Serotonin-Bradykinin Synergism in the Mammalian Capillary Bed.* (31772)

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Recently it has been demonstrated that bradykinin and serotonin (5-HT) have a synergistic action on the rat mesenteric microcirculation(1). This reaction is characterized by intense *venular* spasm followed by capillary stasis and extravasation of red blood cells through defects in the walls of the postcapillary venules. More recently Sicuteri and coworkers(2) have demonstrated a potentiation of bradykinin on pain receptors by 5-HT, in man. In view of these findings, an investigation was undertaken to: 1) determine whether other vasoactive agents (*i.e.*, amines and polypeptides) could act synergistically with 5-HT, on the rat mesenteric microvasculature, to produce a similar reaction; and 2) elucidate the mechanism(s) underlying this serotonin-bradykinin interaction through the use of selected pharmacologic agents.

Inasmuch as sympatholytic agents such as phenoxybenzamine and chlorpromazine have been shown to potentiate cardiovascular responses to bradykinin(3) these same pharmacologic agents were utilized to explore further the microcirculatory synergism. A specific anti-serotonin compound (BOL-148) was employed to discriminate between the musculotropic actions of 5-HT *versus* its possible actions on the endothelial wall(1). Since mast cells: 1) anatomically parallel the mesenteric microvessels, 2) have been implicated in tissue injury(4,5), and 3) have been implicated in local regulation of blood flow and vascular reactivity(6,7) it seemed appropriate to ascertain whether mast cell depletion would modify the 5-HT-bradykinin synergism. A proteolytic enzyme inhibitor, which has been reported to protect in inflammation(8) and to suppress the vasoconstrictor actions of catecholamines and 5-HT on the microcirculation(9,10), was also employed.

Materials and methods. The mesocecum of pentobarbital sodium (Nembutal, 3.0 mg/100 I.M.) anesthetized rats, of the Sprague-Dawley and Wistar strains (120-170 g), was prepared for microscopic observation and utilized according to previously described methods(11,12). Microscopic observations were made at 60-400 × magnification for: a) changes in microvascular diameters (*i.e.*, arterioles, metarterioles, precapillaries and venules); b) signs of vascular injury characterized by the absence or presence of stasis (close packing of RBCs and cessation of flow); and/or 3) vasorhexis (rupture of postcapillary venular walls). A wide variety of vasoactive test agents (amines and polypeptides; dilators and constrictors) were evaluated for possible interaction with 5-HT on the rat mesenteric microvasculature, *i.e.*, bradykinin,† histamine phosphate (Nutritional Biochemicals Corp.), isoproterenol HCl (Isuprel®), eledoisin, l-epinephrine HCl (Adrenalin Chloride, Parke, Davis and Co.), l-norepinephrine bitartrate (Levophed®, Winthrop Laboratories) and angiotensin amide (Hypertensin®).

The sympatholytic agents phenoxybenzamine (Dibenzyline) and chlorpromazine HCl (Thorazine®), the anti-serotonin compound BOL-148, the mast cell depletor-compound 48/80 (Burroughs Wellcome), and the proteolytic enzyme inhibitor ε-amino caproic acid (Mann Research Labs.) were also employed locally and I.V. All doses of the vasoactive test substances are referred to as the base except for epinephrine, as the salt.

To test for possible synergism between

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† Dr. R. Bircher of Sandoz kindly supplied the synthetic polypeptides bradykinin and eledoisin and BOL-148. Angiotensin amide (Hypertensin®) was obtained through the courtesy of Dr. R. Gaunt, Ciba Pharmaceuticals.

TABLE I. Serotonin-Vasoactive Substance Interaction on Rat Mesenteric Microvessels.

Vasoactive test agents (doses in μg)	No. of rats	Microvascular response*			
		Art'le	Metart'le	Precap.	Venule
5-HT- then bradykinin† (200-0.1)	85	+	+	closure	+++
5-HT- then histamine (200-1.0)	50	0	0	open	0
5-HT- then isoproterenol (200-1.0)	10	0	0	"	0
5-HT- then eledoisin (200-1.0)	4	0	0	"	0
5-HT- then epinephrine (200-0.05)	25	++	++	closure	++
5-HT- then norepinephrine (200-0.10)	20	++	++	"	++
5-HT- then angiotensin (200-0.003)	5	++	++	"	++

* +, ++, +++, ++++ = degree of vasoconstriction; 0, 00 = degree of vasodilation.
† This combination of agents produced postcapillary stasis and vasorhexis.

the vasoactive test agents the following protocol was strictly adhered to: 1) each vasoactive test substance was evaluated for its intrinsic musculotropic actions, on the mesocecum, and then washed out for 3-5 minutes. 2) Serotonin (200 μg) was topically applied to the rat mesentery and its microvascular effects were allowed to develop within 30-45 seconds. 3) An interval of some 10-15 seconds was then allowed to elapse after the constrictor effect of 5-HT became manifest, and then bradykinin or one of the other vasoactive test agents was administered to the surface of the rat mesentery and the subsequent vascular effects were scored depending on degree of constriction (+-++++) or dilation (0, 00). The above experimental protocol was similarly followed before or after the topical or I.V. administration of the 4 categories of pharmacologic agents (sympatholytics, anti-serotonin, mast cell depletor and proteolytic enzyme inhibitor).

A double-blind study was utilized to eliminate any subjectivity on the part of the observer.

Results and discussion. The discrete microvascular actions of each of the vasoactive test agents used in this study (dilators: bradykinin, histamine, isoproterenol and eledoisin; constrictors: 5-HT, epinephrine, norepinephrine and angiotensin) have been previously evaluated and documented for the rat mesenteric microvasculature(7,10,12,13-17). Table I indicates that of all the 5-HT-vasoactive test agent combinations tried, only 5-HT and bradykinin produced venular spasm, postcapillary stasis as well as vasorhexis. These data therefore: 1) confirm the previous observations of Zweifach(1) showing a 5-HT-bradykinin synergism on the microcirculation; 2) show that other dilator substances such as the amines histamine and isoproterenol as well as another dilator polypeptide eledoisin fail to induce a similar synergistic reaction with 5-HT on the microcirculation; and 3) demonstrate the failure of amine or polypeptide constrictors to likewise induce a synergistic microcirculatory effect with 5-HT. It is of interest to point out that Sicuteri and his coworkers (18) likewise were unable to induce a 5-HT potentiation on human pain receptors with any other vasoactive peptides such as angiotensin, eledoisin, physalaemin, vasopressin or oxytocin, *i.e.*, none of these substances, other than bradykinin, acted synergistically with 5-HT.

The data in Table II show that topical pretreatment with phenoxybenzamine not only produced widespread microvascular dilation, as described previously(13,16), but prevented the 5-HT-bradykinin synergistic reaction. Interestingly, pretreatment with another sympatholytic-chlorpromazine, which by itself produced similar dilator effects on the microcirculation as phenoxybenzamine, exacerbated the synergistic reaction between 5-HT and bradykinin. Rocha e Silva(3) has shown previously, in the cat, that chlorpromazine potentiated the cardiovascular effects of bradykinin. This may account for the exacerbation seen in our experiments. Although this same worker has reported that phenoxybenzamine also potentiated the vascular effects of bradykinin in the cat, this might not be so in the rat. Topical pretreatment with BOL-148 not only prevented

TABLE II. Influence of Pharmacologic Agents on Microvascular Synergism Between Serotonin and Bradykinin.

Pretreatment agent (dose)	No. of rats showing reaction/total	5-HT- then bradykinin		
		Venular response*	Vasorhexis	Stasis
1. Adrenergic blockers				
a. Phenoxybenzamine (topical, 10-50 μg)	7/7	0	none	none
b. Chlorpromazine (topical, 200 μg)	9/10	++++	extensive	extensive
2. Antiserotonin				
BOL-148 (topical, 100 μg)	2/2	0	none	none
3. Mast cell depletor				
Compound 48/80 (topical, 50 μg)	10/12	+++	some	some
(i.v., 50 μg)†	5/8	0	none	none
4. Proteolytic enzyme inhibitor				
ε-Amino caproic acid (topical, 2 mg)	5/5	0	"	"
(i.v., 50 mg)‡	3/3	0	"	"

* +, ++, +++, ++++ = degree of vasoconstriction; 0 = vasodilation.
† Observations recorded 4½ hr after i.v. administration.
‡ Observations recorded at from 5-45 min after i.v. administration.

the venular constrictor action of 5-HT(13), but in addition abolished the 5-HT-bradykinin synergism. It would appear from these latter observations that the venular constrictor action of 5-HT must be present for the synergistic reaction to take place. Acute *local* mast cell depletion with compound 48/80 failed to prevent the synergistic effect. However, acute *systemic* mast cell depletion abolished the microvascular synergism in 5 of 8 animals tested. This apparent paradoxical phenomenon between local and systemic mast cell depletion may be explained through the overwhelming release of "pharmacological" amounts of histamine from mast cell stores (after I.V. 48/80) within the animal producing a sustained dilation of all muscular microvessels including the important muscular venules. It should be noted that the venular constrictor action of 5-HT was blocked in the 48/80 systemically pretreated animals, whereas 5-HT reactivity was not altered in the locally mast cell depleted situation(6). Both systemic and local administration of ε-amino caproic acid not only suppressed the musculotropic effects of 5-HT(9,10), but completely abolished the 5-HT-bradykinin microvascular synergism.

It would appear from our observations that the venular spasm induced by a combination of 5-HT and bradykinin produces increased intravenular pressure which is transmitted retrograde to the postcapillary collecting venules thereby exerting a distending force on the endothelial cells resulting in vasorhexis. In this context, it would be of interest to determine whether or not a synergistic action occurs between bradykinin and other venular constricting agents such as the synthetic analogues of vasopressin and oxytocin (19,20,21).

Summary and conclusions. The synergistic action of 5-HT and bradykinin in the rat mesenteric microcirculation was shown: a) not to be mediated through other dilator or constrictor amines or vasoactive polypeptides; b) to be dependent upon the venular constrictor action of 5-HT; and c) it was proposed that rhexis of postcapillary collecting venules is brought about by a local increase of intravascular pressure as a consequence of severe venular constriction.

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Insulin Secretion by Isolated Islets in Presence of Glucose, Insulin and Anti-Insulin Serum. (31773)

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Two methods have recently been introduced for measurement of insulin secretion *in vitro*. In one(1), small pieces of pancreas from the rat are incubated with guinea pig anti-insulin serum (GPAIS) which serves two purposes. The antibodies to bovine insulin combine with the secreted hormone before it can be destroyed by lytic substances released from the acinar tissues, and their neutralization during incubation gives a measure of the rate at which insulin is secreted. In the second method(2) individual Islets are isolated after treatment of the rat pancreas with collagenase. Secreted insulin, which is not then exposed to the lytic effects of any acinar tissue, accumulates in the incubation medium and can be measured by conventional methods of immuno assay. By using a combination of these techniques, it is here shown that the insulin content of an incubation medium has no effect upon insulin secretion by isolated Islets of Langerhans.

Materials and methods. Isolated Islets were obtained from the pancreas of the normal rat by the method described by Lacy and Kostianovsky(2) and incubated (5 or 10 islets/1.0 ml) in a bicarbonate-buffered medium equilibrated with oxygen (95%) and carbon dioxide (5%) and containing bovine serum albumin (0.5%, w/v; bovine albumin, Fraction V, Sigma Chemical Co., St. Louis, Mo.). To this medium, as required, were added glucose (30, 150 or 300 mg/100 ml), rat insulin, and GPAIS. The rat insulin (19.5 U/mg; Lot No. R 564; Novo Research Institute, Copenhagen) was dissolved in dilute acetic acid (0.6%, v/v, glacial) to give a stock solution (100 mU/ml) which was stored in the frozen state. The same batch of guinea pig anti-insulin serum (Lot 270) was used throughout. A stock solution (8%, v/v) was prepared in phosphate buffer (0.1 M; pH 7.0) containing bovine albumin (1%, w/v) and stored in the frozen state; no change in insulin binding potency of this diluted GPAIS (86.4 $\pm$ 0.4 mU bovine insulin/ml, n = 7) was observed over the short period (7 days) needed to complete the present studies.

To study *destruction of added insulin* by pancreatic tissue, constant amounts of unlabeled (540 μU/ml, PJ 4600, Eli Lilly and Co., Indianapolis) and ^{131}I-labeled (60 μU/ml; 9.6 mc/mg; In 281-2, Abbott Laboratories, North Chicago, Ill.) bovine insulin