# Bradykinin Interaction with 5-Hydroxytryptamine, Norepinephrine, and Potassium Chloride in Rabbit Aorta (36359)

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Bradykinin and serotonin (5-HT) interact synergistically on the rat mesenteric microcirculation (1), their effect being characterized by intense venular spasm followed by capillary stasis and extravasation of red blood cells through defects in the wall of postcapillary venules. The interaction is not mediated through other dilator or constrictor amines or vasoactive polypeptides and is dependent upon the venular constrictor action of 5-HT (2). In man, 5-HT (iv) induces a marked potentiation of bradykinin on pain receptors (3). Bradykinin has also been found to have a direct vasoconstrictor action when injected into the marginal ear vein of the rabbit (4). Moreover, it has been suggested that 5-HT/bradykinin synergism could be important in the pathogenesis of syndromes such as migraine, other vascular headaches, and angina pectoris, where circulatory disturbances and pain are closely associated (5). In view of these findings on veins and the microcirculation, an investigation was undertaken to: (i) determine whether bradykinin could act synergistically with 5-HT and other vasoactive agents on arterial smooth muscle; (ii) determine whether there is a direct effect of bradykinin; and (iii) elucidate the mechanism(s) involved in such interactions through the use of selected pharmacologic agents.

*Materials and Methods.* Spirally cut aortic strips from 72 fasted (12 hr) albino rabbits (1-2 kg) of either sex were suspended in 20-ml baths containing buffered Ringer's solution (pH 7.6–7.8) maintained at 38° and bubbled with a mixture of $O_2$ and $CO_2$ (95–5%). Isometric contraction against 2 g tension was recorded on a Narco Bio-Systems Physiograph through microdisplacement myograph transducers (Model F-50). Each strip was allowed to equilibrate under 2 g tension for 90 to 120 min prior to experiment. At 15-min intervals during equilibration each chamber was flushed with fresh Ringer's solution several times. Slight adjustments in resting tension (to 2 g) were made during the course of the experiment, if necessary. Three strips (2.5 mm wide and 25 mm long) were prepared from each aorta. Before recording a control response, each strip was challenged with two concentrations of agonist (5-HT or NE at $10^{-8}$ and $10^{-7}$ $M$; KCl at 9 and 12 m$M$) three to four times or until tension development was reproducible. Following the contractile response to each concentration of either 5-HT ($5 \times 10^{-9}$–$5 \times 10^{-6}$ $M$) or NE ($5 \times 10^{-9}$–$10^{-5}$ $M$), a strip was washed three times with fresh Ringer's and allowed 10 to 15 min for relaxation to resting tension. The control response to KCl (3–24 m$M$) was determined cumulatively. Synthetic bradykinin (BRS, Sigma Chemical Co.) was evaluated at three concentrations (1, 3, and 9 $\mu$g/ml). When testing for BRS interaction with 5-HT, NE, or KCl, BRS was added to the tissue chamber 5 min prior to additon of agonist.

The following drugs were tested for antibradykinin activity: acetylsalicylic acid (USP), butylated hydroxyanisole (Nutritional Biochemical Corp.), cyproheptadine HCl, beta-diethylaminoethyldiphenylpropylacetate (SKF-525A), hydrocortisone-21-phosphate, methysergide maleate, phentolamine methanesulfonate, phenylbutazone, and soy-

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bean trypsin inhibitor (Sigma Chemical Co.). Agonists used included serotonin creatinine sulfate (5-HT), *l*-norepinephrine bitartrate (NE), and potassium chloride (KCl). Fresh solutions were usually prepared daily and drug concentration is expressed as the weight of the salt.

*Results and Discussion.* Strips from 30 of the 72 animals used in the study contracted upon exposure to BRS (1, 3, and 9 μg/ml). BRS-sensitive strips contracted slowly (5 to 10 min were required for full tension development) and relaxation with repeated washing required 20 to 30 min. Tension development to 9 μg/ml of BRS varied from 0.5 to 4.7 g. Contractions were dose related, reversible, and tachyphylaxis did not develop. Maximal tension was attained with 9 μg/ml of BRS; and none of the strips responded to BRS below 1 μg/ml (*i.e.*, 0.05 or 0.5 μg/ml). BRS did *not* produce relaxation of normal or KCl-contracted strips.

Acetylsalicylic acid ($10^{-5}$–$10^{-3}$ *M*) and phenylbutazone ($5 \times 10^{-7}$–$10^{-5}$ *M*), nonsteroidal anti-inflammatory agents shown to antagonize BRS-induced contraction of femoral and carotid arteries isolated from the guinea pig (6), were not effective against BRS-sensitive rabbit aortic strips. However, in the presence of a steroidal anti-inflammatory drug, hydrocortisone ($5 \times 10^{-5}$ *M*), tension development to BRS (9 μg/ml) was reduced by 50%. BRS contraction was not attenuated by phentolamine ($10^{-5}$ *M*), methysergide ($10^{-6}$ *M*), cyproheptadine ($10^{-4}$ *M*), atropine ($10^{-5}$ *M*), or SKF-525A ($10^{-4}$ *M*), a potent inhibitor of KCl-induced contraction (7). Butylated hydroxyanisole ($5 \times 10^{-5}$ *M*), an antioxidant-antibradykinin agent (8), almost completely inhibited BRS contraction of the BRS-sensitive aortas. Butylated hydroxanisole or hydrocortisone had no effect on contractions of aortas induced by 5-HT, NE, or KCl. Although only 42% of the strips responded, the contraction apparently represents a direct action of BRS on arterial smooth muscle. No explanation is available for the fact that BRS contracted strips from some, but not all animals. The control responses of BRS-sensitive strips to the other agonists (5-HT, NE, or KCl) were not different from those of the BRS-insensitive strips. Similar results were reported for the direct effect of BRS on isolated canine venous strips (9). The concentration of bradykinin (9 μg/ml) used was higher than the amount present in normal rabbit or human plasma (10). However, it is possible under pathophysiologic conditions that bradykinin plasma levels may equal or exceed

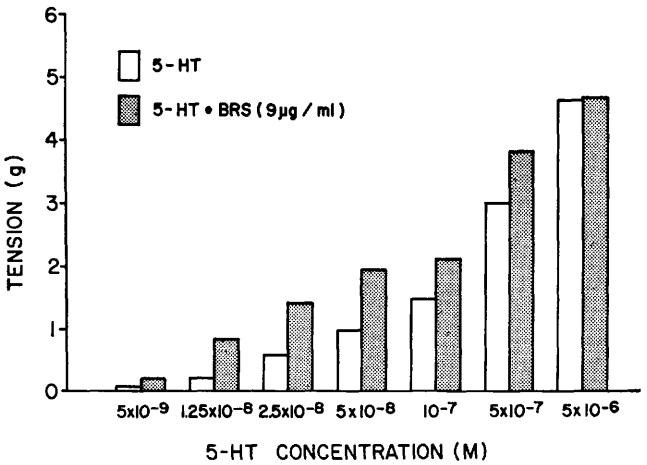


FIG. 1. Contraction of aortic strips to 5-hydroxytryptamine (5-HT) in the presence of 9 μg/ml of bradykinin synthetic (BRS): (each bar) the mean response of 32–40 strips. Standard error varied from 8 to 19% of the mean. Except at $5 \times 10^{-6}$ *M* 5-HT, the response to each concentration of 5-HT + BRS (shaded bars) was significantly greater ($p < .05$) than the response to 5-HT alone (clear bars).

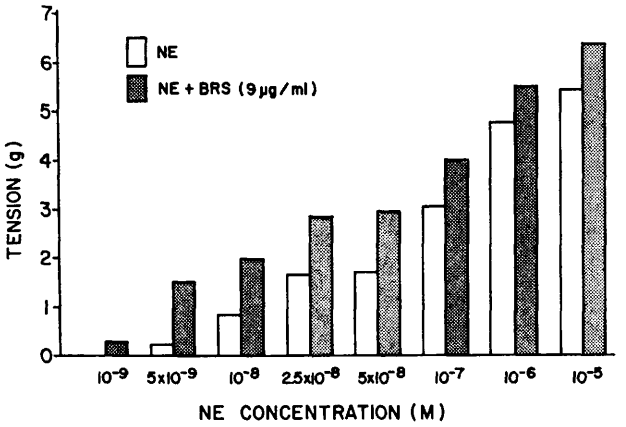


FIG. 2. Contraction of aortic strips to norepinephrine (NE) in the presence of 9 µg/ml of bradykinin synthetic (BRS): (each bar) the mean response of 24–30 strips. Standard error varied from 7 to 14% of the mean. The response to each concentration of NE + BRS (shaded bars) was significantly greater ($p < .05$) than the response to NE alone (clear bars).

the concentration of bradykinin used in this study (11).

The tension developed to 5-HT, NE, or KCl by BRS-sensitive strips in the presence of BRS was measured by subtracting the BRS response in the case of BRS-sensitive strips from the total response. If the response to BRS alone exceeded 1 g tension, the strip was excluded from the interaction experiment. The 30 BRS-sensitive strips that did respond to BRS with contractile force exceeding 1 g were used in experiments to test for agents that inhibited such contractile response.

BRS (9 µg/ml) potentiated the response of each aortic strip to 5-HT (Fig. 1). The potentiation was significant for all concentrations except $5 \times 10^{-6}$ *M* 5-HT, the concentration that induced maximal tension. Methysergide ($5 \times 10^{-8}$ *M*) almost completely blocked the response to 5-HT; however, attenuation was not complete in the presence of BRS (Table I). Several interpretations could apply to this finding: (i) BRS prevented the blockade by methysergide at 5-HT receptor sites; (ii) BRS inhibited the deactivation of 5-HT; or (iii) BRS altered the effector cell in some way that allowed 5-HT contraction to be elicited by what previously was a subthreshold or a less potent stimulus. Further experiments demonstrated that BRS also potentiated contraction of arterial smooth muscle to NE (Fig. 2) and KCl (Fig. 3). The NE- and KCl-induced contractions were potentiated at each concentration and there was a significant increase in maximal tension development to NE but not to KCl. Phentolamine ($5 \times 10^{-7}$ *M*) abol-

TABLE I. Effect of Methysergide (MSG)[a] and Phentolamine (PTA)[b] on BRS-Potentiated[c] Responses of Aortic Strips to 5-HT and NE.

| | | Tension (g) | |
|---|---|---|---|
| 5-HT (*M*) | *N*[d] | MSG | MSG + BRS |
| $5 \times 10^{-9}$ | 12 | 0 (0.01)[e] | 0.13 (0.19)[f] |
| $1.25 \times 10^{-8}$ | 12 | 0 (0.16) | 0.48 (0.82) |
| $2.5 \times 10^{-8}$ | 12 | 0.1 (0.56) | 0.66 (1.43) |
| $5 \times 10^{-8}$ | 12 | 0.2 (1.02) | 0.74 (1.93) |
| NE (*M*) | | PTA | PTA + BRS |
| $5 \times 10^{-9}$ | 15 | 0 (0.18)[g] | 0.12 (1.48)[h] |
| $10^{-8}$ | 15 | 0 (0.88) | 0.18 (2.03) |
| $2.5 \times 10^{-8}$ | 15 | 0 (1.75) | 0.39 (2.77) |
| $5 \times 10^{-8}$ | 15 | 0 (1.90) | 0.66 (2.82) |

[a] Methysergide ($5 \times 10^{-8}$ *M*) added to bath 5 min prior to BRS.

[b] Phentolamine ($5 \times 10^{-7}$ *M*) added 5 min prior to BRS.

[c] Bradykinin synthetic (9 µg/ml) added 5 min prior to 5-HT or NE.

[d] Number of strips.

[e] Response to 5-HT alone is given in parentheses.

[f] Response to 5-HT + BRS in parentheses.

[g] Response to NE alone is given in parentheses.

[h] Response to NE + BRS in parentheses.

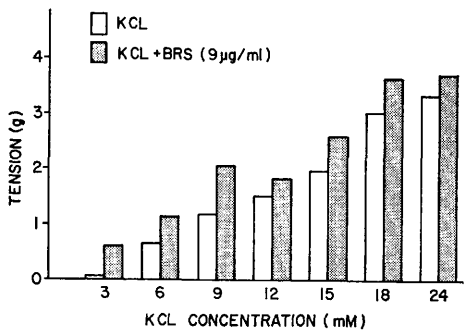


FIG. 3. Contraction to potassium chloride (KCl) in the presence of 9 $\mu$g/ml of bradykinin synthetic (BRS): (each bar) the mean response of 20–28 strips. Standard error varied from 9 to 21% of the mean. Except at 24 m$M$ KCl, the response to each concentration of KCl + BRS (shaded bars) was significantly greater ($p < .05$) than the response to KCl alone (clear bars).

ished the response to NE ($5 \times 10^{-9}$–$5 \times 10^{-8}$ $M$), but blockade was not complete in the presence of BRS (Table I). BHA, found to effectively inhibit the contractile response of strips of BRS, had no effect on BRS-potentiated responses of 5-HT, NE, or KCl (Table II). Such results suggested that BRS may increase vascular tone via two separate mechanisms: (i) by direct stimulation of arterial smooth muscle; and (ii) by an interaction with other agonists, viz., 5-HT, NE, or KCl. In support of a dual mechanism of action for BRS in arterial smooth muscle, SKF-525A attenuated BRS potentiation of contraction induced by 5-HT, NE, and KCl (Table III) but did not inhibit BRS contraction *per se*. SKF-525A does not affect 5-HT- or NE-induced contraction, but does block the response to KCl.

The mechanism by which BRS potentiated the contraction of strips to 5-HT, NE, and KCl may therefore be similar to that for KCl stimulation. BRS-induced contraction of aortic strips *per se*, however, apparently occurred via specific bradykinin receptors present only in the BRS-sensitive aortae.

TABLE II. Effect of Butylated Hydroxyanisole (BHA)[a] on Contractions of Aortic Strips to 5-HT, NE, or KCl in the Presence of Synthetic Bradykinin (BRS).[b]

| 5-HT ($M$) | N[c] | Tension (g) | | $p$ |
|---|---|---|---|---|
| $5 \times 10^{-8}$ | 6 | 1.87[d] | 1.93[e] | NS |
| $5 \times 10^{-8}$ | 6 | 3.55 | 3.71 | NS |
| NE ($M$) | | | | |
| $10^{-8}$ | 9 | 1.95 | 2.03 | NS |
| $10^{-8}$ | 9 | 3.21 | 3.57 | NS |
| KCl (m$M$) | | | | |
| 9 | 6 | 1.80 | 2.04 | NS |
| 18 | 6 | 2.87 | 3.66 | NS |

[a] BHA ($5 \times 10^{-5}$ $M$) added 5 min prior to BRS.
[b] BRS (9 $\mu$g/ml) added 5 min prior to 5-HT, NE, or KCl.
[c] Number of strips exposed to BHA.
[d] Response in presence of BHA + BRS.
[e] Response in presence of BRS.

TABLE III. SKF-525A[a] Inhibition of Synthetic Bradykinin (BRS)[b] Potentiation of 5-HT-, NE-, and KCl-induced Contractions of Aortic Strips.

| 5-HT ($M$) | N[c] | Tension (g) | | $p$ |
|---|---|---|---|---|
| $5 \times 10^{-8}$ | 7 | 0.95[d] | 1.93[e] | <.05 |
| $5 \times 10^{-7}$ | 7 | 2.66 | 3.71 | <.05 |
| NE ($M$) | | | | |
| $10^{-8}$ | 5 | 0.87 | 2.03 | <.05 |
| $10^{-7}$ | 5 | 2.91 | 3.87 | <.05 |
| KCl (m$M$) | | | | |
| 9 | 7 | 0.00 | 2.04 | <.001 |
| 18 | 7 | 0.00 | 3.66 | <.001 |

[a] SKF-525A ($2.6 \times 10^{-5}$ $M$) added 15 min prior to BRS.
[b] BRS (9 $\mu$g/ml) added 5 min prior to 5-HT, NE, or KCl.
[c] Number of strips exposed to SKF-525A.
[d] Response in the presense of SKF-525A + BRS.
[e] Response in the presence of BRS.

*Summary.* Bradykinin was shown to potentiate the contraction of rabbit aortic strips to 5-HT, NE, and KCl. Forty-two percent of the strips tested responded to bradykinin alone. Butylated hydroxyanisole antagonized the direct contractile effect of bradykinin, but did not attenuate bradykinin-potentiated responses of strips to 5-HT, NE, or KCl. SKF-525A did not affect bradykinin contraction, but did significantly decrease the potentiated response resulting from the interaction of bradykinin with 5-HT, NE, or KCl. It was concluded that bradykinin potentiation of arterial smooth muscle contraction was not

mediated through an interaction with adrenergic or serotonergic receptors, but rather through a nonspecific effect at the cell membrane of smooth muscle. It was proposed that bradykinin-induced contraction was mediated through specific bradykinin receptors not present in all the rabbit aortae.

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