Catecholamine-Induced Release of 5-Hydroxytryptamine (5-HT) from Perfused Vasculature of Isolated Dog Intestine

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The ability of exogenously administered epinephrine, norepinephrine, and tyramine and stimulation of sympathetic nerves to release 5-HT from the vasculature of isolated dog intestinal segments has been investigated. These stimuli were all found to produce significant release of 5-HT. Administration of an α-receptor blocking agent (tolazoline) significantly reduced the 5-HT release by norepinephrine but not by epinephrine.

IT HAS BEEN demonstrated recently (1) that isolated segments of dog small intestine release 5-hydroxytryptamine (5-HT) into physiological solution perfusing the vasculature. Stimuli which produced smooth muscle contraction or enhanced motility of the perfused segment increased its release of 5-HT. Such stimuli as acetylcholine, angiotensin, $BaCl_2$, increased intraluminal pressure, and scratching the serosal surface of the gut section all significantly increased 5-HT release. It was felt that such increased 5-HT release could simply be due to mechanical distortion of the tissues produced by the various stimuli.

More recent work indicates that the above explanation may be oversimplified since some stimuli that relax the intestinal musculature also release 5-HT.

METHODS

Adult mongrel dogs of either sex weighing 8 to 15 Kg. were anesthetized with 15 mg./Kg. of sodium thiopental and 250 mg./Kg. of sodium barbital administered intravenously. The small intestine was exposed and a small branch of the superior mesenteric artery with its juxtaintestinal arterial fan was cannulated with polyethylene tubing. The artery was perfused with warmed Krebs bicarbonate solution which was aerated by bubbling with a mixture of 95% oxygen and 5% carbon dioxide. Perfusion pressure, provided by use of a Sigmamotor model T-8 constant-flow peristaltic infusion pump, was maintained at 80–100 mm. Hg and was measured from a T-tube between the pump and the artery by a Statham pressure transducer and recorded on an Offner Dynograph (type RS). Since flow into the artery was held constant, changes of perfusion pressure were then proportional to changes in arterial resistance.

After flow was established through the artery, the associated vein was cannulated so that the venous effluent could be collected. Ligatures were tied around the intestinal segment supplied by the cannulated arterial fan and the segment surgically removed, placed on a cotton pad, and covered with a warm, saline-soaked gauze sponge. The section was then kept warm by use of an incandescent lamp.

In some of the experiments a balloon, attached to a Statham pressure transducer, was tied into the lumen of the intestinal section and intraluminal pressure recorded on either an Offner Dynograph or a Gilson (GME) polygraph.

In one preparation the venous effluent from the dog intestinal segment was superfused over an isolated uterus horn from an oestrus rat.

A constant recording of 5-HT concentration in the venous effluent was obtained by use of a flow-through cell in an Aminco-Bowman spectrophotofluorometer. The excitation monochromator of the spectrophotofluorometer was set at 295 mμ and the fluorescence monochromator was set at 330 mμ where the native fluorescence of 5-HT is maximal at neutral to slightly alkaline pH. Calibration of the instrument was performed with appropriate concentrations of 5-HT dissolved in Krebs solution placed in the cell. An illustration of the preparation employed is provided in Fig. 1.

The 5-HT concentration in the venous effluent was recorded in mcg./ml. of the effluent solution, then this figure was divided by the weight (to the nearest 0.1 Gm.) of the gut segment to give a 5-HT estimation of ng./ml./Gm. of wet tissue. The data are all reported in ng./ml./Gm.

In preparations from 15 dogs, the periarterial sympathetic nerves were isolated and fixed on a stimulating electrode. Nerve stimulation was performed with a Grass Instrument Co. model S4 stimulator. Parameters of stimulation were within the following ranges: frequency 20–30 c.p.s., duration 10–20 msec., and at 6–15 v. for 5 30 sec.

Test drugs were injected in volumes of 0.002–0.1 ml. intra-arterially *via* the arterial cannula. Agents employed were l-epinephrine hydrochloride, l-norepinephrine bitartrate (calculated as the base), tyramine hydrochloride, and tolazoline hydrochloride.

Experiments were so designed that each preparation served as its own control, and statistical comparisons were performed by the Student's t test (2). A P value equal to or less than 0.05 was considered significant.

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RESULTS

The initial observation responsible for this series of experiments is illustrated in Fig. 2. It was noted that stimulation of the periarterial sympathetic nerve of the isolated intestinal section resulted in enhanced contraction of the rat uterus being superfused with the venous effluent from the gut segment.

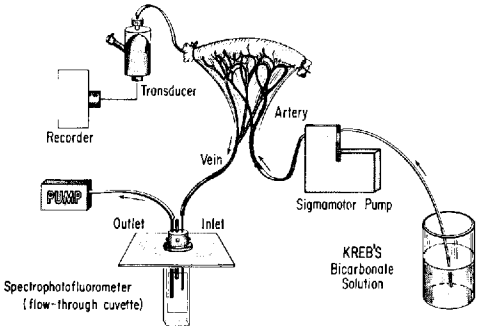

Fig. 1.—A drawing of the isolated intestinal preparation employed. A T-tube was inserted between the pump and the artery to monitor perfusion pressure. Intraluminal pressure was measured from a baloon in the lumen of the isolated intestinal segment. The effluent solution from the cannulated vein could be allowed to superfuse a rat uterus or to flow through the cell for analysis. A quartz baffle plate divides the quartz cell into two compartments, and the solution flows past and around this divider.

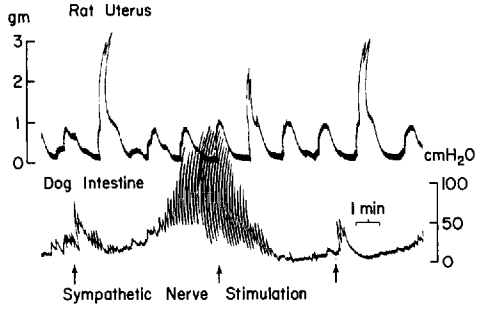

Fig. 2.—Key: top, tracing produced by an isolated rat uterus superfused with venous effluent from the dog intestinal segment; bottom, intraluminal pressure of intestinal segment. Stimulation of the periarterial sympathetic nerves of the intestinal segment produced contractions of the rat uterus which were not blocked by atropine.

Since norepinephrine usually produces only relaxation of the rat uterus, it was felt that the stimulation of the sympathetic nerve and catecholamine administration might release 5-HT.

In preparations from 16 dogs, epinephrine (4 mcg./dose) was found to significantly release 5-HT from the intestinal segment (see Table I). The musculature of the segment was relaxed by the epinephrine, but often enhanced activity of the section was observed after the period of relaxation. This increased activity occurred after the release of 5-HT and may have been produced by the released 5-HT (Figs. 3 and 4). It was noted that repeated injections of epinephrine into the vasculature of the section released progressively diminishing amounts of 5-HT. On some occasions when there was no longer any release of 5-HT by the epinephrine, 2-mcg. doses of acetylcholine were injected, and these treatments invariably released additional 5-HT.

Injected norepinephrine (4 mcg./dose) behaved qualitatively in the same manner as epinephrine (Figs. 3 and 4). In preparations from 10 dogs, the norepinephrine significantly ($p < 0.01$) increased 5-HT release (see Table I).

Since the above catecholamines were found to be active as releasers of 5-HT from the perfused intestinal vasculature, tyramine (200 mcg./dose) was injected to determine if endogenous catecholamines would similarly produce 5-HT release. In preparations from 10 dogs, the administered tyramine resulted in 5-HT release ($p < 0.01$) which is illustrated in Fig. 3 and Table I.

In perfused intestinal segments from 15 dogs, stimulation of the periarterial sympathetic nerves resulted in significant ($p < 0.01$) release of 5-HT (see Table I). The effects of nerve stimulation on intraluminal pressure were no different from that observed following the above agents (Fig. 4). Similarly, after repeated nerve stimulation there was loss of ability to release additional 5-HT, in which case administration of epinephrine, norepinephrine, or tyramine would not usually produce any 5-HT release. On some occasions administration of 2 mcg. of acetylcholine under these conditions would produce some 5-HT release.

A separate study was undertaken to determine if 5-HT release by the catecholamines was dose related. Five isolated intestinal segments were prepared from each of 13 dogs. One segment received first a low (0.2 mcg.) dose of epinephrine followed by a high (0.4 mcg.) dose of epinephrine; a second preparation received the same treatments but in reverse order; a third section received a low (0.3 mcg.) dose of norepinephrine followed by a high (0.6 mcg.) dose of norepinephrine; a fourth seg-

Table I.—Release of 5-HT by Catecholamines and Sympathetic Nerve Stimulation

Stimulus	ng. 5-HT Base/ml./Gm. Before[a]	ng. 5-HT Base/ml./Gm. After[a]	P^b	N^c
Epinephrine, 4 mcg.	5.7 ± 0.8	13.5 ± 1.9	<.01	16
Norepinephrine, 4 mcg.	6.7 ± 0.8	16.7 ± 3.4	<.01	10
Tyramine, 200 mcg.	6.0 ± 1.5	8.9 ± 1.8	<.01	10
Sympathetic nerve stimulation	12.7 ± 1.8	20.8 ± 2.7	<.01	15

[a] Mean ± standard error. [b] Level of significance, Student t test, paired comparison. [c] Number of animals employed in the experiment.

ment received the norepinephrine doses in the reverse order; a fifth segment first received tolazoline hydrochloride (250 mcg./dose) then 0.6 mcg. norepinephrine and 0.4 mcg. epinephrine, the order of the latter two agents being alternated from dog to dog. The treatments were randomly applied to each of the five loops from each dog. The results of these experiments may be seen in Tables II and III.

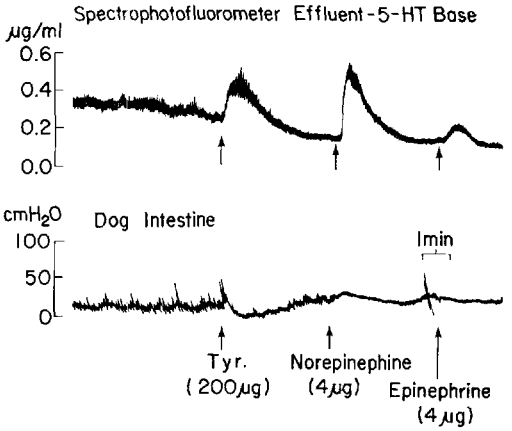

Fig. 3.—Demonstration of release of 5-HT from dog intestinal segment by tyramine (Tyr.), norepinephrine, and epinephrine. Key: top, continuous recording of 5-HT concentration in venous effluent; bottom, intraluminal pressure in dog intestinal section. The relatively small response to epinephrine was due to its being third in this particular series.

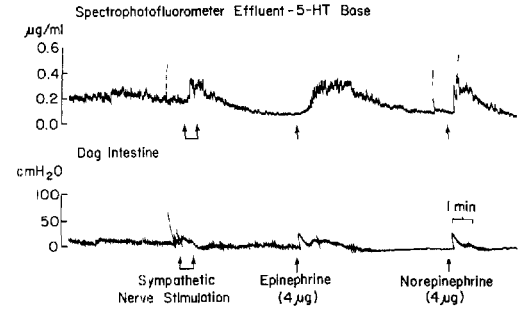

Fig. 4.—Release of 5-HT by 30-sec. stimulation of periarterial sympathetic nerves, epinephrine, and norepinephrine. Key: top, 5-HT content of venous effluent; bottom, intraluminal pressure of intestinal segment.

A dose-response curve was obtained for 5-HT release by the catecholamines and this release appeared to be related to pressor responses observed. It was noted that tolazoline alone was capable of producing pressor responses and releasing 5-HT and is therefore included in Table II.

Comparison of the responses to epinephrine and norepinephrine after tolazoline to the mean responses produced by the same doses of these agents in the absence of tolazoline revealed that while the pressor response to 0.4 mcg. epinephrine was significantly antagonized ($p < 0.05$) there was no statistically significant difference in 5-HT release by this agent whether or not tolazoline was present. On the other hand, the release of 5-HT by 0.6 mcg. norepinephrine was significantly antagonized by tolazoline ($p < 0.05$) but there was no significant antagonism of the norepinephrine pressor response. These data are summarized in Table III.

DISCUSSION

The ability of catecholamines, whether endogenous or added exogenously to release 5-HT from perfused dog intestinal segments does not constitute evidence for the hypothesis that intestinal 5-HT is released only by mechanical distortion of that organ. Since the catecholamines relax the musculature of the dog intestine and also release 5-HT, the above hypothesis must be considerably modified. It is possible that there is more than one method (perhaps there are many) by which the intestine can be stimulated to release 5-HT. Some agents, such as acetylcholine, $BaCl_2$, angiotensin (1), increased intraluminal pressure (1, 3), hydrochloric acid (4), and intraluminal hypertonic sucrose solutions (5) could produce intestinal contractions with incidental, secondary release of 5-HT. Catecholamines, however, may release 5-HT in some other manner, perhaps by a more direct mechanism. The 5-HT released by these latter agents may represent a second source or "pool" of 5-HT, perhaps another binding site in the intestinal mucosa, which is separate or distinct from that released by mechanical deformation. Evidence for this may be represented by the fact that acetylcholine can still release 5-HT after catecholamines have lost the ability to do so; the converse situation has also been observed.

The means by which the catecholamines release 5-HT in the dog intestine is open to speculation.

TABLE II.—EFFECTS OF CATECHOLAMINES AND TOLAZOLINE ON RELEASE AND INCREASE IN PERFUSION PRESSURE, $N = 13$[a]

Stimulus, mcg.	ng. 5-HT Base/ml./Gm. Before[b]	ng. 5-HT Base/ml./Gm. After[c]	P[d]	Rise in Perfusion Pressure,[e] mm. Hg
Epinephrine, 0.2	5.0 ± 0.5	7.2 ± 0.9	<.01	8 ± 4.8
Epinephrine, 0.4	5.1 ± 0.5	8.4 ± 1.3	<.01	14 ± 5.3
Norepinephrine, 0.3	5.1 ± 0.5	7.9 ± 1.1	<.01	17 ± 4.7
Norepinephrine, 0.6	5.2 ± 0.6	8.9 ± 1.2	<.01	23 ± 5.6
Tolazoline, 250[f]	6.7 ± 0.6	9.4 ± 1.0	<.01	12 ± 2.2

[a] Number of animals employed in each experiment. [b] Mean ± standard error before stimulus. [c] Mean ± standard error after stimulus. [d] Level of significance, Student t test, paired comparisons. [e] Mean response ± standard error. [f] Tolazoline included in this table only to show that it is capable of producing effects alone.

TABLE III.—EFFECTS OF 250 mcg. OF TOLAZOLINE ON 5-HT RELEASE AND INCREASE IN PERFUSION PRESSURE, $N^a = 13$

Stimulus, mcg.	Before Tolazoline: Increase 5-HT Base,[b] ng./ml./Gm.	Before Tolazoline: Increase in Perfusion Pressure,[b] mm. Hg	After Tolazoline: Increase 5-HT Base,[b] ng./ml./Gm.	After Tolazoline: Increase in Perfusion Pressure,[b] mm. Hg
Epinephrine, 0.4	3.3 ± 0.9	14 ± 4.6	2.1 ± 2.4	3 ± 1.1[c]
Norepinephrine, 0.6	3.7 ± 1.1	23 ± 5.8	1.7 ± 0.8[c]	13 ± 7.4

[a] Number of animals employed in each experiment. [b] Mean ± standard error. [c] Significantly ($p < 0.05$) decreased after tolazoline.

Perhaps the most plausible explanation would be that of displacement by the catecholamine of 5-HT at its mucosal binding sites. Stacey has stated (6) that tyramine, for example, can inhibit the uptake of 5-HT by blood platelets. Bertler *et al.* (7) have found that injected norepinephrine can replace 5-HT in its pineal gland sympathetic nerve storage sites. Similarly, administered metaraminol causes a pronounced depletion of pineal 5-HT. Born *et al.* (8) have suggested as an intracellular storage site for 5-HT, a 5-HT–ATP complex similar to that proposed for catecholamines in the adrenal medulla. In the fraction of the dog small intestine rich in 5-HT (mucous membrane) there is a molar ratio of approximately 3 to 1 between 5-HT and ATP (9). So it is quite possible that either exogenously administered catecholamines or those released by sympathetic nerve stimulation or tyramine could simply displace 5-HT from its storage granules in the intestinal mucosa.

More evidence will be required to demonstrate a positive role for 5-HT as a neurotransmitter in autonomic innervation of the small intestine. There have been some data presented, however, that may help define any such function. Van Harn found that, in the cat, stimulation of the thoracic sympathetic chain is inhibitory when the jejunum is spontaneously active but may cause an increase in activity in a previously inactive gut (10). It seems possible from the data reported in this communication, that the intestinal stimulation reported by Van Harn could have been due to 5-HT release. Klingman (11) has reported that immunosympathectomized rats have decreased levels of norepinephrine in their intestines, but that there is no alteration in that tissue in DOPA-decarboxylase activity. Since DOPA-decarboxylase and 5-hydroxytryptophan decarboxylase may be the same enzyme (12), it would be interesting to know whether there is a concomitant alteration of 5-HT levels in immunosympathectomized animals. A decrease in gastrointestinal 5-HT following immunosympathectomy could suggest that an intact sympathetic system is required for 5-HT elaboration, storage, or participation in transmission. An increase in 5-HT levels might indicate that there is competition between 5-HT and catecholamines for common storage sites in the intestine.

Ahlquist (13) states that the canine intestine has both α and β adrenergic receptors which produce intestinal inhibition. There may be "receptor sites" in the dog intestine with which catecholamines interact to produce 5-HT release which are different from the usual α and β receptors. The present data indicate that by use of an α-receptor blocking agent, tolazoline, some separation of pressor activity in the intestinal vasculature and 5-HT-releasing activity can be achieved with epinephrine and norepinephrine.

There may be some evidence for a homeostatic role of 5-HT in the intestine, as has been proposed for this substance in the cardiovascular system (14). It has previously been reported (1) that 5-HT release from the dog intestine can occur after either an increase or a decrease in intraluminal pressure. Similarly, agents that produce intestinal constriction (acetylcholine) or relaxation (catecholamines) similarly produce 5-HT release. Van Harn's experiments (10) contain related suggestions. It could be proposed that 5-HT somehow serves a buffer capacity, or as one component of a buffer system for intestinal motility. Any such function, of course, as yet eludes elucidation.

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