Neuroscience Vol. 5, pp. 581 to 586 0306-4522/80/0301-0581$02.00/0

ELECTROPHYSIOLOGICAL EVIDENCE FOR PRESYNAPTIC INHIBITION OF ACETYLCHOLINE RELEASE BY 5-HYDROXYTRYPTAMINE IN THE ENTERIC NERVOUS SYSTEM

R. A. NORTH[1], G. HENDERSON[1], Y. KATAYAMA[2] and S. M. JOHNSON[3]

Neurophysiology Laboratory, Department of Pharmacology, Loyola University Stritch School of Medicine, 2160 South First Avenue, Maywood, IL 60153, U.S.A., and Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, U.K.

Abstract—Intracellular recordings were made *in vitro* from myenteric neurones of guinea-pig ileum. 5-hydroxytryptamine was applied to the preparation either by superfusion or by iontophoresis on to the somatic membrane of the impaled cells. 5-Hydroxytryptamine (50 nM–2 μM) depressed the amplitude of the cholinergic fast excitatory postsynaptic potential in every neurone to which it was applied ($n = 58$). 5-Hydroxytryptamine did not change the amplitude of depolarizations produced by iontophoretic application of acetylcholine which were of a similar time course to the excitatory postsynaptic potential. Lysergic acid diethylamide (1 μM) and methysergide (1–30 μM) mimicked and sometimes blocked the action of 5-hydroxytryptamine.

The results indicate that the inhibition of the peristaltic reflex by 5-hydroxytryptamine may be due to a blockade of transmission within the myenteric plexus brought about by a presynaptic action.

5-HYDROXYTRYPTAMINE (5-HT) has both inhibitory and excitatory effects on isolated preparations of the intestine. Inhibition of the peristaltic reflex is observed when 5-HT is applied to the serosal surface of the guinea-pig ileum (KOSTERLITZ & ROBINSON, 1957) and this was attributed by BÜLBRING & CREMA (1958) to a blockade of ganglionic transmission within the myenteric plexus (see review by KOSTERLITZ & LEES, 1964). Low doses of 5-HT also inhibit the nerve-mediated contractile response of the isolated guinea-pig ileum (GINTZLER & MUSACCHIO, 1974). On the other hand, 5-HT causes a contracture of the intestinal musculature, in part by a direct action on the muscle and in part by causing the release of acetylcholine from myenteric neurones (GADDUM & PICARELLI, 1957; BROWNLEE & JOHNSON, 1963; ADAM-VIZI & VIZI, 1978). A direct excitation of myenteric neurones by 5-HT has been reported in electrophysiological experiments (SATO, TAKAYANAGI & TAKAGI, 1974; DINGLEDINE & GOLDSTEIN, 1975), although inhibition of cell firing is sometimes observed. The present experiments were conducted to study further the mechanism underlying the inhibitory actions of 5-HT in the myenteric plexus. Preliminary reports of these findings have been published (HENDERSON & NORTH, 1975; NORTH & HENDERSON, 1975).

EXPERIMENTAL PROCEDURES

Myenteric ganglia adherent to the longitudinal muscle layer of ileum removed from adult guinea-pigs were superperfused *in vitro*. The perfusion solution was heated to 37°C and had the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2, glucose 11, gassed with 5% CO_2 and 95% O_2. The flow rate of the solution was 1–3 ml/min and the bath volume was 1–2 ml. When drugs were applied by perfusion, this solution was changed to one which differed only in its content of the drug(s). There was a delay of approximately 1 min between the time of this change and the first arrival at the tissue of a new solution; steady state equilibrium with new solutions occurred within 2–3 min.

Ganglia were observed with differential interference contrast microscopy. Individual somata could be discerned, and these were impaled with glass microelectrodes containing potassium chloride (3 M). Membrane resistance was calculated by passing known currents through the recording microelectrode by an active bridge circuit (WP Instruments, M701); full details of the intracellular recording techniques have been published previously (NISHI & NORTH, 1973*a*; NORTH & TONINI, 1977). A glass microelectrode filled with the perfusing solution was used for extracellular focal stimulation; cathodal current pulses (200–500 μs) were applied to the ganglion surface at distances of up to 100 μm from the soma of the impaled cell. In the present study, only those neurones are included which responded to such a stimulus (single pulse) with an excitatory postsynaptic potential (EPSP). Such cells were termed S cells by HIRST, HOLMAN & SPENCE (1974) and are apparently identical with the Type 1 cells described by

[1] Present address of R.A.N. and G.H. is indicated under the title.

[2] Present address: Department of Physiology, Kurume University Medical School, Kurume, Japan.

[3] Present address: Department of Pharmacology, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

Abbreviations: ACh, acetylcholine; EPSP, excitatory postsynaptic potential; 5-HT, 5-hydroxytryptamine; LSD, lysergic acid diethylamide.

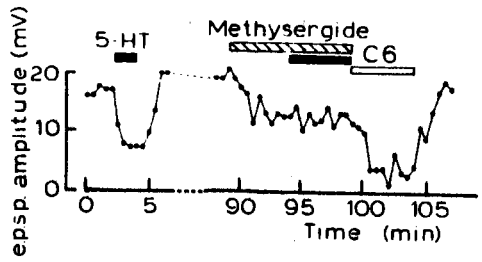

Fig. 1. Depression of excitatory postsynaptic potential amplitude by hydroxytryptamine, methysergide and hexamethonium. During the periods indicated by the filled, hatched and open bars, the perfusing solution contained 5-HT (250 nM), methysergide (1.7 μM) and hexamethonium (100 μM). 5-HT caused a rapid and reversible depression of the EPSP. Methysergide partially depressed the EPSP in the same cell, but completely prevented the action of 5-HT. Hexamethonium reversibly depressed the same EPSP.

Nishi & North (1973*a*). Except where stated, EPSPs were evoked at intervals greater than 30 s. The fast EPSP is reversibly blocked by hexamethonium (Nishi & North, 1973*a*) and (+)-tubocurarine (Hirst *et al.*, 1974), thus implying that it is mediated by acetylcholine.

Acetylcholine (ACh) was applied to cells by iontophoresis from electrodes which contained acetylcholine chloride (1 or 3 M). The tip of this electrode was placed close to the soma (within 5 μm) of the impaled cell and a short (10–100 ms; 10–100 nA) current pulse was passed. With a well-situated electrode a depolarization was produced which had a time course only slightly slower than that of the EPSP (e.g. Fig. 4A), although in many cells the ACh-evoked potential was of considerably longer time course than the EPSP. 5-HT was also applied by iontophoresis directly on to the impaled neurones. In these experiments the 5-HT electrode contained 5-HT creatinine sulphate (20 mM) in sodium acetate buffer (100 mM, pH 5.4). ACh or 5-HT were ejected by preset positive currents from constant current sources. Retaining currents of 3–5 nA were used. Drugs used were 5-HT creatinine sulphate and oxalate (Sigma), methysergide maleate (Sandoz), hexamethonium bromide (Sigma), lysergic acid diethylamide (LSD) (Sandoz) cyproheptadine hydrochloride (Merck, Sharp and Dohme) and phentolamine (Ciba). The concentrations given in this paper are the final bath concentration of the above salts.

RESULTS

The membrane properties of the myenteric neurones were closely similar to those previously described (Nishi & North, 1973*a*; Hirst *et al.*, 1974). The present results on the action of 5-HT are based on intracellular recordings from sixty-two neurones.

Effects of 5-hydroxytryptamine on synaptic potentials

When 5-HT was applied by perfusion, it depressed the amplitude of the EPSP in every one of the fifty-eight cells in which it was tested. This action was rapid in onset (Fig. 1) and reversed with equal rapidity when the 5-HT was washed from the tissue bath. The depression of the EPSP was readily reproducible on a given neurone (e.g. Fig. 2); that is to say, tachyphylaxis to this effect was not marked. The degree of depression of the EPSP was dependent on the concentration of 5-HT (10 nM–2 μM) and high concentrations often completely abolished the EPSP (Fig. 5). The depression of amplitude of the EPSP by 5-HT was independent of any effect which 5-HT had on the membrane of the impaled neurone. 5-HT itself has variable effects on the membranes of myenteric neurones depolarizing some, hyperpolarizing others and leaving many unaffected (Henderson & North, 1975; Wood & Mayer, 1979; Johnson, Katayama & North, 1980). In most of the cells examined in the present study (Type I cells) membrane potential and resistance were not affected by 5-HT in concentrations (up to 250 nM) which depressed the EPSP. Higher concentrations of 5-HT were more likely to either depolarize or hyperpolarize myenteric neurones; however, in these experiments the depression of the EPSP amplitude was not related to the direction or degree if any potential or resistance change of the soma membrane.

Iontophoretic application of 5-HT (30 nA for 30 s) also reversibly depressed the amplitude of the EPSP in four cells. In these cells, this effect was not associated with any direct effect of 5-HT on the membrane of the impaled cell. These experiments indicate that the site of action of 5-HT in depressing the EPSP is likely to be localized to the terminal regions or release sites near the soma of the impaled cell.

Antagonism of the inhibitory action of 5-hydroxytryptamine. The inhibitory action of 5-HT on the amplitude of the EPSP was prevented by prior exposure of the preparation to cyproheptadine (50 μM) in three cells but not affected in two other cells. Methysergide (1–30 μM) blocked the action of 5-HT in depressing the EPSP amplitude (four cells) but methysergide also depressed the EPSP itself (Fig. 1). Similar difficulties were associated with the use of LSD as a 5-HT antagonist. LSD alone (1 μM) caused a reversible depression of the EPSP similar to that caused by 5-HT; this seems likely to represent a presynaptic agonist action of LSD (see Discussion) because in two

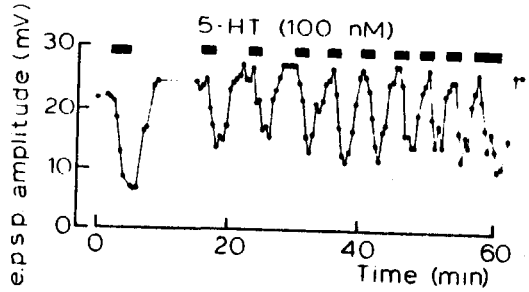

Fig. 2. Depression of the excitatory postsynaptic potential by 5-hydroxytryptamine. Repeated applications of 5-HT caused reproducible depression of the EPSP amplitude, even when repeated at intervals as short as 2 min. Tachyphylaxis was not marked.

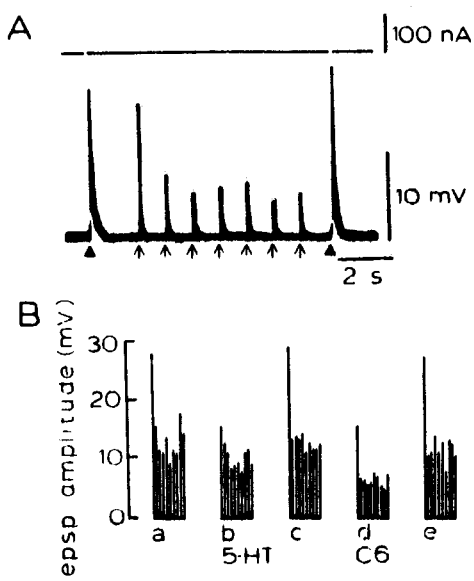

FIG. 3. Excitatory postsynaptic potentials progressively declined in amplitude when evoked at frequencies greater than 0.5 Hz. (A) At the times indicated by the arrows, EPSPs were evoked by focal stimulation of the ganglion surface. At the times indicated by the filled triangles, acetylcholine (50 nA, 50 ms) was applied to the cell soma by iontophoresis (iontophoretic current in upper trace). The EPSP amplitude declined during repetitive stimulation but there was no change in the sensitivity of the cell to iontophoretically applied acetylcholine. (B) In each panel (a–e) 10 EPSPs were evoked at a frequency of 1 Hz. a, control; b, 2 min after changing to a solution containing 5-HT (250 nM); c, 5 min after washing out the 5-HT; d, 2 min after changing to a solution containing hexamethonium (100 μM); e, 5 min after washing out the hexamethonium. Note that 5-HT preferentially depressed the first EPSP in the train, whereas hexamethonium caused an equivalent depression of all the EPSPs.

of these cells it was possible to show that hexamethonium (100 μM) also blocked the EPSP.

Effect of 5-hydroxytryptamine on the slow excitatory postsynaptic potential. It was observed that 5-HT (1 μM) also depressed the non-cholinergic slow EPSP which can be evoked in Type 1 cells by repetitive stimulation (up to 20 pulses at 10 Hz) of the ganglion surface or interconnecting strands. The time course of this effect of 5-HT was similar to that by which it depressed the amplitude of the fast EPSP. A similar depression of the slow EPSP by perfusion with 5-HT has been reported by WOOD & MAYER (1979).

Evidence for a presynaptic site of action of 5-hydroxytryptamine

The observation that 5-HT depressed the amplitude of the EPSP without changing membrane potential or resistance suggests a presynaptic site of action, especially in view of the evidence that the fast EPSP in myenteric neurones is generated mainly at the somatic membrane (NISHI & NORTH, 1973*a*). Two further experiments were performed to examine whether the depression of the EPSP was due solely to a presynaptic action of 5-HT. In the first, the effect of 5-HT was compared on the fast EPSP and on the ACh-evoked potential in the same and different neurones. 5-HT always depressed the amplitude of the EPSP but never reduced the amplitude of the ACh-evoked potential (Figs 4 and 5). Hexamethonium depressed the EPSP and ACh-evoked potential to a similar degree and with a similar time course.

In the second experiment, EPSPs were evoked by repetitive stimulation of presynaptic nerves at a frequency of 1 Hz. At this frequency, the EPSP amplitude progressively declined (see NISHI & NORTH, 1973*a*). This decline in amplitude was not associated with any change in the postsynaptic sensitivity to iontophoretically applied ACh (Fig. 3A) and must therefore represent a presynaptic reduction in the amount of ACh released with successive impulses. 5-HT reduced the amplitude of the first EPSP in a train without affecting the amplitude of second and subsequent EPSPs (Fig. 3B). By contrast, hexamethonium caused a proportionate reduction in amplitude of all EPSPs in a train (Fig. 3B). Such a selective effect of 5-HT on the first EPSP is most easily reconciled with a presynaptic site of action; a similar suggestion was made for the action of noradrenaline (NISHI & NORTH, 1973*a*; NORTH & NISHI, 1974).

DISCUSSION

Inhibitory action of 5-hydroxytryptamine on acetylcholine release

Our results present good evidence that 5-HT acts presynaptically to reduce the amount of ACh released at synapses between neurones within the myenteric plexus. Experiments to test the pharmacological

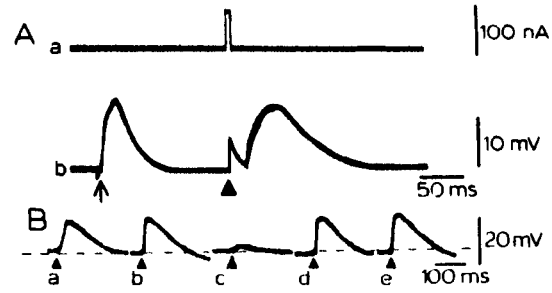

FIG. 4. (A) Comparison of excitatory postsynaptic potential and acetylcholine potential in the same neurone. Upper trace (a), iontophoretic current. Lower trace (b), membrane potential. At the arrow, a single pulse stimulus was applied to the ganglion surface to evoke an EPSP. At the filled triangle, acetylcholine was applied iontophoretically (70 nA, 10 ms) on to the soma membrane. (B) Depolarizations caused by acetylcholine iontophoresis in a different myenteric neurone. a, control; b, after 5 min perfusion with 5-HT (1 μM); c, after 2 min perfusion with hexamethonium (100 μM); d, after washing out the hexamethonium; e, after 5 min perfusion with noradrenaline (3 μM).

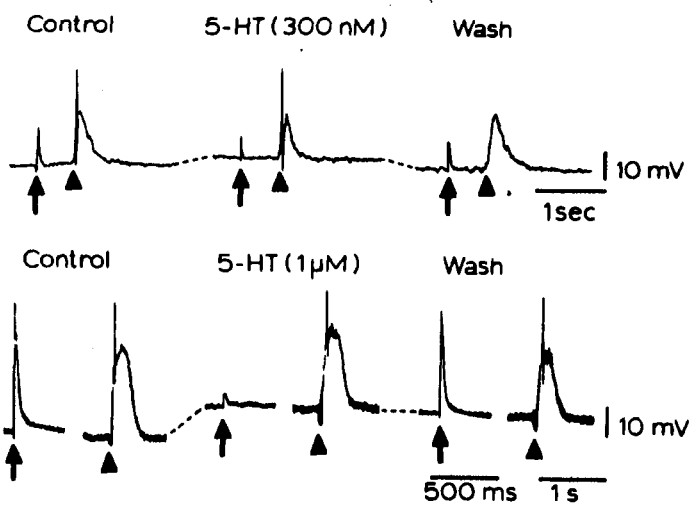

FIG. 5. Depression of the excitatory postsynaptic potential by 5-hydroxytryptamine without change in the acetylcholine potential in two different myenteric neurones. At the arrows, an EPSP was evoked by a single focal stimulus applied to the ganglion surface. At the filled triangles, acetylcholine was applied iontophoretically (100 nA, 50 ms) on to the soma membrane. Upper trace: left panel, control. Centre panel, after 6 min perfusion with 5-HT (300 μM). The EPSP amplitude is much depressed but the ACh potential is unchanged. There was also a slight membrane depolarization. Right panel, after 15 min wash out of 5-HT. Lower trace: left panel, control. Centre panel, after 5 min perfusion with 5-HT (1 μM). The EPSP was almost completely abolished whilst the ACh potential was unchanged. There was a 10 mV membrane depolarization in the presence of 5-HT. Right panel, after 10 min wash-out of 5-HT. The EPSP amplitude has fully recovered; the membrane potential change did not recover. Action potentials are attenuated by the pen-recorder.

specificity of this effect were inconclusive. Methysergide appeared to block this action of 5-HT, but itself caused a depression of EPSP amplitude. Methysergide also caused a depression of the amplitude of other much slower non-cholinergic synaptic potentials in the preparation (S. M. Johnson, Y. Katayama & R. A. North, unpublished observation). Furthermore, methysergide was equally effective in preventing the depression of the EPSP caused by noradrenaline (R. A. North & G. Henderson, unpublished observations). In any event, methysergide does not block the neuronal excitatory actions of 5-HT in the small intestine (Bülbring & Gershon, 1968; Drakontides & Gershon, 1968; Furness & Costa, 1979) and its use as a 5-HT antagonist in this preparation could profitably be abandoned. LSD had marked agonist actions, not dissimilar to those through which it depresses noradrenaline release by an action on prejuctional α-receptors (Hughes, 1973). Cyproheptadine was partially effective in only some experiments. In view of the finding that noradrenaline causes an equally strong presynaptic inhibition at cholinergic synapses within the myenteric plexus (Nishi & North, 1973*b*; Hirst & McKirdy, 1974), it might be considered that the present actions of 5-HT are mediated by α-adrenoceptors. It is noteworthy that 5-HT does not cause presynaptic inhibition in the superior cervical ganglion of the rabbit (Wallis & North, 1978) although noradrenaline does (Christ & Nishi, 1971).

The present results indicate that presynaptic inhibition at excitatory cholinergic synapses between neurones within the myenteric plexus is a likely explanation for the earlier observations that serosal application of 5-HT inhibits the peristaltic reflex (Bülbring & Crema, 1958). Such an inhibition of transmitter release may also occur in cholinergic terminals within the smooth muscle, as it does in the case of noradrenaline (Paton & Vizi, 1969; Kosterlitz, Lydon & Watt, 1970). This would explain the findings of Gintzler & Musacchio (1974) that low concentrations of 5-HT depress the nerve-mediated contractile response of the longitudinal muscle, in a fashion similar to the inhibition caused by noradrenaline and morphine.

Other actions of 5-hydroxytryptamine

On the other hand, it is well known that 5-HT causes the release of ACh from this preparation (Gaddum & Picarelli, 1957; Brownlee & Johnson, 1963; Adam-Vizi & Vizi, 1978). It does this by a tetrodotoxin-sensitive process (Adam-Vizi & Vizi, 1978); that is, by inducing action potentials in the neurones which release ACh at nerve-muscle junctions. Depolarization of some myenteric neurones to the threshold potential by 5-HT has been observed by Wood & Mayer (1979) and by the present investigators (unpublished observations). In this case, 5-HT must have at least two actions on the cholinergic neurones in the myenteric plexus, quite probably at

different sites. First, there is a depolarization which can reach threshold for action potential generation, and second, there is an inhibition of the amount of ACh released by each action potential. There is ample precedent for 5-HT having more than one action on a single neurone (GERSCHENFELD & PAUPARDIN-TRITSCH, 1974; COTTRELL, 1977).

Possible mechanisms of presynaptic inhibition

The mechanism which underlies the presynaptic inhibition observed in the present experiments is not known. It could be a depolarization of presynaptic terminals in accordance with classical ideas (TAKEUCHI & TAKEUCHI, 1962; ECCLES, SCHMIDT & WILLIS, 1963). Or it could be a membrane hyperpolarization and conductance increase (KAWAI & NIWA, 1977) as appears to operate for the action of morphine on myenteric neurones (NORTH & TONINI, 1977). Different populations of myenteric neurones are both depolarized and hyperpolarized by 5-HT (NORTH & HENDERSON, 1975; WOOD & MAYER, 1979; JOHNSON, KATAYAMA & NORTH, 1980). One should also bear in mind the possibility that 5-HT reduces the amplitude of the EPSP by reducing the number of presynaptic fibres excited by the focal stimulating electrode, perhaps by hyperpolarization of the nerve fibres.

Possible functional implications

There is good evidence from the work of Gershon and his colleagues that the myenteric plexus contains intrinsic neurones which can take up, synthesize, store and release 5-HT (see review by FURNESS & COSTA, 1979). It is possible that 5-HT released under physiological circumstances from myenteric neurones may act through axoaxonal synapses or simply by diffusion into the vicinity of nerve terminals to inhibit cholinergic transmission within the plexus and thereby (see above) inhibit the peristaltic reflex. Such a functional presynaptic inhibitory effect occurs with noradrenaline when the extrinsic sympathetic nerves are stimulated (HIRST & MCKIRDY, 1974); it may be difficult to show a similar functional role for 5-HT because the neurones which may contain it are intrinsic to the myenteric plexus (see, *inter alia*, GERSHON, DREYFUSS, PICKEL, JOH & REIS, 1977) and would be difficult to stimulate in isolation.

5-HT is contained within distinct neuronal groups in the mammalian central nervous system. When applied by iontophoresis to single neurones it may cause excitation or inhibition of neuronal firing (BLOOM, HOFFER, SIGGINS, BARKER & NICOLL, 1972). These effects are generally of a rather long time course and it is not at all clear whether they represent direct postsynaptic actions on the neurone whose activity is recorded, or presynaptic action to reduce ongoing synaptic inputs. In the rat locus coeruleus, SEGAL (1979) adduced evidence for a presynaptic action of 5-HT because it reduced the response of neurones to noxious inputs more than it depressed their spontaneous firing rates. A more convincing case for a presynaptic action was provided by HEADLEY, DUGGAN & GRIERSMITH (1978) who found that 5-HT reduced the response of cat dorsal horn cells to noxious inputs whilst having little effect on the responses of the same cells to non-noxious inputs, an effect which may be functionally related to descending raphe–spinal pathways (*inter alia* DUGGAN & GRIERSMITH, 1979). The present results provide direct electrophysiological evidence that 5-HT does cause presynaptic inhibition in the enteric nervous system, a tissue which actually has several close similarities to the central nervous system (see GERSHON & BURSZTAJN, 1978).

Acknowledgements—This work was supported by USPHS Grants DA01730, NS06672 and the Schweppe Foundation (to R.A.N.) and by USPHS Grant DA00662 (to Professor H. W. KOSTERLITZ).

REFERENCES

ADAM-VIZI V. & VIZI E. S. (1978) Direct evidence of acetylcholine releasing effect of serotonin in the Auerbach plexus. *J. Neural Transm.* **2**, 127–138.

BLOOM F. E., HOFFER B. J., SIGGINS G. R., BARKER J. L. & NICOLL R. A. (1972) Effects of serotonin on central neurons: microiontophoretic administration. *Fedn Proc Fedn Am. Socs exp. Biol.* **31**, 97–106.

BROWNLEE G. & JOHNSON E. S. (1963) The site of 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. *Br. J. Pharmac.* **21**, 306–322.

BÜLBRING E. & CREMA A. (1958) Observations concerning the action of 5-hydroxytryptamine on the peristaltic reflex. *Br. J. Pharmac.* **13**, 444–457.

BÜLBRING E. & GERSHON M. D. (1968) 5-hydroxytryptamine participation in the vagal inhibitory innervation of the stomach. *J. Physiol., Lond.* **192**, 832–846.

CHRIST D. D. & NISHI S. (1971) Site of adrenaline blockade in the superior cervical ganglion of the rabbit. *J. Physiol., Lond.* **213**, 107–117.

COTTRELL G. A. (1977) Identified amine-containing neurones and their synaptic connexions. *Neuroscience* **2**, 1–18.

DINGLEDINE R. & GOLDSTEIN A. (1975) Single neuron studies of opiate action in the guinea-pig myenteric plexus. *Life Sci.* **17**, 59–62.

DRAKONTIDES A. B. & GERSHON M. D. (1968) 5-hydroxytryptamine receptors in the mouse duodenum. *Br. J. Pharmac. Chemother.* **33**, 480–492.

Duggan A. W. & Griersmith B. T. (1979) Inhibition of spinal transmission of nociceptive information by supraspinal stimulation in the cat. *Pain* **6**, 149–161.

Eccles J. C., Schmidt R. F. & Willis W. D. (1963) The mode of operation of the synaptic mechanism producing presynaptic inhibition. *J. Neurophysiol.* **26**, 523–528.

Furness J. B. & Costa M. (1979) On the possibility that an indoleamine is a neurotransmitter in the gastrointestinal tract. *Biochem. Pharmac.* **28**, 565–572.

Gaddum J. H. & Picarelli Z. P. (1957) Two kinds of tryptamine receptor. *Br. J. Pharmac.* **9**, 240–248.

Gerschenfeld H. M. & Paupardin-Tritsch D. (1974) Ionic mechanisms and receptor properties underlying the responses of molluscan neurones to 5-hydroxytryptamine. *J. Physiol., Lond.* **243**, 427–454.

Gershon M. D. & Bursztajn S. (1978) Properties of the enteric nervous system: limitation of access of intravascular macromolecules to the myenteric plexus and muscularis externa. *J. comp. Neurol.* **180**, 467–488.

Gershon M. D., Dreyfuss C. F., Pickel V. M., Joh T. H. & Reis D. J. (1977) Serotonergic neurons in the peripheral nervous system: identification in gut by immunohistochemical localization of tryptophan hydroxylase. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3086–3089.

Gintzler A. R. & Musacchio J. M. (1974) Interaction between serotonin and morphine in the guinea-pig ileum. *J. Pharmac. exp. Therap.* **189**, 484–492.

Headley P. M., Duggan A. W. & Griersmith B. T. (1978) Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. *Brain Res.* **145**, 185–189.

Henderson G. & North R. A. (1975) Presynaptic action of 5-hydroxytryptamine in the myenteric plexus of the guinea-pig ileum. *Br. J. Pharmac.* **54**, 265P.

Hirst G. D. S., Holman M. E. & Spence I. (1974) Two types of neurone in the myenteric plexus of the guinea-pig duodenum. *J. Physiol., Lond.* **236**, 303–326.

Hirst G. D. S. & McKirdy H. C. (1974) Presynaptic inhibition at a mammalian peripheral synapse. *Nature, Lond.* **250**, 430–431.

Hughes J. (1973) Inhibition of noradrenaline release by lysergic acid diethylamide. *Br. J. Pharmac.* **49**, 706–708.

Johnson S. M., Katayama Y. & North R. A. (1980) Multiple actions of 5-hydroxytryptamine on myenteric neurones of the guinea-pig ileum. *J. Physiol., Lond.* (in press).

Kawai N. & Niwa A. (1977) Hyperpolarization of the excitatory nerve terminals by inhibitory nerve stimulation in lobster. *Brain Res.* **137**, 365–368.

Kosterlitz H. W. & Lees G. M. (1964) Pharmacological analysis of intrinsic intestinal reflexes. *Pharmac. Rev.* **16**, 301–339.

Kosterlitz H. W., Lydon R. J. & Watt A. J. (1970) The effects of adrenaline, noradrenaline and isprenaline on inhibitory α- and β-receptors in the longitudinal muscle of the guinea-pig ileum. *Br. J. Pharmac.* **39**, 398–413.

Kosterlitz H. W. & Robinson J. A. (1957) Inhibition of the peristaltic reflex of the isolated guinea-pig ileum. *J. Physiol., Lond.* **136**, 249–262.

Nishi S. & North R. A. (1973*a*) Intracellular recording from the myenteric plexus of the guinea-pig ileum. *J. Physiol., Lond.* **231**, 471–491.

Nishi S. & North R. A. (1973*b*) Presynaptic action of noradrenaline in the myenteric plexus. *J. Physiol., Lond.* **231**, 29–30P.

North R. A. & Henderson G. (1975) Action of morphine on guinea-pig myenteric plexus and mouse vas deferens studied by intracellular recording. *Life Sci.* **17**, 63–66.

North R. A. & Nishi S. (1974) Properties of ganglion cells in the myenteric plexus of the guinea-pig ileum determined by intracellular recording. *Proceedings of the IV International Symposium Gastrointestinal Motility*, pp. 667–676. Mitchell, Vancouver.

North R. A. & Tonini M. (1977) The mechanism of action of narcotic analgesics in the guinea-pig ileum. *Br. J. Pharmac.* **61**, 541–549.

Paton W. D. M. & Vizi E. S. (1969) The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea-pig ileum longitudinal muscle strips. *Br. J. Pharmac.* **25**, 10–28.

Sato T., Takayanagi I. & Takagi K. (1974) Effects of acetylcholine releasing drugs on electrical activities obtained from Auerbach's plexus in the guinea-pig ileum. *Jap. J. Pharmac.* **24**, 447–451.

Segal M. (1979) Serotonergic innervation of the locus coeruleus from the dorsal raphe and its action on responses to noxious stimuli. *J. Physiol., Lond.* **286**, 401–416.

Takeuchi A. & Takeuchi N. (1962) Electrical changes in pre- and post-synaptic axons of the grant synapse of *Loligo*. *J. gen. Physiol.* **45**, 1181–1193.

Wallis D. I. & North R. A. (1978) Intracellular recording of responses of rabbit superior cervical ganglion cells to 5-hydroxytryptamine applied by iontophoresis. *Neuropharmacology* **17**, 1023–1028.

Wood J. D. & Mayer C. J. (1979) Serotonergic activation of tonic-type enteric neurons in guinea-pig small bowel. *J. Neurophysiol.* **42**, 582–592.

(*Accepted* 14 *October* 1979)