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THE RELAXING EFFECT OF BRADYKININ ON INTESTINAL SMOOTH MUSCLE

BY

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Bradykinin has been found to be a potent stimulant of the adrenal medulla (Lecomte, Troquet & Dresse, 1961 ; Feldberg & Lewis, 1964) and sympathetic ganglia (Lewis & Reit, 1965). This effect seems to be caused by an interaction of the polypeptide with non-nicotinic receptors in ganglionic synapses (Trendelenburg, 1966) and could explain the potentiation of the hypotensive (Rocha e Silva, Corrado & Ramos, 1960 ; Lloyd, 1962 ; Nakano, 1965) and bronchoconstrictor (Collier, James & Piper, 1965) actions of bradykinin by adrenergic blockade or by adrenalectomy.

It was of interest to know whether some " sympathomimetic " effects of bradykinin observed in isolated preparations could also be explained by a release of catecholamines ; the weak cardiostimulant action of bradykinin on the isolated guinea-pig heart has been shown to be direct and not the result of a local release of catecholamines or an interaction of the polypeptide with adrenergic receptors (Antonio, 1966). In this investigation the relaxation of the isolated rat duodenum was studied, as another effect of bradykinin which resembles sympathetic stimulation. The duodenum was subjected to different ionic environments in an attempt to investigate how such relaxation is produced ; epinephrine and papaverine were used for comparison.

METHODS

Adult rats of either sex were killed by a blow on the head and bled. The abdomen was opened and the duodenum was removed. The preparation was set up in a 10 ml. organ bath containing Tyrode solution at 37° C and continuously bubbled with air. The duodenum was tied to a light frontal-writing aluminium lever with a total load on the tissue of approximately 1 g. Isotonic responses, magnified five times, were recorded on smoked paper. The agonists were added directly to the organ bath in volumes never exceeding 0.2 ml. Antagonists were dissolved in the perfusing Tyrode solution and the dose-response curves of the agonist were obtained after 30 min of contact of the antagonist with the preparation.

Unless otherwise stated, the composition of the Tyrode solution (in g/l. of glass-distilled water) was: NaCl, 8.00 ; KCl, 0.20 ; $CaCl_2$, 0.20 ; $MgCl_2.6H_2O$, 0.20 ; $NaHCO_3$, 1.00 ; $NaH_2PO_4.H_2O$, 0.055 ; and glucose 1.00. Potassium depolarizing solutions were made up by replacing 30–100% of the NaCl with equimolar amounts of KCl. In the calcium-free Tyrode solution no other ion was used to replace calcium.

Drugs: Synthetic bradykinin (SBR 640, Sandoz, Switzerland), l-epinephrine bitartrate (Mann Research Laboratories, U.S.A.), papaverine hydrochloride (Lafi, Brazil), acetylcholine chloride (Roche, Brazil), propranolol hydrochloride (Imperial Chemical Industries, England), phentolamine hydrochloride (Regitine, Ciba, Switezerland), atropine sulphate (Merck, Darmstadt, Germany). All doses refer to the salts.

RESULTS

Different sensitivity of the rat duodenum to bradykinin and epinephrine. Influence of sympatholytic agents

The polypeptide is about 200 times more potent than epinephrine in relaxing the rat duodenum (Fig. 1); bradykinin relaxation is not significantly affected by sympatholytic agents, whereas the effect of epinephrine is abolished by the combination of α- and β-sympatholytic agents (Regitine and propranolol). Regitine or propranolol alone are known not to abolish the response to epinephrine.

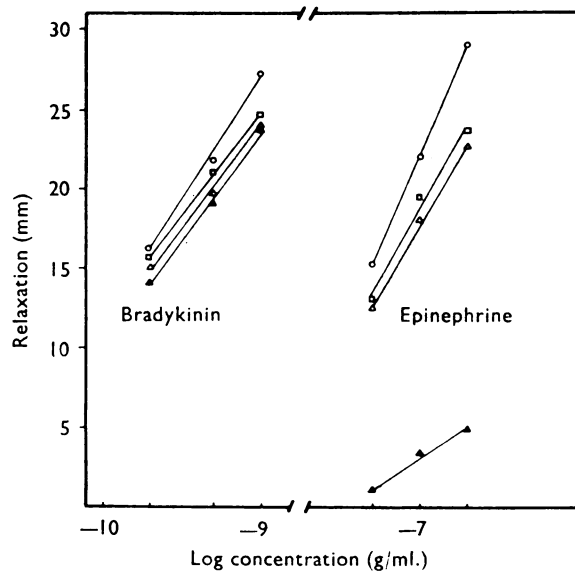

Fig. 1. Influence of the sympatholytic agents Regitine and propranolol on the relaxing effects of bradykinin and epinephrine on the rat duodenum. The absolute relaxing effect (in mm) is plotted against the logarithm of the dose of the agonists. Each point is the average of six experiments, excepting the control lines and the lines for the combination Regitine + propranolol which were obtained from twelve experiments. ○——○, Control; □——□, propranolol 2×10^{-7}; Δ—— Δ Regitine 2×10^{-7}; ▲——▲, propranolol 1×10^{-7} + Regitine 1×10^{-7}.

Modification of the rat duodenum "tonus" and of the bradykinin effect by changing the calcium concentration of the Tyrode solution

When the duodenum, in low-calcium Tyrode solution, is exposed to bradykinin, a contraction can follow the relaxing effect; the contraction is seen only when higher doses of bradykinin are used and it becomes smaller as the concentration of calcium is increased; papaverine and epinephrine induce only relaxation (Fig. 2). When the ratios between contraction and relaxation are plotted against calcium concentration, as shown in Fig. 3, it can be seen that contraction once more predominates in the low-calcium medium. Incidentally, the effect of calcium itself when added to the organ-bath also depends on the concentration of calcium in the Tyrode solution; it can be seen in Fig. 2 that in a 0.1 mM solution the addition of calcium produces contraction whereas in concentrations of 0.4 mM or higher it produces relaxation.

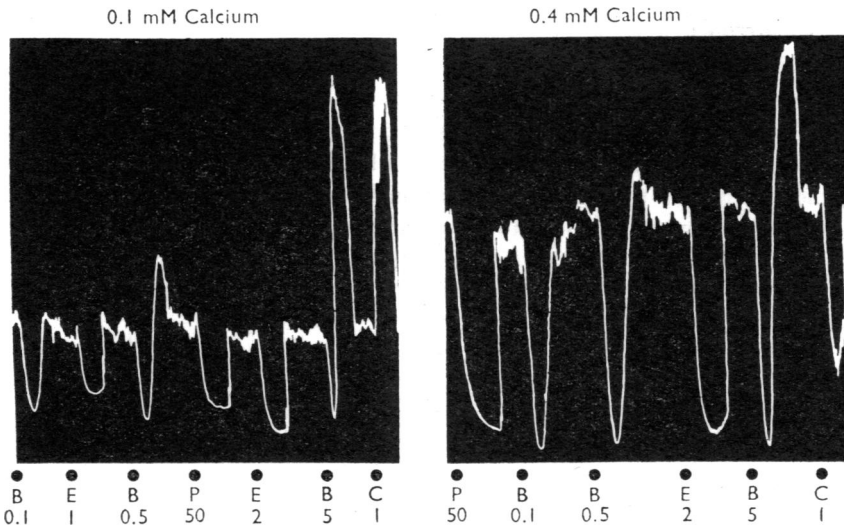

Fig. 2. Effects of bradykinin (B), epinephrine (E), papaverine (P) and of 1 mg of $CaCl_2$ (C) on the same rat duodenum in two different calcium concentrations. Numbers below B, E and P represent doses in μg in the 10-ml. bath. Note that C produces contraction in the 0.1 mM calcium Tyrode solution and relaxation in the 0.4 mM calcium solution.

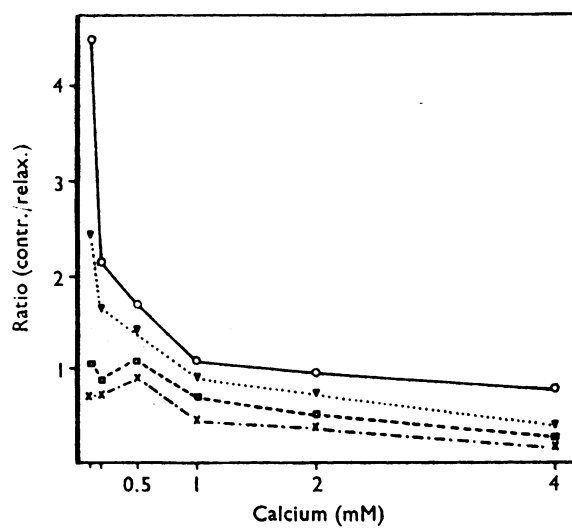

Fig. 3. Influence of calcium concentration of the Tyrode solution on the effect on the rat duodenum of bradykinin (○——○, 5×10^{-7} g/ml.; ▽ ····· ▽, 5×10^{-8} g/ml.; □– – –□, 5×10^{-9} g/ml; × —·— ×, 5×10^{-10} g/ml.). The ratio between the absolute values (in mm.) of contraction and relaxation is plotted against the calcium concentration. The contraction was measured in mm from the maximal relaxed position. Each point is the average of six experiments.

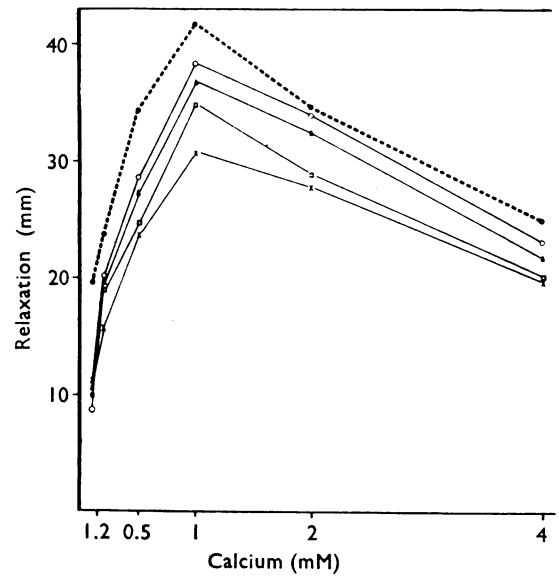

Fig. 4. Influence of calcium concentration of the Tyrode solution on the relaxing effect of bradykinin (○——○, 5×10^{-7} g/ml; ▲——▲, 5×10^{-8} g/ml.; □——□, 5×10^{-9} g/ml.; ×——×, 5×10^{-10} g/ml.) in the rat duodenum. The maximal relaxation (. . .) was the effect obtained by adding epinephrine (2.0 μg/ml. to the bath. Each point is the average of six to eighteen experiments. It should be recalled that the maximal relaxation is a good approximation of the rat duodenum " tonus ".

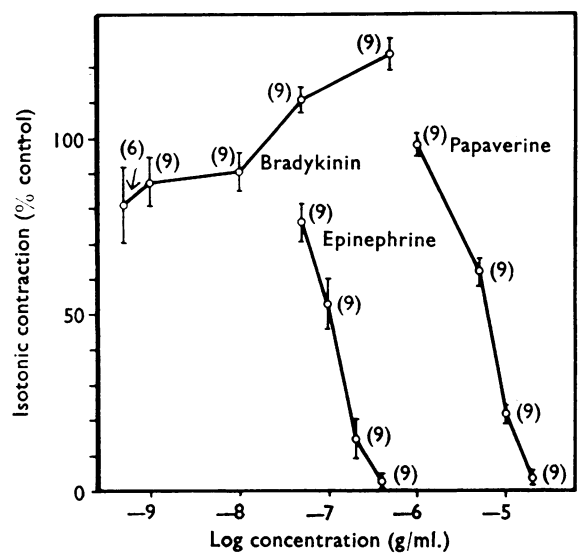

Fig. 5. The rat duodenum in calcium-free Tyrode solution. Influence of increasing doses of bradykinin, epinephrine and papaverine on the contraction elicited by 0.2 mg $CaCl_2$ (10 ml. bath). In ordinates are the percentage changes in calcium contractions plotted against the logarithm of the concentration of bradykinin, epinephrine and papaverine, added 30 sec before calcium. Numbers in parentheses indicate the number of experiments; individual points indicate mean ± standard error of the mean.

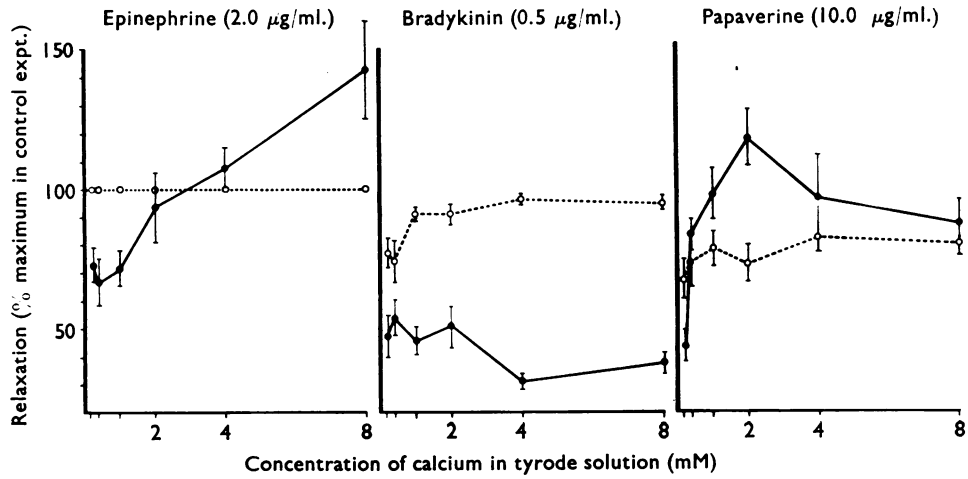

Fig. 6. Effect of acetylcholine (1 μg/ml.) on the response of the rat duodenum to ephinephrine, bradykinin and papaverine at different calcium concentrations. The relaxation obtained with 2.0 μg/ml. of epinephrine in the absence of acetylcholine was taken as the maximum (100% relaxation); all other values are related to this maximum effect. ○, control responses to the agonists; ●, responses in the presence of acetylcholine. Each point represents the mean ± standard error of the mean of seven experiments.

The absolute relaxing effect of bradykinin, epinephrine and papaverine is a function of the tonus in the duodenum, which in turn depends on the calcium concentration of the Tyrode solution (Figs. 2 and 4). Fig. 4 shows the absolute values for the relaxing effect of bradykinin in different calcium concentrations compared with the "maximal relaxation" which was obtained with the supramaximal dose of 2.0 μg of epinephrine/ml.

Rat duodenum in calcium-free Tyrode solution

When the duodenum is immersed in a calcium-free medium it still retains a high tone and spontaneous activity which declines slowly as the preparation is repeatedly washed out. After 60–90 min the tone is completely lost and the spontaneous activity is very small; at this stage no drug action can be detected except when calcium or barium are added, which evoke an atropine-resistant contraction even in these circumstances. Fig. 5 shows that epinephrine and papaverine, but not bradykinin, are able to block such calcium contractions.

Modification by acetylcholine of the rat duodenum tonus and bradykinin effect

When acetylcholine is added to the perfusion fluid in a concentration of 1 μg/ml., the preparation contracts vigorously and assumes a new base line which is roughly proportional to the calcium concentration of the Tyrode solution. Fig. 6 shows that the effect of supramaximal doses of bradykinin and epinephrine are differently affected by acetylcholine; the relaxing effect of bradykinin is reduced by acetylcholine at all calcium concentrations, whereas that of epinephrine is reduced for the low calcium concentrations and increased for the Tyrode solutions with a higher concentration of calcium. The effect of the dose of papaverine 10.0 μg/ml. is also shown for comparison. The results

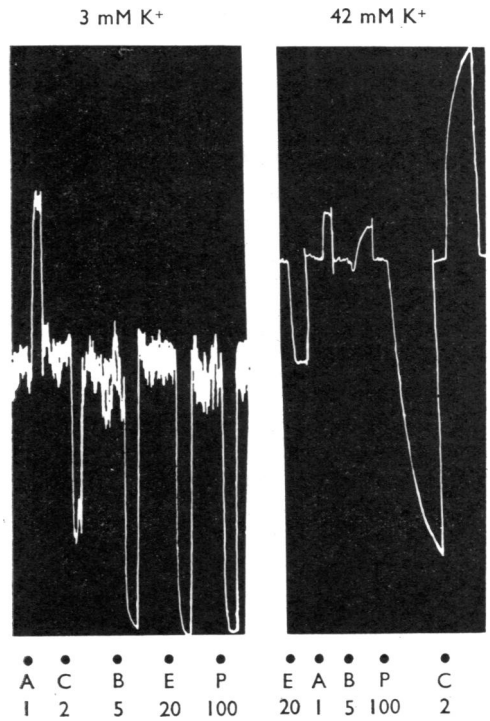

Fig. 7. Influence of potassium depolarizing solution (42 mM K^+) on the response of the rat duodenum (normal calcium concentration) to acetylcholine (A), bradykinin (B), $CaCl_2$ (C), epinephrine (E) and papaverine (P). Numbers represent dose in μg added to the 10 ml. bath, except for $CaCl_2$ (dose, 2 mg).

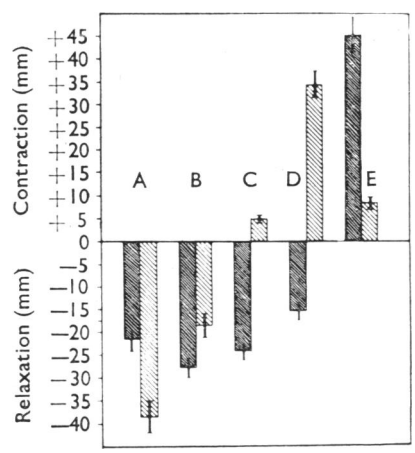

Fig. 8. Influence of potassium depolarizing solution (42 mM K^+) on the responses of the rat duodenum (in 0.5 mM calcium Tyrode) to papaverine (A, 1×10^{-5} g/ml.), epinephrine (B, 2×10^{-6} (g/ml.), bradykinin (C, 5×10^{-7} g/ml.), calcium (D, 2×10^{-4} g/ml.), and acetylcholine (E, 1×10^{-7} g/ml.). Experiments conducted as shown in Fig. 7. Each column is the mean ± standard error of the mean of six experiments. It should be noted that the horizontal bar (0 axis) was always higher at 42 mM than at 3 mM potassium as shown in Fig. 7; the absolute effects of the drugs were measured starting from the base line at the moment the drug was added. ▨, 3 mM K^+; ▧, 4.2 mM K^+.

in Fig. 6 are the percentage changes in relation to the maximal relaxation in control experiments for each calcium concentration; as mentioned before, the relaxation caused by epinephrine 2.0 μg/ml. was taken as the maximal effect.

Modification of bradykinin effect by depolarization with potassium solutions

When more than 30% of the NaCl of the Tyrode solution is replaced by an equimolar amount of KCl, the duodenum contracts strongly and remains contracted and without spontaneous activity (Fig. 7); the new base line depends on the calcium concentration of the perfusing solution. In this condition, the relaxing effect of bradykinin is completely lost—only a small and slow contraction is seen when bradykinin is added. The effect of epinephrine is reduced but not lost, while papaverine seems to be more potent than before in the depolarized preparations. The effect of acetylcholine is reduced. In the potassium-depolarized preparations, the addition of calcium always produces contraction. The changes obtained when only 30% of the NaCl is replaced by KCl are summarized in Fig. 8. These changes are in fact the result of an excess of potassium, because the replacement of 30% of the NaCl by an equimolar amount of sucrose does not interfere with the normal effects.

DISCUSSION

Most smooth muscle preparations are stimulated by bradykinin, but the rat duodenum is relaxed by very small amounts of the polypeptide (Horton, 1959). The sensitivity of the rat duodenum to bradykinin is of the same order as that of the rat uterus (10^{-9}–10^{-10} g/ml.); the relaxation is rapid in onset and is very similar to that evoked by epinephrine; on a weight basis the polypeptide is about 200 times more potent than epinephrine.

Other inhibitory effects of bradykinin on the gut include the depression of the peristaltic reflex in the intact animal (Levy, 1963; Türker, Kirau & Kaymakçalan, 1964) and of the isolated guinea-pig ileum (Beleslin, Bogadnovic & Radmanovic, 1964). In Levy's experiments the inhibitory effect of bradykinin was not affected by adrenergic blockade. Accordingly, we cannot ascribe the relaxing effect of bradykinin on the rat duodenum to a release of catecholamine or an interaction with adrenergic receptors, for the following reasons: (a) it is not blocked by a combination of the sympatholytic agents propranolol and phentolamine in doses which abolish epinephrine relaxation; (b) no tachyphylaxis is developed; (c) the adrenergic neurone-blocking agent bretylium or the previous treatment of the rat with reserpine do not interfere with bradykinin relaxation (Antonio, Ricciopo Netto & Corrado, 1967); (d) depending on the calcium concentration of the Tyrode solution, the relaxing effect of bradykinin is followed by a contraction, which is not the case with epinephrine.

Our observations concerning the relaxing effect of epinephrine on the rat duodenum are in agreement with the existence of both α- and β-adrenergic receptors in the intestinal smooth muscle (Furchgott, 1959; Ahlquist & Levy, 1959; Levy, 1959; Lum, Kermani & Heilman, 1966). Türker *et al.* (1954) reported a blockade of bradykinin relaxation in the rat duodenum with pronethalol, a result which has not been confirmed in our experiments with the use of propranolol.

The contraction which usually follows the relaxing effect of bradykinin depends on the calcium concentration of the Tyrode solution: it is maximal in low calcium solutions and decreases as the calcium concentration is increased. In this respect bradykinin

resembles calcium itself when the latter is added directly into the organ-bath. It should be remembered, however, that in the complete absence of calcium (calcium-free Tyrode), bradykinin as well as epinephrine, papaverine and acetylcholine have no visible effect on the rat duodenum, while calcium still produces a contraction; the contraction elicited by calcium is blocked by epinephrine and papaverine, but not by bradykinin. Calcium is the only physiologically occurring ion which causes shortening when injected in low concentrations into the muscle fibre (Heilbrunn & Wiercinski, 1947; Niedergerke, 1955), and so is reasonable to assume that such contraction is probably caused by the entrance of calcium into the cell and its interaction with the contractile machinery. Our results show that epinephrine but not bradykinin interfere with this calcium entry.

The relaxing effect of bradykinin is completely lost when the duodenum is perfused with depolarizing KCl solutions; only a slow and small contraction not related to the dose remains. Moreover, in the normal preparation, bradykinin is unable to block the contraction elicited by acetylcholine, pilocarpine, prostigmine, serotonin, barium or histamine (Duriasz-Rowińska, 1963). We have also seen that in the presence of acetylcholine even a 1,000-fold increase in bradykinin concentration is unable to relax the preparation to its greatest extent. All these observations show clearly that bradykinin relaxes only the preparation with its normal tone; the polypeptide lacks a true antispasmodic activity, because it is unable to prevent the contraction elicited by several classes of drugs or to relax a preparation strongly contracted by depolarizing solutions.

The intimate mechanism by which bradykinin relaxation is brought about deserves further investigation; we know that the relaxing effect is very short-lasting and quite often followed by a contraction; the short duration of this relaxing effect can explain the lack of inhibition of calcium entry and the contraction elicited by stimulants; it does not explain, however, the absence of relaxation produced by bradykinin in preparations depolarized with KCl or its strong reduction when the duodenum is perfused with Tyrode solution containing acetylcholine.

SUMMARY

1. The relaxing effect of bradykinin on the isolated rat duodenum has been compared with that of epinephrine. Weight for weight the polypeptide is about 200 times as potent as epinephrine.

2. No tachyphylaxis is developed to either epinephrine or bradykinin.

3. The combination of propranolol (1×10^{-7} g/ml.) and phentolamine (1×10^{-7} g/ml.) abolishes the responses to epinephrine but does not interfere with bradykinin relaxation.

4. It is concluded that the rat duodenum has both α- and β-adrenergic receptors and that the effect of bradykinin is not the result of a local release of catecholamines or an interaction with these adrenergic receptors.

5. In low calcium Tyrode solutions the relaxing effect of bradykinin is very short-lasting and is followed by a contraction proportional to the dose of the polypeptide; the contraction is not seen in normal or high calcium Tyrode solutions.

6. Epinephrine and papaverine, but not bradykinin, prevent the contraction induced by calcium in the rat duodenum immersed in calcium-free Tyrode solution.

7. The relaxing effect of bradykinin, but not those of epinephrine and papaverine, is strongly reduced in the duodenum contracted by acetylcholine (1×10^{-6} g/ml.).

8. The relaxing effect of bradykinin, but not those of epinephrine and papaverine, is abolished in the duodenum depolarized by high KCl Tyrode solutions.

9. It is concluded that bradykinin, unlike epinephrine and papaverine, lacks a true antispasmodic activity; the polypeptide relaxes the preparation with its normal tone but not the preparation actively contracted by depolarizing agents.

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