Br. J. Pharmac. Chemother. (1967), **30**, 229–239.

RELEASE OF 5-HYDROXYTRYPTAMINE FROM ISOLATED DOG INTESTINE BY NICOTINE

BY

T. F. BURKS* AND J. P. LONG

From the Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa, U.S.A.

(*Received September* 9, 1966)

It has been shown by Burks & Long (1966a, 1966b, 1967) that several agents, including acetylcholine (ACh), barium chloride, angiotensin, catecholamines and morphine, are capable of enhancing the release of 5-hydroxytryptamine (5-HT) from perfused dog intestine. Schievelbein, Werle & Eckert (1963) reported that oral or parenteral administration of nicotine in dogs resulted in release of body 5-HT, especially from the chromaffin cells of the intestinal mucosa, shown by removing the gastrointestinal tract before injection. Schievelbein (1965) later showed that nicotine can release 5-HT from rabbit thrombocytes. Hansson, Masuoka & Clark (1963) reported that nicotine administered to guinea-pigs produced a decrease in intestinal 5-HT without altering brain, heart or spleen 5-HT levels, but this decrease was later reported to be non-significant (Hansson, Masuoka & Clark, 1964).

Since we had evidence that parasympathetic stimulation could release 5-HT from the isolated dog intestine (Burks & Long, 1966a) and since nicotine has been reported to release intestinal 5-HT *in vivo* (Schievelbein *et al.*, 1963) it was decided to attempt stimulation of 5-HT release from the isolated dog intestine by administration of nicotine.

METHODS

Isolated dog intestinal segments

Mongrel dogs of either sex, weighing 8–12 kg, were anaesthetized with barbitone sodium (250 mg/kg, intravenously) and thiopentone sodium (15 mg/kg, intravenously). Juxta intestinal mesenteric arteries were cannulated and perfused with warmed Krebs bicarbonate solution gassed by bubbling 95% O_2–5% CO_2 in the manner previously described (Burks & Long, 1966a). Perfusion pressure was provided by a Sigmamotor model T-8 constant flow peristaltic infusion pump and was maintained at 80–100 mm Hg. The small vein draining the perfused intestinal segment was cannulated and the segment surgically removed. For recording changes in pressure, a small balloon was tied into the lumen of the intestinal sections and connected to a Statham pressure transducer (P23AA) linked with Offner type RS Dynograph. The clear, bloodless effluent from the venous cannula was pumped through a flow-through cuvette placed in an Aminco-Bowman spectrophotofluorometer for continuous estimation of 5-HT concentration in the effluent and was recorded using an Offner Dynograph. The excitation monochromator of the spectrophotofluorometer was set at 295 mμ

* Present address: Department of Pharmacology, School of Medicine, The University of New Mexico, Albuquerque, New Mexico, U.S.A.

and the fluorescence monochromator set at 330 mμ, the wavelengths at which the native fluorescence of 5-HT is maximal at neutral or slightly alkaline pH. The specificity of this method of 5-HT estimation has been established (Burks & Long, 1966a, 1966b, 1967). The technique is illustrated in Fig. 1.

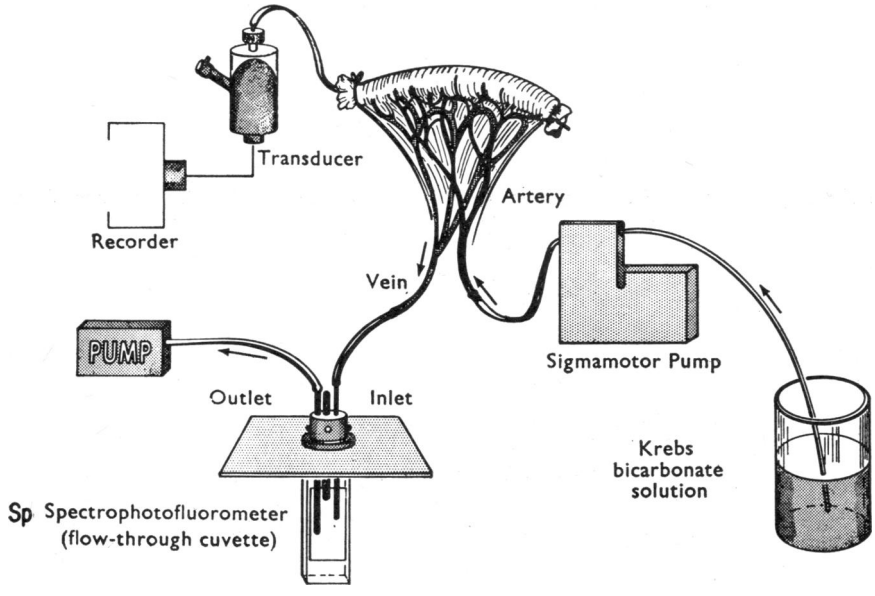

Fig. 1. Drawing of isolated dog small intestine preparation. The warmed, gassed Krebs solution is perfused through the intestinal vasculature and emerges from the venous cannula. This clear venous effluent flows into the quartz cuvette, around a quartz baffle dividing the cuvette, and is pumped out *via* the outlet tube. Intraluminal pressure of the gut segment is measured from a balloon placed in the intestinal lumen.

Drugs, in volumes of 0.01–0.1 ml. were injected into the arterial cannula and were also tested in the flow-through cuvette to ascertain their lack of fluorescence. Intraluminal pressure of the segment provided a measure of intestinal motility. Any autonomic ganglia present in this isolated preparation are assumed to be parasympathetic.

Calcium deficient perfusions

The Ca^{++}-free solutions were similar to the control Krebs bicarbonate solution except that Na^{+} was substituted for Ca^{++}. No effort was made to eliminate trace amounts of Ca^{++} occurring as a contaminant of other chemicals. The Ca^{++} concentration in the control Krebs solution was 2.5 mM. In these experiments the isolated dog small intestinal segment was perfused for 10–20 min with the Ca^{++}-free solution, drugs were tested by intra-arterial administration and responses on intestinal tone and 5-HT content of the venous effluent recorded, then perfusion was continued with control Krebs solution containing Ca^{++}. After 5–20 min of perfusion with the Krebs solution, the drugs were again tested and the responses noted.

In situ *intestinal segments*

These sections were prepared in a manner similar to the above except that parasympathetic vagal tracts to the gut section were not interrupted. Mesenteric arteries were cannulated and perfused with Krebs solution at perfusion pressure approximating each animal's arterial blood pressure. Two small balloons were introduced into the intestinal lumen through a small incision in the

intestine distal to the perfused segment. One balloon was situated in the perfused section of intestine and the other was placed 15–30 cm proximal to this section. Both vagi were sectioned in the neck and a bipolar electrode was placed on the dorsal vagus nerve in the thoracic cavity at the level of the diaphragm. Intraluminal pressure from the balloons was measured by Statham pressure transducers (P23BB) and recorded on an Offner Dynograph. The vagus nerve was stimulated with a Grass Instrument Co. model S4 stimulator at a frequency of 5–10 c/s, a pulse duration of 25 msec at 10 V for 30 sec. Several control responses were obtained and then hexamethonium (C6) was administered intra-arterially to the perfused section and the stimulation repeated.

Drugs

Nicotine tartrate, hexamethonium bromide (C6), atropine sulphate, dimethylphenylpiperazinium iodide (DMPP), noradrenaline hydrochloride, morphine sulphate, acetylcholine bromide and 5-hydroxytryptamine (5-HT) creatinine sulphate. All agents were dissolved in distilled water; doses were calculated as salts unless otherwise stated.

Statistical analyses were performed by use of the Student's t test, paired comparisons (Goldstein, 1964). Values of P equal to or less than 0.05 were considered to be significant.

RESULTS

Isolated intestinal segments

Nicotine (5 μg) and DMPP (2 μg) administered intra-arterially significantly enhanced release of 5-HT into the venous effluent of the perfused intestinal segments and also stimulated the intestinal smooth muscle (Table I). Figure 2 shows a typical record of the 5-HT release by nicotine. In experiments with preparations from eight dogs, nicotine (10 μg) increased the content of 5-HT in the perfusate from 4.8 ± 1.8 to 9.0 ± 1.7 μg/ml./g and increased the intraluminal pressure of the intestinal sections 50 ± 6.1 cm H_2O. It was presumed that the ganglionic stimulants exerted their effects on 5-HT release and gut motility *via* the liberation of ACh at the post-ganglionic parasympathetic nerve terminals; therefore, the ability of atropine to antagonize the effects of equipotent doses of nicotine and ACh was tested. Acetylcholine (0.4 μg) and nicotine (5 μg) produced similar intestinal spasms; after atropine (5 μg, intra-arterially) the smooth muscle stimulating and 5-HT releasing properties of nicotine and ACh were antagonized (Table 1).

TABLE 1

EFFECTS OF NICOTINE, ACETYLCHOLINE (ACh) AND DIMETHYLPHENYL PIPERAZINIUM (DMPP) ON RELEASE OF 5-HYDROXYTRYPTAMINE (5-HT) AND MUSCLE TONE IN ISOLATED DOG INTESTINAL SEGMENTS AND INFLUENCE OF ATROPINE ON RESPONSES TO NICOTINE AND ACh

Values are the means and standard errors of the maximum responses during each experiment. * Significantly decreased from control response at $P<0{\cdot}01$

Treatment	Expts. (no.)	Increase in release of 5-HT into venous effluent (ng/ml./g)	Increase in intraluminal pressure (cmH_2O)
5 μg nicotine	12	$4{\cdot}6 \pm 0{\cdot}3$	$66 \pm 15{\cdot}9$
5 μg nicotine + 5 μg atropine	10	$0{\cdot}6 \pm 0{\cdot}3$*	$7 \pm 2{\cdot}6$*
0·4 μg ACh	13	$3{\cdot}4 \pm 1{\cdot}0$	$65 \pm 12{\cdot}4$
0·4 μg ACh + 5 μg atropine	11	$0{\cdot}3 \pm 0{\cdot}1$*	$12 \pm 8{\cdot}1$*
2 μg DMPP	8	$3{\cdot}2 \pm 0{\cdot}7$	$47 \pm 7{\cdot}9$

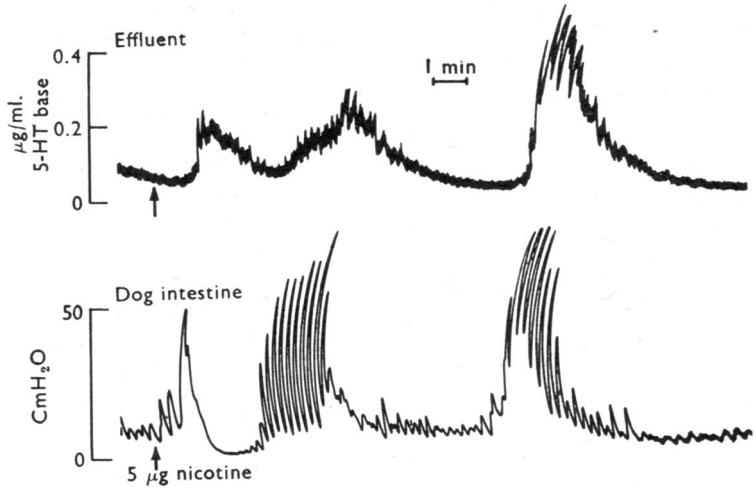

Fig. 2. A typical response to nicotine by the isolated intestinal segment. The upper tracing represents the 5-HT content of the venous effluent, the lower tracing is intraluminal pressure of the gut section. Multiple responses similar to this occurred in 40–50% of the sections administered nicotine.

Calcium-free perfusion

Perfusion of isolated intestinal segments with Ca^{++}-free solution completely eliminated spasmogenic responses to nicotine, and the responses of the intestinal muscle to 5-HT and morphine were greatly reduced (Table 2). The diminution in response to ACh, although statistically significant, was not as great as seen with the other agonists. Figure 3 shows that reactivity of the intestinal smooth muscle and enhanced release of 5-HT were generally restored after 10 min perfusion with Krebs solution containing Ca^{++}. In most cases there was reduced release of 5-HT during the Ca^{++}-free perfusion period. The ability of noradrenaline to enhance release of 5-HT (Fig. 3) has been previously reported (Burks & Long, 1966b).

TABLE 2

EFFECTS OF CALCIUM-FREE PERFUSION OF ISOLATED DOG INTESTINAL SEGMENTS ON RESPONSES OF INTESTINAL MUSCLE TO NICOTINE, 5-HYDROXYTRYPTAMINE (5-HT), MORPHINE AND ACETYLCHOLINE (ACh)

Values are the means and standard errors of the maximum responses during each perfusion period of each experiment. * Significant at $P<0{\cdot}01$

Stimulus	Expts. (no.)	Intraluminal pressure of isolated intestinal sections (cm H_2O) During Ca^{++}-free perfusion	Intraluminal pressure of isolated intestinal sections (cm H_2O) During perfusion with Krebs solution
Nicotine, 20μg	10	0±0	59±10·5*
5-HT, 20 μg	19	4±2·1	68± 5·3*
ACh, 2 μg	18	45±6·8	72± 5·7*
Morphine, 50 μg	7	1±0·7	46± 7·9*

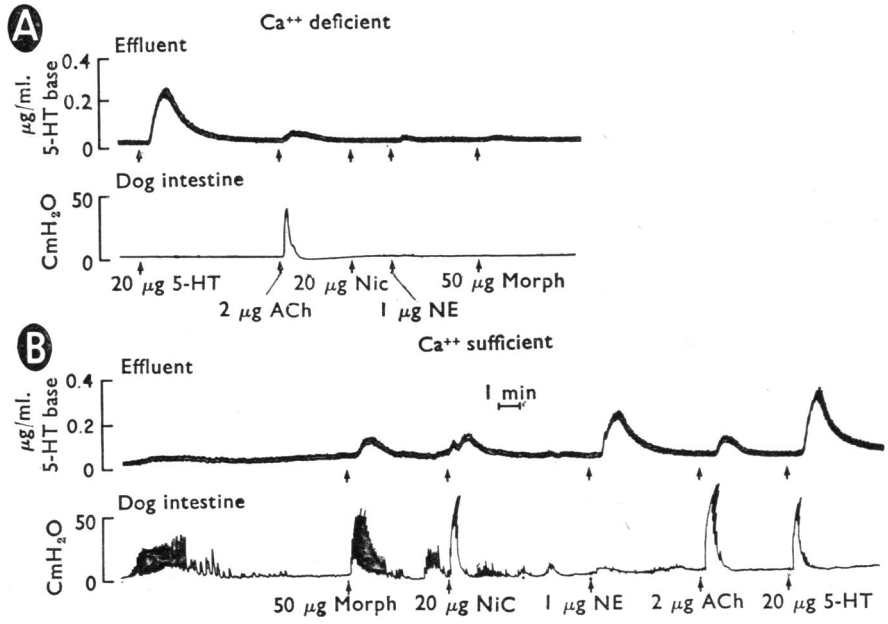

Fig. 3. Influence of calcium-deficient perfusion on intestinal reactivity to 5-hydroxytryptamine (5-HT), acetylcholine (ACh), nicotine (Nic), noradrenaline (NE) and morphine (Morph). A. Perfusion of tissue with Ca++-free solution. The upper tracing is 5-HT content of the venous effluent, lower tracing is intraluminal pressure of the gut segment. Note that the injected 5-HT appears in the venous effluent and that muscular responses are observed only with ACh. B is a continuation of A, the perfusion solution was Krebs with Ca++. Upper tracing is 5-HT content of venous effluent, lower tracing is intraluminal pressure. Note that NE released 5-HT without producing smooth muscle contraction.

Effects of C6 and high doses of nicotine

Since atropine could have masked any direct (non-ganglionic) effects of nicotine and DMPP on 5-HT release, the actions of C6, a selective and potent ganglionic blocking agent (Quilliam & Shand, 1964), were investigated. After recording the effects of nicotine (10 μg) and DMPP (2 μg), hexamethonium (100 μg) was given and the nicotine and DMPP were repeated. A large dose of nicotine (500 μg) was then given and test doses of nicotine and DMPP were repeated. The rather unexpected results are shown in Table 3 and Fig. 4. Neither the contractor response of the intestine to DMPP nor that

TABLE 3

INFLUENCE OF HEXAMETHONIUM (C6) AND HIGH DOSE OF NICOTINE ON SPASMOGENIC RESPONSES OF ISOLATED DOG INTESTINAL SEGMENTS TO LOW DOSE OF NICOTINE AND DIMETHYLPHENYLPIPERAZINIUM (DMPP)

Values are the means and standard errors of the maximum responses during each experiment. * Significant at $P<0.01$

Stimulus	Expts. (no.)	Increase in intraluminal pressure (cm H_2O)		
		No blocking agent present	After 100 μg C6	After 500 μg nicotine
Nicotine, 10 μg	12	47±4·9	54±4·3	1±0·5*
DMPP, 2 μg	12	60±4·3	59±6·4	7±2·2*

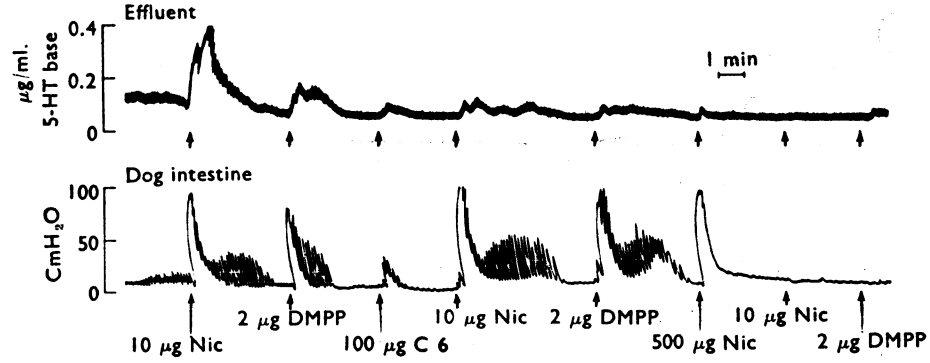

Fig. 4. Influence of hexamethonium (C6) on response of isolated intestinal segment to nicotine (Nic) and dimethylphenylpiperazinium (DMPP). Upper tracing is 5-HT content of venous effluent; lower tracing is intraluminal pressure of intestinal section. Administration of C6 did not diminish responses to Nic and DMPP, but the high dose of Nic blocked both. The progressive diminution of 5-HT release was often observed and is probably a result of tissue depletion of 5-HT stores.

to nicotine was reduced by C6. After the large dose of nicotine (500 μg), however, the responses to DMPP (2 μg) and nicotine (10 μg) were significantly reduced. Higher doses of C6 (up to 1–2 mg, intra-arterially) also failed to block the intestinal contractor responses to nicotine and DMPP. The apparent decrease in the ability of nicotine and DMPP to enhance release of 5-HT from the intestinal sections after C6 (Fig. 4) was probably not due to any effect of the C6, but was more likely due to gradual loss or depletion of 5-HT from its intestinal stores during the experiment. If several injections of nicotine, for example, were given to an intestinal section, there occurred progressively smaller amounts of 5-HT released by each stimulus. Similar observations have been reported for release of 5-HT by ACh (Burks & Long, 1966a) and by catecholamines (Burks & Long, 1966b).

In situ *intestinal sections*

The ability of C6 (100 μg, intra-arterially) to antagonize intestinal responses to vagus nerve stimulation *in situ* was tested. Several control responses to vagal stimulation were obtained in the region of the intestine being perfused with Krebs solution and also in a site several cm proximal to this section. Hexamethonium (100 μg, intra-arterially) was then given to the perfused section. After any response to the injected C6 subsided, the

TABLE 4

EFFECT OF 100 μg HEXAMETHONIUM (C6) ON LOCAL RESPONSES OF *IN SITU* DOG INTESTINAL SEGMENTS TO THORACIC VAGUS STIMULATION

Values are the means and standard errors of the maximum responses occurring within 5 min of nerve stimulation. * Difference significant at $P<0.05$

		Increase in intraluminal pressure (cm H_2O)		
Site	Expts. (no.)	Before C6	After C6	Difference
Perfused section	4	32±8	10± 2	22±7*
Proximal section	4	24±9	28±12	4±4

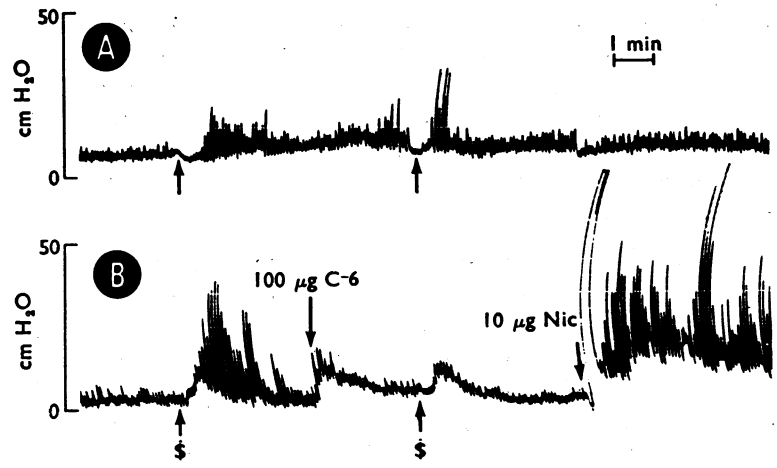

Fig. 5. Influence of hexamethonium (C6) on response of *in situ* intestinal sections to vagus nerve stimulation (S). The upper tracing (A) is intraluminal pressure of an intestinal site some 20 cm proximal to the perfused section, the intraluminal pressure of which is shown in the lower tracing. Only the section shown in the lower tracing (B) received 100 μg C6 and only this section exhibited antagonism to vagus stimulation. Note that the section which received C6 is still reactive to nicotine.

vagus nerve was again stimulated. The results are shown in Table 4 and Fig. 5. The intestinal response to stimulation of the vagus was antagonized in all the four dogs tested, but there was no effect of C6 on the response in the untreated site proximal to the perfused section. In one instance (Fig. 5), nicotine (10 μg, intra-arterially) was given to the perfused section after C6 and still produced intestinal contraction.

DISCUSSION

In these experiments nicotine and DMPP, both powerful stimulants of autonomic ganglia, produced enhanced release of 5-HT into the venous effluent of isolated perfused dog intestinal sections. These agents also induced strong contractions of the intestinal smooth muscle; this was often a prolonged response, especially observed with nicotine. Since nicotine and DMPP are generally thought to exert their major pharmacological effects on ganglia, this effect would be expected to be on the parasympathetic ganglia present in this isolated preparation and the final response would be expected to be mediated by ACh. The experiments with nicotine stimulation and atropine blockade of nicotine stimulation of intestinal muscle and 5-HT release were quite consistent with those expectations, especially since it has already been demonstrated that ACh can release 5-HT from isolated intestinal segments and that this response is blocked by atropine (Burks & Long, 1966a). The effects of perfusion of the intestinal sections with calcium-free solutions were also consistent with the idea that the response to nicotine was one of simple ganglionic stimulation. The ganglia may be especially sensitive to Ca^{++} deprivation and this would explain the absolute loss of response to nicotine during the Ca^{++}-free perfusion but only partial loss of reactivity to ACh. Morphine and 5-HT were also only poorly active during the infusion of Ca^{++}-free solution, suggesting that at least part of their effects on intestinal muscle are mediated by autonomic ganglia.

The failure of C6 to block the actions of nicotine and DMPP suggests that the major action of nicotine is *not* on the parasympathetic ganglia of the intestine, especially if the view of the actions of nicotine and C6 on autonomic ganglia offered by Pelikan (1960) is correct. He suggests that nicotine affects the release of ACh from the presynaptic nerve terminals whilst C6 competes with ACh for the postsynaptic receptor sites in the ganglion. On this hypothesis, there is no ready explanation for the failure of C6 to antagonize nicotine stimulation of the isolated dog intestine.

It is possible that nicotine and DMPP could act in this preparation by stimulation of extraganglionic nervous structures or by direct action on the cholinergic receptors. Other workers have reported evidence that nicotine may directly affect the post-ganglionic nervous structures of various organs. Schaeppi & Dodd (1965) claim that in the pig iris sphincter, nicotine induces discharge of cholinergic transmitter by an excitatory action of post-ganglionic nerve fibres; these investigators used quite large doses of drugs. Wright & Shepherd (1965) reported that nicotine and DMPP contracted the non-ganglionic human colonic muscle from a case of Hirschsprung's disease and suggested that nicotine can stimulate post-ganglionic parasympathetic nerve fibres. Khan, Mantegazza & Piccinini (1965) also suggested that nicotine can affect guinea-pig atria at extra-ganglionic sites, but these actions could be abolished by C6. Evans & Schild (1953) found that the plexus free circular muscle of cat jejunum would still react to nicotine. Other workers have reported that C6 fails to block some actions of DMPP in organs which contain ganglia (Chiang & Leaders, 1965). Still others report block of nicotine by C6 (Fishlock & Parks, 1966).

The failure of C6 in our experiments to antagonize nicotine was not due to incomplete ganglionic block, since higher doses of C6 failed to block nicotine, but the effects of vagus nerve stimulation in the *in situ* experiments were antagonized. Nicotine often exhibited a multiple response on the isolated segment and the possibility of some mode of action in addition to stimulation of cholinergic ganglia could exist. Stimulation of post-ganglionic cholinergic fibres and 5-hydroxytryptaminergic fibres could possibly explain these actions of nicotine.

Perhaps a more likely reason for the failure of C6 to block the responses of the isolated intestine to nicotine and DMPP would be the fact that the latter two agents possess the ability to stimulate a diverse population of slightly differing cholinergic ganglia. Under the perfusion conditions and in the particular tissue studied, there may be a significant fraction of these ganglia which for some reason are exceptionally resistant to C6 blockade. Similar observations involving C6, atropine and inhibitors of cholinesterase, have been reported for the stellate ganglion (Long & Eckstein, 1961). A less plausible explanation could be the possible direct combination of nicotine and DMPP with the cholinergic receptors of the intestinal smooth muscle. Such a direct effect would be antagonized by atropine but not by C6. The ability of high doses of nicotine to prevent responses to nicotine and DMPP, however, probably results from depolarization of the ganglia.

Some of the stimulating effects of nicotine on the dog small intestine may be mediated by 5-HT. Ellis & Rasmussen (1951) studied the atropine-resistant nicotine stimulation of the muscularis mucosae of the dog intestine and concluded that the stimulatory action of nicotine was due to some factor other than ACh, noradrenaline or histamine; they

suggested that the response could be due to darmstoff or some similar substance. The fact that combinations of atropine and dihydroergotamine blocked the action of nicotine is noteworthy since Gaddum & Hameed (1954) have found that dihydroergotamine is, in some instances, a fairly potent antagonist of 5-HT. Burks & Long (1966b) found that noradrenaline releases 5-HT from the perfused isolated dog intestine and it may be postulated that the ability of the catecholamines to produce contractions of the muscularis mucosae of the dog small intestine is related to the release of 5-HT by these agents. The 5-HT liberated by the catecholamines and by ACh (Burks & Long, 1966a) is presumed to arise from the 5-HT storage sites in the intestinal mucosa ; since the 5-HT appears in the venous effluent, it must necessarily come into intimate contact with the muscularis mucosa after its liberation and would, therefore, be expected to exert some effect on this muscle layer.

Since in the present study the stimulating effects of both nicotine and 5-HT on the intestinal smooth muscle were almost completely abolished during perfusion of the isolated intestinal sections with Ca^{++}-free solution, while the response to ACh was less affected (although significantly reduced), it would be attractive to propose that at least part of the intestinal contractor response to nicotine is mediated by 5-HT. The effect of the 5-HT released by nicotine might be exerted primarily on the muscularis mucosae. If this is indeed the case, there are at least five means by which nicotine could release 5-HT and thereby produce some " indirect " stimulation of muscularis mucosae or other muscle. (1) The 5-HT release could result simply from the mechanical deformation of the tissues following nicotine stimulation. (2) Nicotine could produce stimulation of " 5-hydroxytryptaminergic " neurones which might exist in the intestinal wall. (3) Nicotine could directly release 5-HT, perhaps by displacement of 5-HT at its storage granules. (4) Nicotine could stimulate adrenergic neurones and produce liberation of noradrenaline, which in turn could release 5-HT. (5) Nicotine could stimulate cholinergic neurones, either by stimulating the parasympathetic ganglia in the intramural plexuses or by combining with post-ganglionic cholinergic elements, and the ACh so liberated could release 5-HT. The last possibility would appear to be the most likely since atropine effectively reduced both the stimulating effect of nicotine and its ability to enhance release of 5-HT (Table 1). Other possibilities of mechanism of release of 5-HT by nicotine also exist, some more complex than the simple schemes mentioned here. The parallel effects of the Ca^{++}-free perfusion on responses of the intestinal segments to nicotine and 5-HT could also simply indicate a general failure of " stimulus-secretion " in the absence of Ca^{++}, especially since noradrenaline also failed to enhance release of 5-HT in the absence of Ca^{++} (Fig. 3). The requirement for Ca^{++} in secretion of catecholamines from the adrenal medulla evoked by histamine and 5-HT has been demonstrated by Poisner & Douglas (1966) ; the failure of 5-HT, nicotine, noradrenaline and morphine to stimulate the intestine during the Ca^{++}-free perfusions may have been due to failure of these agents to provoke secretion of 5-HT, ACh or other substances.

It is unlikely that nicotine and 5-HT share common receptor sites in the dog intestine since it has been demonstrated by other workers (Rocha e Silva, Valle & Picarreli, 1953 ; Gaddum & Hameed, 1954 ; Johnson, 1964) that in the guinea-pig intestine, nicotine and 5-HT have separate receptors on the parasympathetic ganglia.

The fact that nicotine enhances release of 5-HT from isolated sections of dog small intestine does not necessarily indicate that 5-HT participates in the intestinal contractor response to nicotine, but such a possibility should not be excluded in the absence of more definitive experiments.

SUMMARY

1. Both nicotine and dimethylphenylpiperazinium (DMPP) enhanced release of 5-hydroxytryptamine from dog small intestine segments perfused with Krebs bicarbonate solution. The injected agents also initiated contractions of the intestinal smooth muscle.

2. The enhanced 5-HT release and intestinal contraction produced by nicotine and acetylcholine (ACh) were significantly antagonized by atropine.

3. Perfusion of the isolated intestinal sections with calcium-free solution reversibly abolished the smooth muscle responses to nicotine and diminished responses to 5-HT and morphine; there was a smaller but still significant diminution of the responses to ACh.

4. The increase in intestinal motility by 10 μg nicotine and 2 μg DMPP was not antagonized by 100 μg hexamethonium (C6); in fact there was a non-significant increase in the response to nicotine after C6 treatment. Both nicotine and DMPP were significantly antagonized on the isolated intestinal sections by 500 μg doses of nicotine.

5. The ability of 100 μg C6 to depress the parasympathetic ganglia in an *in situ* intestinal section was demonstrated by locally antagonizing intestinal responses to vagal stimulation.

6. Suggestions are presented that nicotine and DMPP could enhance 5-HT release and motility in this preparation by stimulation of extraganglionic nervous structures, by direct action on the cholinergic receptors or, perhaps more likely, by stimulating a population of ganglion cells which are exceptionally resident to C6 blockade.

The authors express their gratitude to Miss Andrea Davis and Mrs. Sharon Heintz for their excellent technical assistance. This work was supported in part by research grants from the Council of Tobacco Research, U.S.A., The American Medical Association-ERF Fund and U.S. Public Health Grant no. 5-F1-GM-31, 186-02. This work was performed while one of us (T. F. B.) was a U.S. Public Health Service Fellow.

REFERENCES

BURKS, T. F. & LONG, J. P. (1966a). 5-Hydroxytryptamine release into dog intestinal vasculature. *Am. J. Physiol.*, **211**, 619–625.

BURKS, T. F. & LONG, J. P. (1966b). Catecholamine-induced release of 5-hydroxytryptamine from perfused vasculature of isolated dog intestine. *J. Pharm. Sci.*, **55**, 1383–1386.

BURKS, T. F. & LONG, J. P. (1967). Release of intestinal 5-hydroxytryptamine by morphine and related agents. *J. Pharmac. exp. Ther.* In press.

CHIANG, T. S. & LEADERS, F. E. (1965). Mechanism for nicotine and DMPP on the isolated rat atria-vagus nerve preparation. *J. Pharmac. exp. Ther.*, **149**, 225–232.

ELLIS, S. & RASMUSSEN, H. (1951). The atropine-fast nicotine stimulation of the rabbit's intestine and of the muscularis mucosae of the dog's intestine. *J. Pharmac. exp. Ther.*, **103**, 259–268.

EVANS, D. H. L. & SCHILD, H. O. (1953). The reactions of plexus-free circular muscle of cat jejunum to drugs. *J. Physiol.*, **119**, 376–399.

FISHLOCK, D. J. & PARKS, A. G. (1966). The action of nicotine on the circular muscle of the human ileum and colon *in vitro*. *Br. J. Pharmac. Chemother.*, **26**, 79–86.

GADDUM, J. H. & HAMEED, K. A. (1954). Drugs which antagonize 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **9**, 240–248.

GOLDSTEIN, A. (1964). *Biostatistics, An Introductory Text*, p. 59. Macmillan, New York.

HANSSON, E., MASUOKA, D. T. & CLARK, W. G. (1963). Effect of nicotine on the catecholamine and the serotonin level in some tissues. *Proc. West. Pharmacol. Soc.*, **6**, 36–37.

HANSSON, E., MASUOKA, D. T. & CLARK, W. G. (1964). Effect of nicotine on catecholamine and serotonin levels *in vivo*. *Arch. int. Pharmacodyn.*, **149**, 153–160.

JOHNSON, E. S. (1964). An independent nerve-pathway for 5-hydroxytryptamine in the guinea-pig ileum. *J. Pharm. Pharmac.*, **16**, 760–763.

KHAN, M., MANTEGAZZA, P. & PICCININI, F. (1965). Effect of low temperatures on the responses of guinea-pig isolated atria to nicotine and to sympathetic and parasympathetic stimulation. *Br. J. Pharmac. Chemother.*, **25**, 119–125.

LONG, J. P. & ECKSTEIN, J. W. (1961). Ganglionic actions of neostigmine methylsulfate. *J. Pharmac. exp. Ther.*, **133**, 216–222.

PELIKAN, E. W. (1960). The mechanism of ganglionic blockade produced by nicotine. *Ann. N.Y. Acad. Sci.*, **90**, 52–69.

POISNER, A. M. & DOUGLAS, W. W. (1966). The need for calcium in adrenomedullary secretion evoked by biogenic amines, polypeptides, and muscarinic agents. *Proc. Soc. exp. Biol. Med.*, **123**, 62–64.

QUILLIAM, J. P. & SHAND, D. G. (1964). The selectivity of drugs blocking ganglionic transmission in the rat. *Br. J. Pharmac. Chemother.*, **23**, 273–284.

ROCHA E SILVA, M., VALLE, J. R. & PICARELLI, Z. P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. *Br. J. Pharmac. Chemother.*, **8**, 378–388.

SCHAEPPI, U. & DODD, M. (1965). Non-ganglionic effects of nicotine in the pig iris sphincter. *Life Sci.*, **4**, 1157–1160.

SCHIEVELBEIN, H. (1965). Zum mechanismus der freisetzung von-5-hydroxytryptamin durch nicotin. *Arch. int. Pharmacodyn.*, **155**, 311–316.

SCHIEVELBEIN, H., WERLE, E. & ECKERT, I. (1963). Experimentelle untersuchungen zur wirkung des lobelins bei der nicotin-entwöhnung. *Arzneimittel Forsch.*, **13**, 211–214.

WRIGHT, P. G. & SHEPHERD, J. J. (1965). Response to drugs of isolated human colonic muscle from a case of Hirschsprung's disease. *Lancet*, **2**, 1161–1164.