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Further evidence for the existence of two receptor sites for bradykinin responsible for the diphasic effect in the rat isolated duodenum

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1 Low doses of bradykinin (below 10 nM), as well as of K^+ (below 10 mM) induced relaxation, whereas higher doses caused contraction of the rat duodenum.

2 The relaxant responses induced by bradykinin and K^+ were not affected by ouabain (1 μM), but pre-incubation with 5.9 mM K^+ abolished the responses to that ion but not those to bradykinin.

3 The contractile and relaxant components of the response to bradykinin (but not those to K^+) increased with the time elapsed after mounting of the preparation, and this was due to stretching by the load of the recording system.

4 Specific and reversible desensitization (tachyphylaxis) was observed with the contractile response (but not the relaxation) induced by bradykinin.

5 Des-Arg^9-bradykinin, an analogue specific for B_1-receptors, was much less active than bradykinin, and elicited only a contractile response.

6 Among four bradykinin potentiating peptides that were tested, potentiator C enhanced the relaxation only, whereas BPP5a and captopril potentiated only the contraction and BPP9a potentiated both types of response to bradykinin.

7 Our results support the hypothesis that the relaxant and contractile components of the rat duodenum's response to bradykinin are due to actions at different receptor sites, which can be distinguished by their properties (desensitization) and their different apparent affinities for agonists and for potentiating peptides.

Introduction

Relaxation is the typical response induced by bradykinin in the rat duodenum, this response being changed into a diphasic effect (relaxation followed by contraction) by lowering the calcium concentration in the medium (Antonio, 1968). However, Faber & van der Meer (1973) observed that a high bradykinin concentration in normal medium could also induce a diphasic response.

The relaxant effect of low doses of bradykinin is usually not observed in the guinea-pig resting isolated ileum but can be induced in the acetylcholine-contracted organ (Hall & Bonta, 1973). In this case higher concentrations of bradykinin also give rise to diphasic responses. A similar pattern of behaviour was described by Ishii & Shimo (1979) for the responses of the taenia coli to increased external K^+ concentration: addition of low K^+ concentrations induced relaxation whereas diphasic responses were elicited by higher K^+ concentrations. The mechanism proposed by these authors for the K^+-induced relaxation was an enhancement of the Na^+/K^+-ATPase activity resulting in increased electrogenic exchange of sodium and potassium, and consequently membrane hyperpolarization.

Aarsen & van Caspel-de Bruyn (1970), based on electrophysiological studies with guinea-pig taenia coli, suggested that two different receptor sites might be responsible for the relaxant and contractile components of the response to bradykinin. This hypothesis was also proposed for rat duodenum and guinea-pig ileum, based on the findings that bradykinin concentrations that cause relaxation are much lower than those eliciting contraction, and that a difference could be observed in the enhancement of those responses by potentiating agents (Camargo & Ferreira, 1971; Hall & Bonta, 1974). This proposal, however, is not accepted by those other authors who found that both components of the response were

Table 1 Bradykinin potentiating agents

Name	*Structure*
Potentiator C (Pot C)	pGlu-Gly-Leu-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro
BPP9a	pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro
BPP5a	pGlu-Lys-Trp-Ala-Pro
Captopril	$HS\text{-}CH_2\text{-}\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}H\text{-}CO\text{-}Pro$

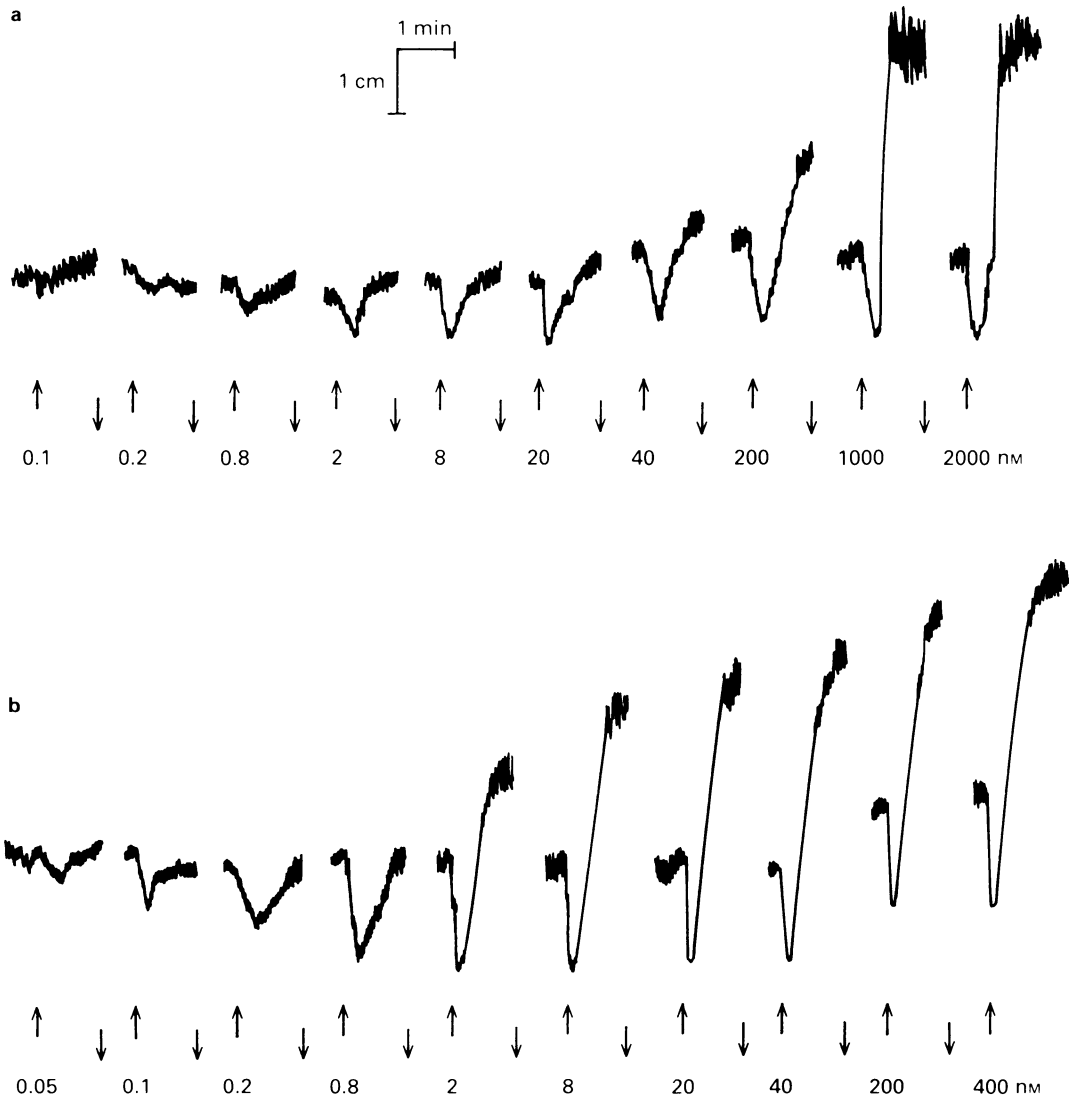

Figure 1 Effect of time after mounting of the rat duodenum on the responses to increasing doses of bradykinin. (a) During the first hour and (b) during the fourth hour after the 30 min equilibration period. Upward arrows indicate addition of the agent and downward arrows washing with fresh medium.

equally potentiated by two potentiating peptides (Üfkes *et al.*, 1976) and that the potentiating peptide BPP5a and bradykinin act at different receptor sites (Aarsen, 1977). On the other hand, based on a comparison of the order of potency between bradykinin and its analogues and estimates of the affinity of specific and competitive antagonists, Regoli & Barabé (1980) characterized two kinds of receptors (B_1 and B_2) for kinins in different smooth muscle preparations.

To investigate further the mechanism of the diphasic response of the rat duodenum to bradykinin and to K^+, we have studied how the pattern of response is affected by: (a) the time elapsed after mounting of the preparation; (b) different synthetic bradykinin potentiating peptides; and (c) agents which interfere with the functioning of the Na^+/K^+-pump. We have also studied the effect on the rat duodenum of a bradykinin analogue (des-Arg^9-bradykinin) which is believed to be a specific agonist for B_1-receptors.

Methods

Wistar rats of either sex, weighing between 190 and 220 g, were killed by a blow on the head. After bleeding, the abdomen was opened and the duodenum removed. The preparation was suspended in a 5 ml chamber containing Tyrode solution kept at 37 °C and bubbled with air. The composition of the Tyrode solution was (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.49, NaH_2PO_4 0.36, $NaHCO_3$ 11.9, D-glucose 5. Isotonic recordings were made, under 1 g load, on smoked drums using frontal levers with 6 fold amplification. The organs were equilibrated for 30 min before the beginning of the experiments. During this time, and also during longer resting periods, the medium was replaced by fresh solution every 5 min. The effect of time after mounting of the preparation, on the responses was studied by comparing the results obtained during the first and the fourth hour after the end of the 30 min equilibration period.

Administration of K^+ and of low bradykinin concentrations (which produced relaxation) were made at 5 min intervals. When higher concentrations of bradykinin were used to elicit contractile responses, the interval between administrations was 15 min, to avoid tachyphylaxis. In the experiments in which K^+ was administered, the dose indicated (in mM) is that excess over the concentration already present in the Tyrode solution (2.7 mM). The relaxant component of the response was measured from the baseline to the deepest point of the relaxation and the contractile component from the baseline to the highest point of the response.

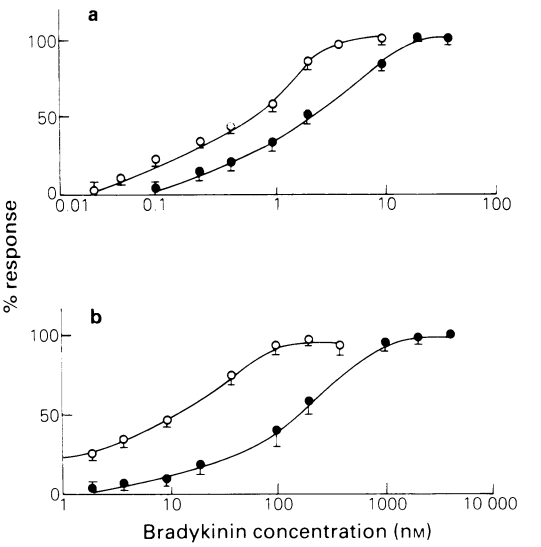

Figure 2 Log dose-response curves for the relaxant (a) and the contractile (b) components of the response to bradykinin. The curves were obtained within the first (●) and the fourth (○) hours after mounting of the preparation.

Bradykinin, des-Arg^9-bradykinin and the potentiating peptides listed in Table 1 were synthetic products made in this laboratory (Sabia *et al.*, 1977) and captopril (Squibb) was a kind gift from the Squibb Institute for Medical Research.

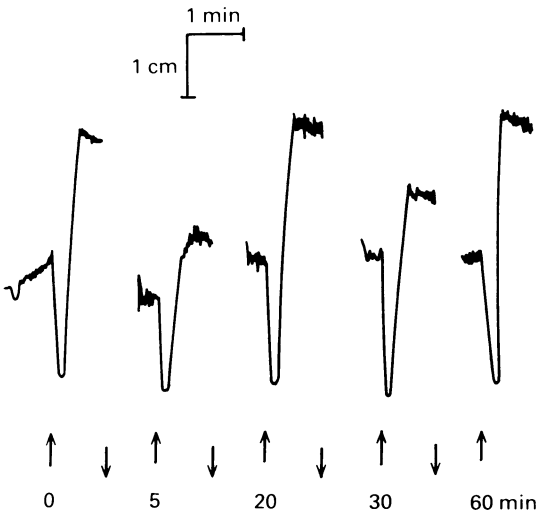

Figure 3 Responses of the rat duodenum to addition of bradykinin (19 nM), at different time intervals. Additions (upward arrows) were made at times 0, 5, 20, 30 and 60 min, and downward arrows indicate washing with fresh medium.

Results

Effect of time after mounting of the preparation on the response to bradykinin and to potassium

The responses of the rat isolated duodenum to bradykinin were markedly dependent on the concentration range studied. When administered within the first hour of the preparation, low doses of bradykinin induced only relaxation, which was dose-dependent in the concentration range of 0.1–20 nM (Figures 1a and 2a). At bradykinin concentrations above 10 nM the response became diphasic, consisting of a maximal relaxation followed by contraction (Figure 1a). The contractile component was dose-dependent in the range of 10–1000 nM (Figures 1a and 2b).

The two components of the response to bradykinin were also different with respect to desensitization (tachyphylaxis) which was observed for the contraction but not for the relaxation. When the interval between administrations was below 15 min there was loss of the contractile component, while the relaxant component was unaffected (Figure 3). The desensitization was reversible and no loss of the contractile component was observed when the interval between doses was 15 min or more.

The responses of the duodenum to bradykinin were also affected by the time elapsed after mounting of the preparation; the duodenum became more sensitive to bradykinin with time, showing the diphasic response at lower concentrations of the agonist (Figures 1b and 2). This increase in sensitivity is evident on comparing the pD_2 values estimated from the dose-response curves for the two components of the response, obtained during the first and fourth hours after mounting (Table 2).

In control experiments in which no load was applied to the preparation during the interval between the first and fourth hours, the dose-response curves to bradykinin did not change significantly with time.

Very similar behaviour was observed for the responses to an increased K^+ concentration in the medium. In freshly mounted preparations, small increases (below 10 mM) evoked only relaxation, while concentrations in the range 10–40 mM caused diphasic responses and higher concentrations elicited mostly contractile responses. With regard to the effect of time after mounting of the preparation, however, the responses to K^+ behaved differently from those to bradykinin. Although some increase in the amplitude of the relaxation component was observed (Figure 4), there were no significant changes in the pD_2 values for either component (Table 2).

We have also studied the responses to des-Arg^9-bradykinin, an analogue which is specific for B_1-bradykinin receptors (Regoli & Barabé, 1980). During the first hour, no response to this analogue was observed even when it was applied in concentrations much higher than those effective in other organs in which B_1-receptors are present. However, during the fourth hour, a dose-dependent contractile response was elicited in the concentration range 0.1 to 30 μM, and the pD_2 value is shown in Table 2. Only in 5% of these experiments was a relaxant component of the response observed which, however, was not dose-dependent.

Effect of ouabain on the relaxation induced by bradykinin and K^+

The relaxation induced by small increases of the extracellular K^+ concentration in other smooth muscle preparations has been attributed to membrane hyperpolarization as a result of stimulation of the Na^+/K^+-ATPase (Webb & Bohr, 1978; Ishii & Shimo, 1979). To test whether this mechanism might apply also for the relaxation induced by bradykinin or K^+ in the rat duodenum, we have studied the effect on these responses of ouabain, a potent inhibitor of the Na^+/K^+-ATPase. We found that ouabain, in concentrations that are reportedly effective in blocking the Na^+/K^+-ATPase, did not affect the relaxant effect of either agent (Figure 5). However, increasing the K^+ concentration in the medium from its normal value (2.7 mM) to 5.9 mM, which stimulates the Na^+/K^+-ATPase, blocked the relaxant response to K^+ but not that to bradykinin (Figure 6).

Table 2 pD_2 values for the contractile and relaxant components of the rat duodenum's responses to bradykinin (Bk) and to potassium in the first and fourth hours after mounting of the preparation

	First hour			*Fourth hour*		
	Bk	des-Arg^9-Bk	K^+	Bk	des-Arg^9-Bk	K^+
Relaxation	8.72 ± 0.08	NR	2.95 ± 0.05	9.30 ± 0.12	NR	3.04 ± 0.08
Contraction	6.69 ± 0.05	NR	1.71 ± 0.09	8.03 ± 0.07	5.9 ± 0.6	1.53 ± 0.05

N.R. indicates that no response was detected to the agonist. Values are means ± s.e.means of 6–8 experiments.

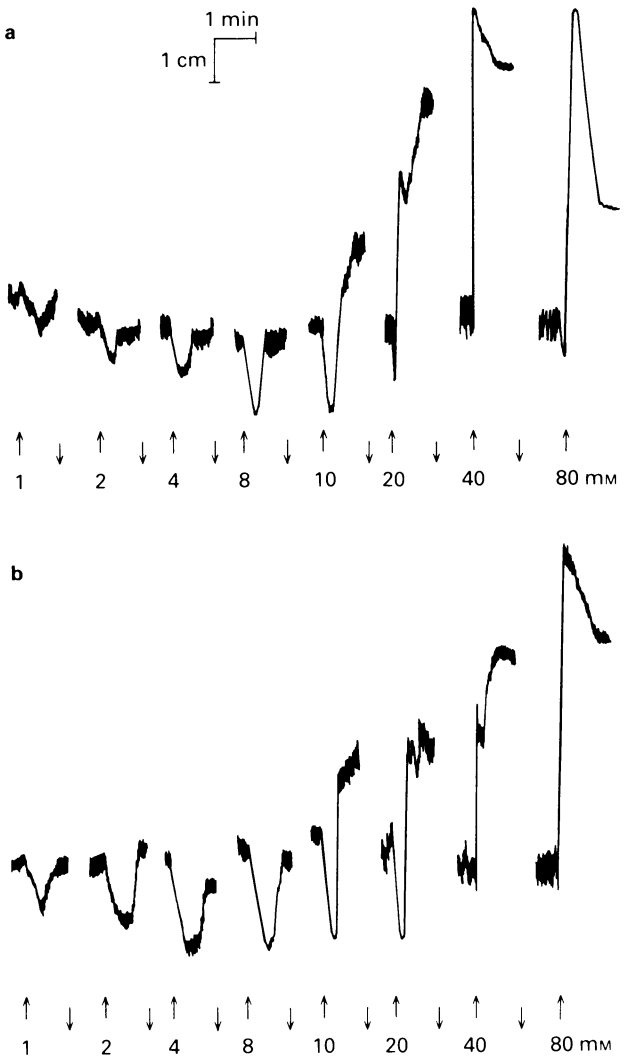

Figure 4 Effect of time after mounting of the rat duodenum on the responses induced by increasing external K^+ concentrations. (a) During the first hour after the 30 min equilibration period. (b) During the fourth hour. The K^+ concentrations applied to the organ bath are given in mM.

Effect of bradykinin potentiating peptides and captopril on the relaxant and contractile responses to bradykinin

The four bradykinin potentiating compounds that were studied (listed in Table 1) had different effects on the contractile and the relaxant components of the response of the rat duodenum to bradykinin.

The relaxation induced by low doses of bradykinin in recently mounted preparations was potentiated by potentiator C and by BPP9a (Figure 7), but not by BPP5a or by captopril (data not shown). Potentiator C elicited two fold potentiation at 0.4 μM, while 2 μM BPP9a was needed to produce the same effect. This contrasts with the greater bradykinin potentiating effect of BPP9a, relative to potentiator C, in other smooth muscles (Tominaga *et al.*, 1975).

Although captopril is a strong potentiator of the contractile response induced by bradykinin in other smooth muscle preparations (Ondetti *et al.*, 1977), it had no effect on the relaxant response of the duodenum.

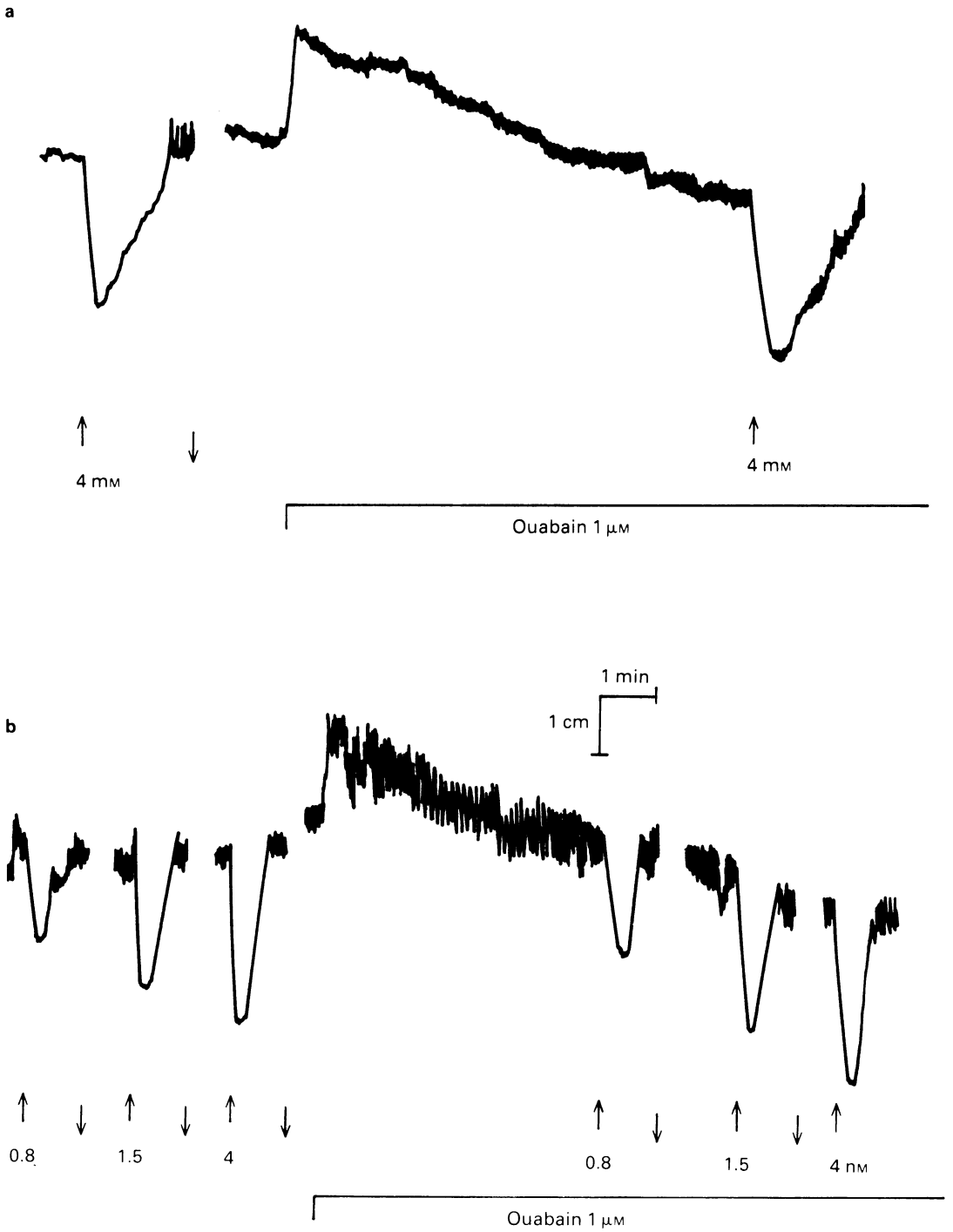

Figure 5 Effect of ouabain on the isotonic recording of the relaxation induced by K^+ (a) or by bradykinin (b) on the rat duodenum, in the absence and in the presence of 1 μM ouabain. Numbers below upward arrows indicate the molar concentration of the agent.

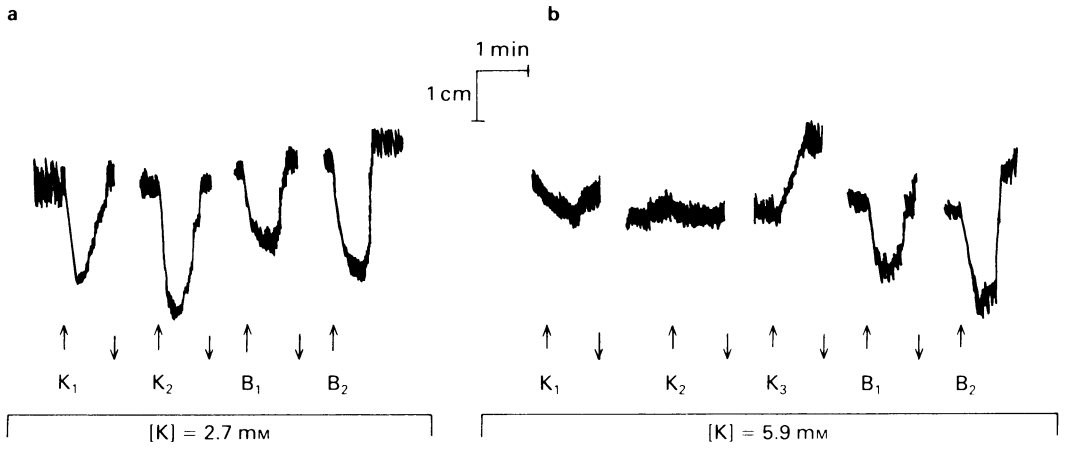

Figure 6 Effect of varying the K^+ concentration of the bathing medium on the relaxations induced by subsequent increases in K^+ concentration ($K_1 = 2$ mM; $K_2 = 4$ mM; $K_3 = 8$ mM) and by bradykinin ($B_1 = 7.5$ nM; $B_2 = 15.0$ nM) in the rat duodenum. (a) Normal medium (2.7 mM K^+). (b) After 30 min equilibrium in Tyrode solution containing 5.9 mM K^+.

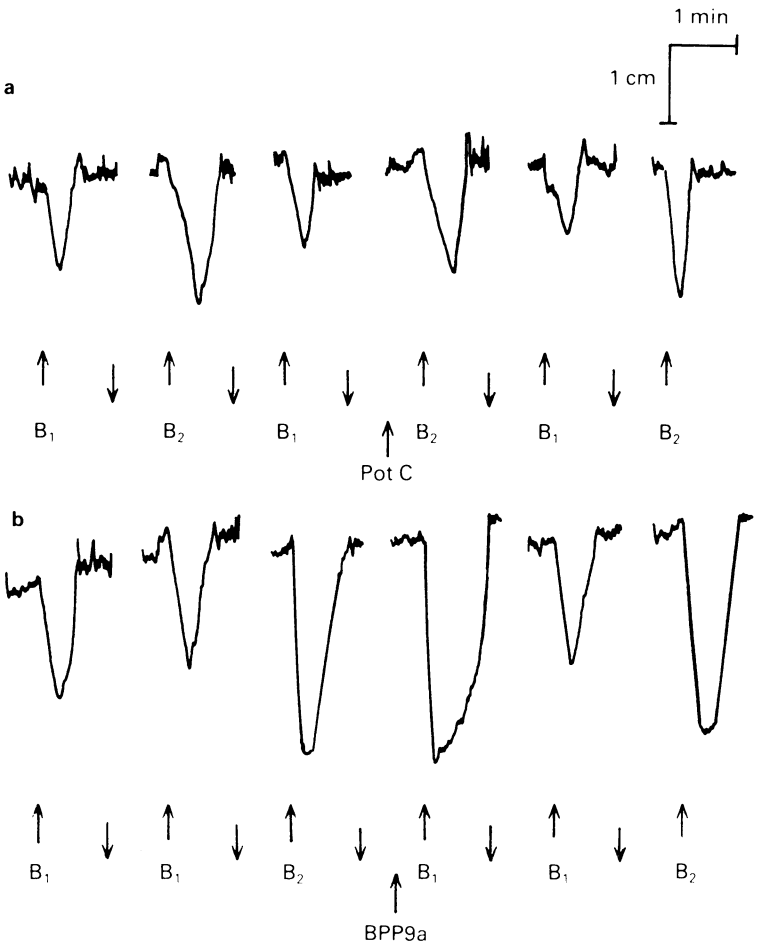

Figure 7 Potentiating effect of addition of (a) 0.4 μM potentiator C (Pot C) and (b) 2 μM BPP9a on the relaxant response of bradykinin ($B_1 = 3.8$ nM; $B_2 = 7.5$ nM) in the rat duodenum. Potentiators were added to the bath 30 s before bradykinin.

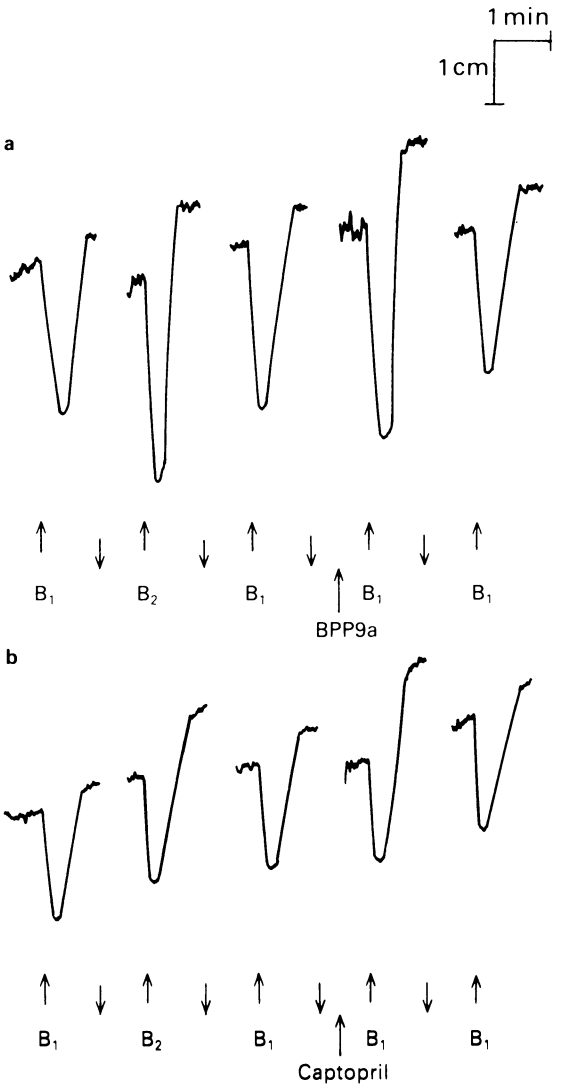

Figure 8 Potentiating effect of 2 μM BPP9a (a), and 0.5 μM captopril (b) on the contractile responses induced in the rat duodenum by bradykinin ($B_1 = 7.5$ nM; $B_2 = 15.0$ nM) in normal Tyrode solution four hours after mounting the preparation.Potentiators were added 30 s before bradykinin.

The effect of the potentiating agents on the contractile response of the duodenum was studied in organs which had been mounted for four hours or more, since these preparations contracted when stimulated by low doses of bradykinin. The contractile component of the response was not affected by potentiator C, but was potentiated by BPP9a, captopril (Figure 8) and BPP5a (data not shown).

Discussion

Relaxation is the typical response induced by low doses of bradykinin in the rat isolated duodenum in normal medium. Adrenergic mediation of this response was ruled out by Antonio (1968) and Hall & Bonta (1973).

Relaxation was also obtained in this preparation by small increases in the K^+ concentration of the medium (Figure 4), as has also been observed for smooth muscles from other tissues such as arteries and taenia coli. In these tissues, the relaxation in response to K^+ has been ascribed to membrane hyperpolarization due to activation of an electrogenic Na^+-pump, mediated by Na^+/K^+-ATPase within the smooth muscle membrane (Webb & Bohr 1978; Ishii & Shimo, 1979). To test whether this mechanism also applies in the rat duodenum we have studied the effect of K^+ and bradykinin under conditions commonly used to inhibit (ouabain) or stimulate (increased K^+) the Na^+/K^+-ATPase. The relaxation to K^+ was abolished by pre-incubation in a medium containing a higher than normal (5.9 mM) concentration of that ion (Figure 6). This result is similar to that observed in the taenia coli (Ishii & Shimo, 1979) and suggests that in the rat duodenum the Na^+/K^+-pump may also be involved in the relaxant response to K^+. However, ouabain, which inhibited K^+-induced relaxation in the taenia coli, was ineffective in rat duodenum (Figure 5). This may be due to insensitivity of the Na^+/K^+-ATPase of the rat duodenum to ouabain, since resistance of the enzyme to inhibition by this glycoside in other rat tissues has been demonstrated (Allen & Schwartz, 1969; Webb & Bohr, 1978; Karaki & Weiss, 1981).

The relaxation induced by bradykinin was not affected either by ouabain (Figure 5) or by increased K^+ in the medium (Figure 6), indicating that a mechanism other than stimulation of Na^+/K^+-ATPase may be involved in this case.

When higher doses of bradykinin are used, a diphasic response is elicited which consists of relaxation followed by contraction (Figure 1). However, this diphasic response can also be induced by low doses of bradykinin if the external Ca^{2+} concentration is reduced (Antonio, 1968). Under this experimental condition an increased Na^+ conductance occurs (Brading *et al.*, 1969) which could explain the development of the contractile response to bradykinin with low doses. The importance of Na^+ conductance for bradykinin activity has already been demonstrated (Aarsen, 1977). An increase in the bradykinin sensitivity of the guinea-pig ileum was also observed under different experimental conditions as described by Shimuta *et al.* (1981). These authors found that sensitivity of the guinea-pig isolated ileum to bradykinin increased as a function of

time after mounting of the preparation, which was attributed to stretching of the tissue by the load of the recording system, with consequent membrane depolarization due to an increased rate of K^+ loss and Na^+ exchange (Bülbring, 1955; Born & Bülbring, 1956; Freeman-Narrod & Goodford, 1962). The similar time-dependent increase in bradykinin sensitivity in the rat duodenum (Figures 1 and 2; Table 2) also appears to be due to stretching, since it did not occur in unloaded preparations, and might be explained by the same mechanism proposed for the guinea-pig ileum. This was a specific effect for bradykinin, since no changes in the pD_2 for K^+ occurred with time.

The observations that pD_2 values were significantly different for the relaxant and contractile responses induced by bradykinin (Table 2) and the fact that desensitization was observed only for the contractile component of the response (Figure 3) give support to the idea that different receptors may be involved in the two components of the response. This hypothesis is also supported by the finding that des-Arg^9-bradykinin produced only a contractile response, suggesting that this analogue can activate only one type of receptor. This might suggest that the receptors responsible for the contractile response could be similar to the B_1-receptors which are thought to occur in arteries and which are specifically activated by des-Arg^9-bradykinin (Regoli & Barabé, 1980). However, this cannot be the case as B_1-receptors have a greater affinity for des-Arg^9-bradykinin than for bradykinin, and the concentrations of that analogue needed to produce contraction of the duodenum were several orders of magnitude larger than those of bradykinin (Table 2).

The presence of two types of receptors for bradykinin has also been suggested by Camargo & Ferreira (1971) based on observations that bradykinin potentiating factor (BPF) potentiated only the contractile response but not the relaxation induced by the peptide in low-calcium medium. The results obtained in normal medium with pure synthetic bradykinin potentiators, under our experimental conditions (relaxant responses in freshly mounted preparations and contractile effects after four hours of stretching), showed further differences between the effects of the potentiating peptides on the two types of response. Among the longer chain peptides, potentiator C potentiated only the relaxant responses (Figure 7) while BPP9a affected both the relaxant and contractile responses (Figures 7 and 8). On the other hand, captopril (Figure 8) and BPP5a potentiated only the contractile responses. Our observation concerning the effect of BPP5a agrees with that found by Camargo & Ferreira (1971) but is at variance with that of Ufkes *et al.* (1976), probably because of the lower concentration of the potentiator used by the latter authors.

The discrimination of the two components of the response by their sensitivity to different potentiating peptides clearly indicates that the potentiation elicited by these peptides cannot be due solely to inhibition of bradykinin breakdown by tissue kininases. As proposed by other authors (Aarsen & van Caspel-de Bruyn, 1970; Camargo & Ferreira, 1971; Hall & Bonta, 1974; Shimuta *et al.*, 1981), at least part of the effect of these compounds must be due to an interaction with bradykinin receptors. Our results indicate that, in the rat duodenum, the receptors responsible for the relaxant and for the contractile effects can be distinguished by their apparent affinity for different potentiating peptides: the longer chain potentiator C acts only on the former, whereas the shorter chain peptides (BPP5a and captopril) act only on the latter, and BPP9a acts on both types of receptors.

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