerve varicosities and no identifiable muscle contamination (White & Leslie, 1982), was suspended in Krebs-Henseleit medium (approximately 0.9 mg protein ml^{-1}) containing (mM) NaCl 111, $NaHCO_3$ 26.2, NaH_2PO_4 1.2, KCl 4.7, $CaCl_2$ 1.8, $MgCl_2$ 1.2, glucose 11 and aerated with 95% O_2: 5% CO_2 to maintain a pH of 7.4. In some cases the varicosities were suspended in a Ca^{2+}-free medium otherwise identical to the above. The suspensions were preincubated for 30 min at 37 °C with periodic aeration before studies of the release of ATP were performed.

Detection of ATP release

The release of ATP was detected directly and continuously in 0.5 ml aliquots of crude P_2 myenteric varicosity preparations by monitoring the light produced by the reaction of ATP with firefly luciferin-luciferase present in the incubation medium, as described previously (White, 1978; White & Leslie, 1982). Drugs and ATP standards were injected into the incubation medium using a Hamilton syringe. Release of ATP was expressed as maximum concentration of ATP achieved in the cuvette mg^{-1} of varicosity protein.

Pretreatment of animals with 6-hydroxydopamine

In some experiments, animals were pretreated with 6-hydroxydopamine (250 mg kg^{-1}, i.p.) in 1 mg ml^{-1} ascorbate and killed 24 h later. Controls received no treatment.

Determination of noradrenaline content of myenteric varicosities

Noradrenaline was extracted and assayed using h.p.l.c. with electrochemical detection as described by White & Al-Humayyd (1983). The minimum detectable level of noradrenaline was 0.15 pmol in 20 µl of perchloric acid. Noradrenaline content of myenteric varicosities was expressed as pmol mg^{-1} protein.

Protein

Protein was determined by the method of Hartree (1972).

Materials

d-Luciferin, crude firefly extract (FLE-50), disodium ATP, noradrenaline HCl, 5-hydroxytryptamine (creatinine sulphate complex), 6-hydroxydopamine HBr, tubocurarine chloride, 5-methoxytryptamine and picrotoxin were purchased from Sigma. Metergoline was supplied by Farmitalia, quipazine maleate by Miles Laboratories, cocaine hydrochloride by May and Baker, methysergide hydrogenmaleinate by Sandoz, morphine sulphate by B.D.H., tetrodotoxin citrate by Calbiochem, phenoxybenzamine by Smith, Kline & French, and spiroperidol by Janssen Pharmaceuticals. All other chemicals were reagent grade.

Results

Release of ATP by 5-hydroxytryptamine

Addition of 5-HT to myenteric varicosities released ATP as indicated by the production of light from the ATP-luciferin-luciferase reaction (Figure 1). Release of ATP was greatly diminished when varicosities were suspended and incubated in a medium lacking $CaCl_2$ (Figure 1). Also, the addition of 1 mM EGTA to chelate residual extracellular Ca^{2+} abolished the 5-HT-induced release of ATP. Restoration of $CaCl_2$ to varicosities preincubated in a Ca^{2+}-free medium restored the 5-HT-induced release of ATP to levels higher than control, indicating that preincubation in Ca^{2+}-free medium had not irreversibly damaged the varicosities.

Dose-response relationship for the release of ATP by 5-hydroxytryptamine

Figure 2 shows the maximum concentration of ATP achieved in the cuvette mg^{-1} protein plotted against the log of the 5-HT concentration administered. Significant release of ATP was observed with as little

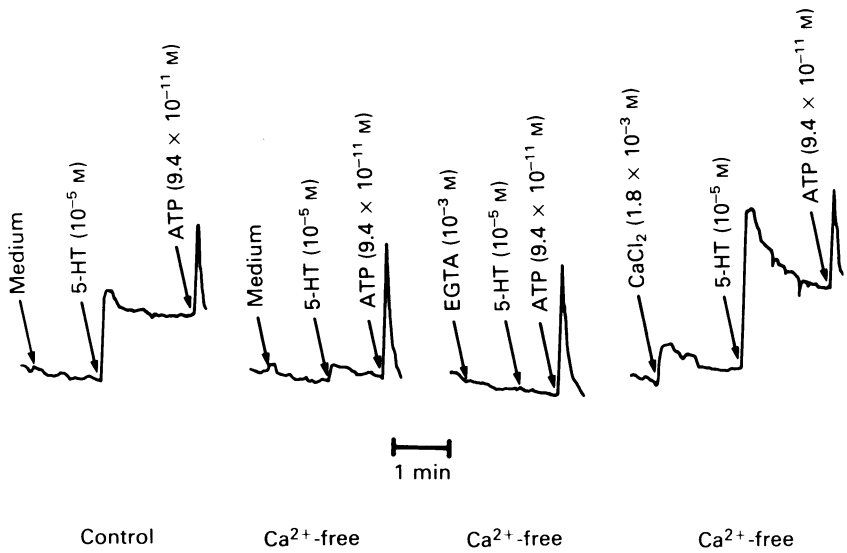

Figure 1 Effect of extracellular Ca^{2+} on 5-hydroxytryptamine (5-HT)-induced release on ATP from myenteric varicosities. Myenteric varicosities were suspended in normal medium or in medium lacking $CaCl_2$ (Ca^{2+}-free) and preincubated for 30 min. Release of ATP was determined for normal varicosities, for the Ca^{2+}-free suspension, for the Ca^{2+}-free suspension to which 1 mM EGTA had been added to chelate residual Ca^{2+}, and for the Ca^{2+}-free suspension in which $CaCl_2$ was restored to 1.8 mM. As an internal standard, ATP in Ca^{2+}-free medium was injected. Results similar to those shown were observed in four experiments.

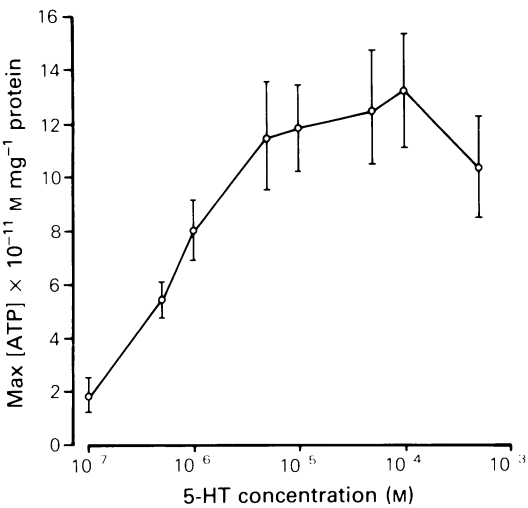

Figure 2 Dose-response curve for the release of ATP evoked from myenteric varicosities by 5-hydroxytryptamine (5-HT). Release of ATP is expressed as the maximum concentration of ATP achieved in the cuvette mg^{-1} varicosity protein. Each point represents the mean and vertical lines s.e. mean of 6 experiments.

as 10^{-7} M 5-HT and maximal release was obtained at concentrations exceeding 5×10^{-6} M. The EC_{50} for 5-HT-induced release of ATP from myenteric varicosities was approximately 7×10^{-7} M.

The effects of 5-hydroxytryptamine agonists or partial agonists on ATP release

5-Methoxytryptamine (10^{-4} M), a potent 5-HT agonist at cholinergic neurones in the ileum (Fozard & Mobarok-Ali, 1978), did not release ATP from myenteric varicosities, nor did it antagonize the release of ATP evoked by 10^{-6} M 5-HT (Figure 3, Table 1). However, 5-methoxytryptamine appeared to prolong the release of ATP evoked by 5-HT (Figure 3).

Quipazine has been reported to be a partial agonist at excitatory 5-HT receptors in the myenteric plexus, where it first excites and subsequently blocks activation of the receptors (Hong & Pardo, 1966; Huidobro-Toro & Foree, 1980). In the present study, quipazine (5×10^{-6} M) released ATP and totally abolished the subsequent release of ATP by 10^{-6} M 5-HT (Figure 3, Table 1).

Methysergide (10^{-4} M) caused a small release of

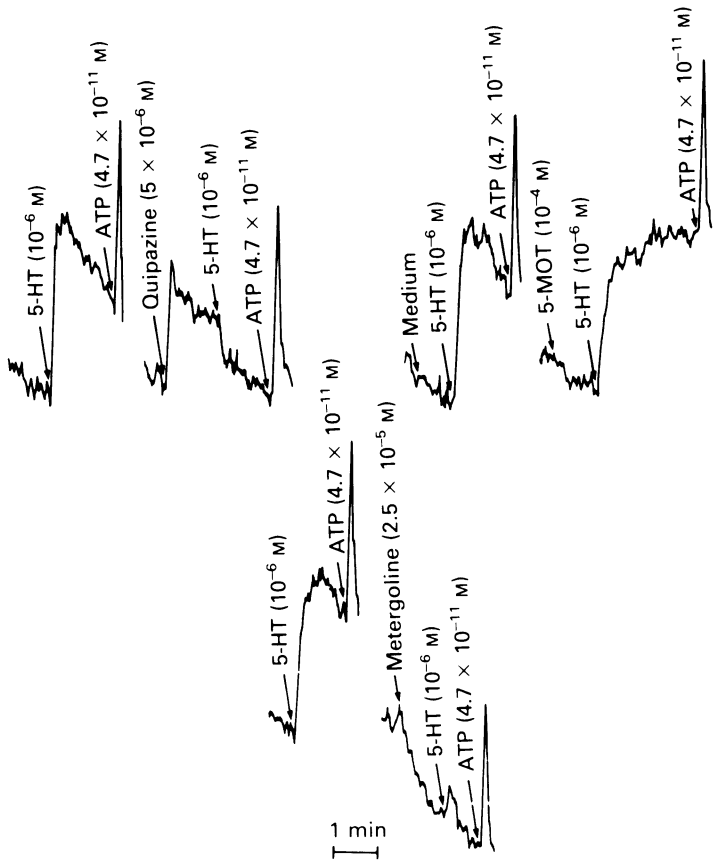

Figure 3 Effects of quipazine, 5-methoxytryptamine (5-MOT) and metergoline on ATP release by 5-hydroxytryptamine (5-HT). Ten μl of drugs in incubation medium were injected into 0.5 ml of synaptosomal suspension to give the final concentrations indicated. Similar results were observed in at least 3 experiments.

ATP and reduced the release of ATP evoked by 10^{-6} M 5-HT to 70% of the control value (Figure 4, Table 1).

The effects of various antagonists on 5-hydroxytryptamine-evoked release of ATP

Metergoline (2.5×10^{-5} M) appeared to diminish the resting release of ATP from myenteric varicosities (Figure 3) and antagonized the subsequent release of ATP by 10^{-6} M 5-HT to 21% of its control value (Figure 3, Table 1).

Tubocurarine has been shown to antagonize both the 5-HT-induced and nicotinic responses in rabbit isolated superior cervical ganglia (Wallis & Nash, 1980). A relatively high concentration of (+)-tubocurarine (7×10^{-5} M) reduced the 5-HT-evoked release of ATP from myenteric varicosities to 15% of its control value (Figure 4, Table 1).

Cocaine (10^{-6}–10^{-5} M) has been shown to be a competitive antagonist of the excitatory actions of 5-HT on sympathetic noradrenergic nerves in the rabbit heart and on cholinergic nerves in the guinea-pig ileum (Fozard *et al.*, 1979). A high concentration of cocaine (10^{-4} M) completely abolished the 5-HT-induced release of ATP (Figure 5, Table 1).

None of the following drug treatments released ATP or antagonized the 5-HT-induced release of ATP from myenteric varicosities (Table 1). Picrotoxin (3.5×10^{-6} M), which has been shown to antagonize the excitatory actions of 5-HT at parasympathetic (Saum & DeGroat, 1973), superior cervical (DeGroat & Lalley, 1973), and nodose ganglia (Simonds & DeGroat, 1980) in the cat, had no effect on 5-HT-evoked release of ATP in the present study. Spiroperidol (10^{-6} M), which is a competitive an-

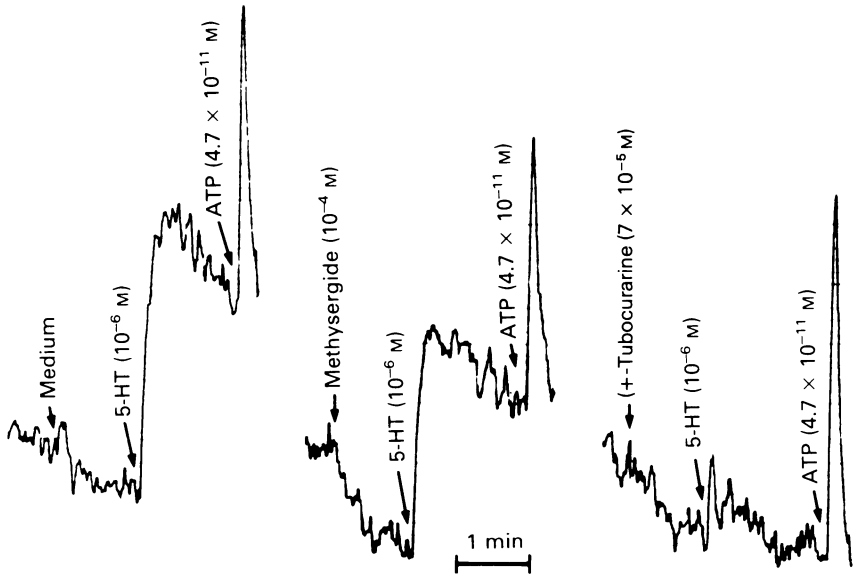

Figure 4 Effects of methysergide and tubocurarine on ATP release by 5-hydroxytryptamine (5-HT). Ten μl of drugs in incubation medium were injected into 0.5 ml of synaptosomal suspension to give the final concentrations indicated. Similar results were observed in at least 4 experiments.

Table 1 Effects of 5-hydroxytryptamine agonists and antagonists on release of ATP from myenteric varicosities

		Release of ATP (max [ATP] per cuvette $\times 10^{-11}$ M mg^{-1} protein)			
	n	*Drug*	*5-HT* (10^{-6} M)	*Drug + 5-HT*	*% of 5-HT-induced release*
5-Methoxytryptamine (10^{-4} M)	4	not detectable	5.48 ± 1.34	4.68 ± 0.80	85
Quipazine (5×10^{-5} M)	5	6.69 ± 0.88	9.69 ± 2.57	not detectable*	0
Methysergide (10^{-4} M)	5	0.79 ± 0.48	4.40 ± 0.48	3.09 ± 0.52*	70
Metergoline (2.5×10^{-5} M)	3	not detectable	7.75 ± 1.35	1.65 ± 0.22*	21
(+)-Tubocurarine (7×10^{-5} M)	4	not detectable	4.91 ± 0.35	0.74 ± 0.34*	15
Cocaine (10^{-5} M)	3	not detectable	7.61 ± 1.57	4.35 ± 0.44	57
Cocaine (10^{-4} M)	3	not detectable	8.06 ± 1.42	not detectable*	0
Picrotoxin (3.5×10^{-6} M)	4	not detectable	4.27 ± 0.84	4.44 ± 0.59	104
Spiroperidol (10^{-6} M)	4	not detectable	10.87 ± 2.07	10.26 ± 1.77	94
Morphine (1.32 ± 10^{-6} M)	3	not detectable	9.22 ± 0.44	9.81 ± 0.63	106
Tetrodotoxin (8×10^{-7} M)	4	not detectable	4.93 ± 0.51	4.09 ± 0.19	83

Each value is the mean ± s.e. mean of the number of experiments indicated (n).
* Significantly different from corresponding 5-HT-induced release ($P < 0.05$, paired t test).

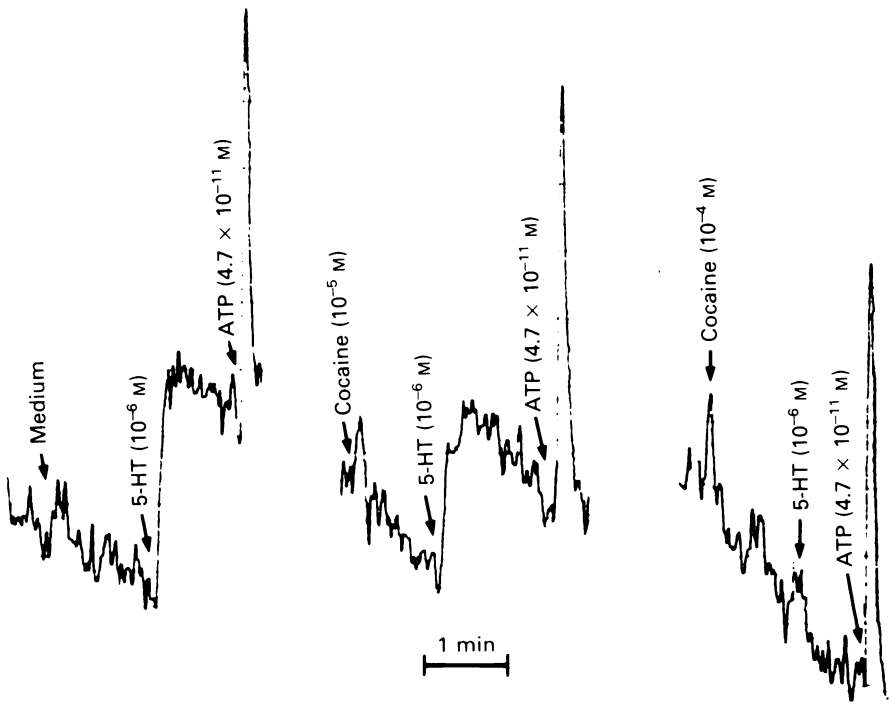

Figure 5 Effect of cocaine on ATP release by 5-hydroxytryptamine (5-HT). Ten μl of cocaine in incubation medium were injected into 0.5 ml of synaptosomal suspensions to give the final concentrations indicated. Similar results were observed in 3 experiments.

tagonist of 5-HT mediated contractions in rat aorta or jugular vein (Cohen *et al.*, 1981), was without effect. 5-HT-evoked release of ATP was unaffected by morphine (1.3×10^{-6} M) or by phenoxybenzamine (3.7×10^{-7} M), antagonists at so-called 'M'- and 'D'-type 5-HT receptors, respectively (Gaddum & Picarelli, 1957). Finally, the Na^+-channel blocker, tetrodotoxin (8×10^{-7} M), which has previously been shown to abolish the veratridine-induced release of ATP from myenteric varicosities (White & Leslie, 1982), did not significantly diminish 5-HT-evoked ATP release.

Effect of 6-hydroxydopamine pretreatment on the release of ATP by 5-hydroxytryptamine

Quayyum (1976) has shown that pretreatment of guinea-pigs with 6-hydroxydopamine (250 mg kg^{-1} i.p. for 24 h) abolishes noradrenaline histofluorescence in the myenteric plexus. We have previously shown that the acetylcholine-evoked release of ATP from myenteric varicosities was diminished by pretreatment with 6-hydroxydopamine (White & Al-Hummayyd, 1983), indicating that much of the ATP might have been released from noradrenergic varicosities in the preparation. Pretreatment of guinea-pigs with 6-hydroxydopamine for 24 h reduced the noradrenaline content of myenteric varicosities to 13% and reduced the 5-HT-evoked release of ATP from myenteric varicosities to 56% of the control values (Table 2).

Discussion

5-HT released ATP from nerve varicosities isolated from the myenteric plexus of guinea-pig ileum by a Ca^{2+}-dependent mechanism. The EC_{50} for release of ATP was 7×10^{-7} M 5-HT, which is only slightly higher than the EC_{50} values previously found for the 5-HT-induced stimulation of guinea-pig ileal preparations (4.5×10^{-7} M 5-HT, Fozard *et al.*, 1979; 3×10^{-7} M 5-HT, Huidobro-Toro & Foree, 1980). Release of ATP appeared to be mediated by specific actions at presynaptic 5-HT receptors on the myenteric varicosities since the partial agonist, quipazine, released ATP and release by 5-HT was diminished by a number of 5-HT antagonists such as quipazine,

Table 2 Effect of 6-hydroxydopamine (6-OHDA) pretreatment on noradrenaline content and 5-hydroxytryptamine-induced release of ATP from myenteric varicosities

	Noradrenaline content (pmol mg^{-1} protein)	*% control*	*Release of ATP by* 5×10^{-5} M 5-HT (max [ATP] per cuvette × 10^{-11} M mg^{-1} protein)	*% control*
Control	41.0 ± 4.6	–	10.4 ± 1.70	–
6-OHDA	5.22 ± 1.41*	12.7	5.8 ± 0.9*	55.8

Guinea-pig were pretreated with 250 mg kg^{-1} 6-OHDA i.p. and killed 24 h later. Noradrenaline contents and release of ATP were determined as described in Methods. Each value is the mean ± s.e. mean of 4 experiments.
* Significantly different from respective control ($P < 0.05$, unpaired t test).

methysergide, metergoline, (+)-tubocurarine and cocaine. On the other hand, 5-HT-induced release of ATP was not antagonized by picrotoxin, spiroperidol, phenoxybenzamine or morphine. Nor was 5-HT-induced release of ATP depressed by tetrodotoxin, suggesting that release was not initiated by the opening of Na^+-channels in the isolated myenteric varicosities. It is possible that 5-HT depolarized the varicosities by increasing the membrane resistance rather than by increasing the conductance of the varicosities to Na^+ (North, 1982).

Release of ATP by 5-HT was substantially diminished, but not abolished, by prior *in vivo* treatment of the guinea-pigs with 6-hydroxydopamine (250 mg kg^{-1} i.p. for 24 h). This treatment, which selectively destroys catecholaminergic nerves (Jonsson, 1980), abolishes noradrenaline histofluorescence in the myenteric plexus (Quayyum, 1976) and does not apparently produce non-selective destruction of myenteric varicosities (White & Al-Humayyd, 1983), reduced the noradrenaline content of the varicosities to 13% of control values, indicating extensive destruction of noradrenergic varicosities. Therefore, it would appear that much, but probably not all, of the ATP released from myenteric varicosities by 5-HT originates from noradrenergic varicosities present in the preparation. The residual, non-adrenergic release of ATP could be co-released with some other neurotransmitter or be released from 'purinergic' varicosities in the preparation. A previous study has shown that much, but not all, of the acetylcholine-evoked release of ATP from myenteric varicosities also originates from noradrenergic varicosities (White & Al-Humayyd, 1983).

In this respect, it is interesting that 5-HT has been shown to stimulate isolated sympathetic ganglia (Mantagazzini, 1966) and to release noradrenaline from sympathetic nerve terminals in the heart (Fozard & Mwaluko, 1976; Humphrey *et al.*, 1983). Moreover, Erde *et al.* (1982) have recently demonstrated the existence of axo-axonic, 5-HT-noradrenergic synapses in the myenteric plexus of guinea-pig ileum. Since much of the 5-HT-evoked release of ATP observed in the present study appears to have originated from noradrenergic varicosities derived from the myenteric plexus, 5-HT may elicit a similar release of noradrenaline from myenteric varicosities.

Wallis (1981) has reviewed the diverse characteristics of neuronal 5-HT receptors in the peripheral nervous system and has classified these receptors for several preparations on the basis of their responses to a variety of 5-HT agonists and antagonists. Unfortunately, the 5-HT-evoked release of ATP from myenteric varicosites does not fall into any of these classifications. For instance, our findings that 5-methoxytryptamine had no agonist activity and that methysergide did have some antagonist actions on ATP release are not consistent with 'type α' excitatory receptors reported to reside on myenteric plexus neurones (Wallis, 1981). The 5-HT-induced release of ATP from myenteric varicosities has many similarities to 'type A' receptor mediated excitation described for sympathetic ganglia cells (Wallis, 1981) and appears to involve to a large extent noradrenergic varicosities, but our observation that metergoline blocked 5-HT-induced ATP release is not consistent with this classification.

One can speculate as to possible functions for ATP released by 5-HT from myenteric varicosities. Released ATP could function as a co-transmitter with noradrenaline, as has been shown for guinea-pig vas deferens (Fedan *et al.*, 1981), or as an inhibitory modulator of noradrenaline release through actions at presynaptic 'P_1'-adenosine receptors on noradrenergic varicosities. On the other hand, Watt (1982) has shown that the predominant inhibitory action of ATP applied to guinea-pig ileum is an indirect one on intramural excitatory cholinergic nerves. Perhaps by releasing ATP from noradrenergic and possibly non-adrenergic myenteric neurones, 5-HT modulates its own excitatory actions on cholinergic interneurones in the myenteric plexus of guinea-pig ileum. In any event, the 5-HT-induced, Ca^{2+}-dependent release of

ATP from myenteric varicosities is consistent with a possible neurotransmitter, co-transmitter or modulatory role for ATP in the myenteric plexus of guinea-pig ileum.

We thank Diana Webb for her expert technical assistance. This work was supported by grants from the Medical Research Council of Canada and the Government of Saudi Arabia.

References

BAUER, V. & KURIYAMA, H. (1982). The nature of non-cholinergic, non-adrenergic transmission in longitudinal and circular muscles of guinea pig ileum. *J. Physiol.*, **332,** 375–391.

BURNSTOCK, G. (1979). Past and current evidence for the purinergic nerve hypothesis. In *Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides.* ed. Baer, H.P. & Drummond, G.I., pp 3–28. New York: Raven.

COHEN, M.L., FULLER, R.W. & WILEY, K.S. (1981). Evidence for 5-HT_2 receptors mediating contraction in vascular smooth muscle. *J. Pharmac. exp. Ther.*, **218,** 421–425.

DEGROAT, W.C. & LALLEY, P.M. (1973). Interaction between picrotoxin and 5-hydroxytryptamine in the superior cervical ganglion of the cat. *Br. J. Pharmac.*, **48,** 233–244.

ERDE, S.M., GERSHON, M.D. & SHERMAN, D.L. (1982). Serotonergic neurons of the small intestine: distribution and interactions with other neurons. *Soc. Neurosci. Abstr.*, **8,** 551.

FEDAN, J.S., HOGABOOM, G.K., O'DONNELL, J.P., COLBY, J. & WESTFALL, D.P. (1981). Contributions by purines to the neurogenic response of the vas deferens of the guinea pig. *Eur. J. Pharmac.*, **69,** 41–53.

FOZARD, J.R. & MOBAROK-ALI, A.T.M. (1978). Receptors for 5-hydroxytryptamine on the sympathetic nerves of the rabbit heart. *Naunyn-Schmiedebergs Arch. Pharmac.*, **301,** 223–235.

FOZARD, J.R., MOBAROK-ALI, A.T.M. & NEWGROSH, G. (1979). Blockade of serotonin receptors on autonomic neurones by (−)-cocaine and some related compounds. *Eur. J. Pharmac.*, **59,** 195–210.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmac.*, **57,** 115–125.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two types of tryptamine receptor. *Br. J. Pharmac.*, **12,** 323–328.

GERSHON, M.D. (1979). Serotonin. *Neurosci. Res. Prog. Bull.*, **17,** 414–424.

HARTREE, E.F. (1972). Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal. Biochem.*, **48,** 422–427.

HONG, E. & PARDO, E.G. (1966). On the pharmacology of 2-(1-piperazinyl) quinoline. *J. Pharmac. exp. Ther.*, **153,** 259–265.

HUIDOBRO-TORO, J.P. & FOREE, B. (1980). Dual agonist-antagonist effects of 5-hydroxytryptamine (5-HT) in the guinea pig ileum: evidence for a selective receptor desensitization effect. *Eur. J. Pharmac.*, **61,** 335–345.

HUMPHREY, P.P.A., FENIUK, W. & WATTS, A.D. (1983). Prejunctional effects of 5-hydroxytryptamine on noradrenergic nerves in the cardiovascular system. *Fedn. Proc.*, **42,** 218–222.

JONSSON, G. (1980). Chemical neurotoxins as denervation tools in neurobiology. *Ann. Rev. Neurosci.*, **3,** 169–187.

MANTAGAZZINI, P. (1966). Pharmacological effects of idolealkylamines and precursor amino acids on the central nervous system. In *Handbook of Experimental Pharmacology* Vol. XIX, *5-Hydroxytryptamines and Related Indolealkylamines.* ed. Erspamer, V. pp. 424–470. Berlin: Springer Verlag.

NORTH, R.A. (1982). Electrophysiology of the enteric nervous system. *Neuroscience*, **7,** 315–325.

QUAYYUM, M.A. (1976). Fluorescence histochemical study of Auerbach's plexus of the ileum of 6-hydroxydopamine-treated guinea pig. *Acta Anat.*, **96,** 497–512.

SAUM, W.R. & DEGROAT, W.C. (1973). The actions of 5-hydroxytryptamine on the urinary bladder and vesical autonomic ganglia in the cat. *J. Pharmac. exp. Ther.*, **185,** 70–83.

SAWYNOK, J. & JHAMANDAS, K.H. (1976). Inhibition of acetylcholine release from cholinergic nerves by adenosine, adenine nucleotides and morphine. Antagonism by theophylline. *J. Pharmac. exp. Ther.*, **197,** 379–390.

SIMONDS, W.F. & DEGROAT, W.C. (1980). Antagonism by picrotoxin of 5-hydroxytryptamine-induced excitation of primary afferent neurons. *Brain Res.*, **192,** 592–597.

WALLIS, D. (1981). Neuronal 5-hydroxytryptamine receptors outside the central nervous system. *Life Sci.*, **29,** 2345–2355.

WALLIS, D.I. & NASH, H.L. (1980). The action of methylated derivatives of 5-hydroxytryptamine at ganglionic receptors. *Neuropharmacology*, **19,** 465–472.

WATT, A.J. (1982). Direct and indirect effects of adenosine 5′-triphosphate on guinea-pig ileum. *Br. J. Pharmac.*, **77,** 725–730.

WESTFALL, D.P., HOGABOOM, G.K., COLBY, J., O'DONNELL, J.P. & FEDAN, J.S. (1982). Direct evidence against a role of ATP as the nonadrenergic, non-cholinergic inhibitory neurotransmitter in guinea pig tenia coli. *Proc. natn. Acad. Sci. U.S.A.*, **79,** 7041–7045.

WHITE, T.D. (1978). Release of ATP from a synaptosomal preparation by elevated extracellular K^+ and by veratridine. *J. Neurochem.*, **30,** 329–336.

WHITE, T.D. (1982). Release of ATP from isolated myenteric varicosities by nicotinic agonists. *Eur. J. Pharmac.*, **79,** 333–334.

WHITE, T.D. & AL-HUMAYYD, M. (1983). Acetylcholine releases ATP from varicosities isolated from guinea pig myenteric plexus. *J. Neurochem*, **40,** 1069–1075.

WHITE, T.D. & LESLIE, R.A. (1982). Depolarization-induced release of adenosine 5′-triphosphate from isolated varicosities derived from the myenteric plexus of the guinea pig small intestine. *J. Neurosci.*, **2,** 206–215.

(Received March 21, 1984. Revised August 22, 1984.)