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Selectivity of bradykinin analogues for receptors mediating contraction and relaxation of the rat duodenum

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1 Bradykinin produces a biphasic response in the rat duodenum that consists of a relaxation ($pD_2 = 8.44$) followed by a contraction ($pD_2 = 6.91$).

2 The B_1 agonist des-Arg^9-bradykinin produced a contraction ($pD_2 = 7.16$) but no relaxation. Des-Arg^9-[Leu^8]-bradykinin, which is a B_1 antagonist in other systems produced contraction ($pD_2 = 7.65$) in the rat duodenum.

3 Four bradykinin analogues that are preferential B_2 agonists in other tissues had a biphasic effect with pD_2 values in the range 7.22–8.68 for relaxation and 6.26–6.91 for contraction.

4 [Thi^{5,8},D-Phe^7]-bradykinin, which is a B_2 antagonist in most other systems produced relaxation in the rat duodenum, with a pD_2 of 7.49.

5 It is concluded that the contractile component of the response to bradykinin in rat duodenum may be mediated by a subtype of the B_1 receptor and the relaxant component by a receptor of the B_2 subtype.

Introduction

The response of the rat duodenum to bradykinin is biphasic: low concentrations of the agonist (below 10 nM) produce relaxation, whereas higher concentrations cause a relaxation followed by contraction (Antonio, 1968; Faber & van der Meer, 1973). The differential effects of bradykinin potentiating peptides on the two components of the response, the finding that only the contractile component is subject to desensitization (tachyphylaxis), and the observation that des-Arg^9-bradykinin elicits only a contractile response, have led to the proposal that two different receptor types may mediate the two components of the biphasic effect (Camargo & Ferreira, 1971; Boschcov *et al.*, 1984). However, binding studies with plasma membrane preparations could not detect more than one class of binding sites and led to the suggestion that one type of bradykinin receptor is able to activate different effector mechanisms in the rat duodenum (Liebmann *et al.*, 1987).

Extensive structure-activity relationship studies employing synthetic peptide analogues, and carried out in numerous smooth muscle preparations, have led to the conclusion that the receptors for bradykinin may be classified into two types. B_1 receptors are characterized by their preferential activation by des-Arg^9-bradykinin, and are specifically inhibited by des-Arg^9-[Leu^8]-bradykinin (Regoli & Barabé, 1980). B_2 receptors are insensitive to des-Arg^9-[Leu^8]-bradykinin but are selectively inhibited by analogues such as [Thi^{5,8},D-Phe^7]-bradykinin that contain a substitution by D-phenylalanine in position 7 of the bradykinin amino acid sequence (Vavrek & Stewart, 1985; Stewart & Vavrek, 1986).

In the present paper we present the results of an analysis of the response of the rat duodenum to analogues of bradykinin that have been shown to be

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selective agonists or antagonists for either B_1 or B_2 receptors. Our results indicate that the relaxation and contraction components of the response to bradykinin are due to stimulation of the B_2 and B_1 receptor types, respectively.

Methods

The rat duodenum preparation was set up as previously described (Boschcov *et al.*, 1984). Briefly, the duodenum from rats of either sex (190–220 g body weight) was suspended in a 5 ml chamber with a salt solution of the following composition (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 5.0. The bath solution was maintained at 37°C and bubbled with a mixture of CO_2 (5%) and O_2 (95%). The organs were submitted to a 1 g load and their isotonic contractions were recorded after a 30 min equilibration period.

The dose-response curves were obtained by administering the lower concentrations of the analogues (that produced only relaxation) at 10 min intervals and washing the preparation after a 90 s contact time. When higher concentrations were used, to elicit contractile responses, the interval between administrations was 30 min to avoid the tachyphylaxis that is observed for this component of the response. 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. The dose-response curves were analysed by linear regression of the double-reciprocal plot, from which ED_{50} values were obtained. The measurement of maximum responses relative to that of bradykinin was made by direct comparison of the effects of supramaximal concentrations.

All the peptides used in this study were synthetic products made in this laboratory, with the exception of [Thi^{5,8},D-Phe^7]-bradykinin which was kindly provided by Prof. J.M. Stewart, from the University of Colorado School of Medicine, Denver, CO, U.S.A.

Results

In our preparations, bradykinin induced a biphasic response; the ED_{50} was 3.6×10^{-9} M for the relaxation and 1.2×10^{-7} M for the contractile response.

The B_1 agonist des-Arg^9-bradykinin did not produce relaxation to any significant extent but was more potent than bradykinin in inducing contraction of the rat duodenum. The contractile response to this peptide also differed from that of bradykinin in that it was only very slowly reversed by washing of the preparation. In the duodenum contracted by bradykinin, baseline tension was fully restored within 5 min after washout, whereas the tonus caused by des-Arg^9-bradykinin usually had not returned to baseline 30 min after washout (Figure 1a). Interestingly in a tissue contracted by des-Arg^9-bradykinin, the administration of bradykinin elicited a biphasic response that was followed by fast relaxation after washing (Figure 1b). Tyr-bradykinin was even more effective than bradykinin in producing full relaxation of tissues previously contracted by des-Arg^9-bradykinin, after washout.

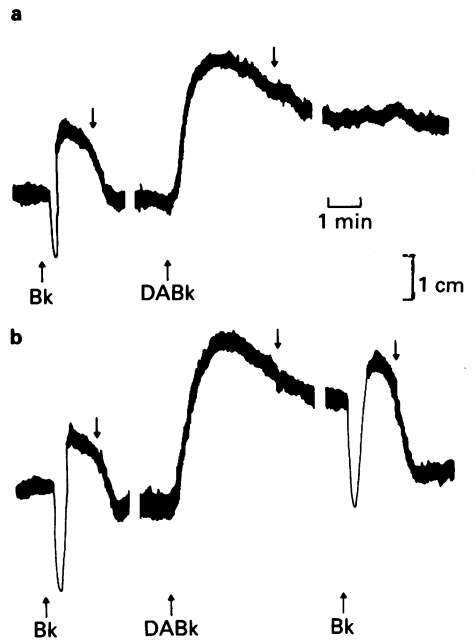

Figure 1 Responses of two rat duodenum preparations to 200 nM bradykinin (Bk) and to 200 nM des-Arg^9-bradykinin (DABk). Downward arrows indicate washings of the preparation with fresh medium. Interruptions of the tracings indicate a 30 min interval.

Cumulative dose-response curves for the contractile effect of des-Arg^9-bradykinin showed that this analogue is a full agonist, and yielded linear double-reciprocal plots from which an ED_{50} of 6.9×10^{-8} M ($pD_2 = 7.16 \pm 0.10$) was obtained.

Attempts to block the contractile effect of bradykinin with the B_1 antagonist des-Arg^9-[Leu^8]-bradykinin were compromised by the surprising finding that this compound was a powerful agonist for

Figure 2 Response of a rat duodenum preparation to increasing concentrations of des-Arg^9-[Leu^8]-bradykinin, ranging from 4 to 128 nM. Additions to the bath were made at 30 min intervals and the preparations were washed 1.5 min after each addition.

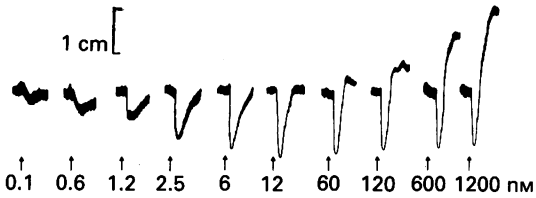

Figure 3 Responses of a rat duodenum preparation to increasing concentrations of Lys-Lys-bradykinin, ranging from 0.1 to 1200 nM. Additions to the bath were made at 10 min intervals up to 60 nM and at 30 min intervals thereafter. The preparations were washed 1.5 min after each addition.

producing contraction (Figure 2). This peptide produced no significant relaxant effect, and linear double-reciprocal plots for its contractile effect yielded an ED_{50} of 2.2×10^{-8} M ($pD_2 = 7.65 \pm 0.07$). However, the maximum response elicited by this analogue was only 40% of that caused by bradykinin, indicating that des-Arg^9-[Leu^8]-bradykinin is not a full agonist, although it is more potent than bradykinin when the respective ED_{50} values for producing contraction are compared.

Since no specific B_2 agonist is available, we studied the effects of several bradykinin analogues that are known to act at both types of receptor but with some preference for the B_2 type. Bradykinin itself has an apparent preference for B_2 receptors, as is indicated by pD_2 values of greater than 8 for B_2 receptor systems (such as the cat and guinea-pig ileum) and of the order of 6 for B_1 receptor systems (such as the rabbit aorta) (Regoli & Barabe, 1980; Regoli *et al.*, 1986). Besides bradykinin, we studied the responses of the rat duodenum to four synthetic bradykinin agonist analogues with modifications at the N-terminus (analogues 2–4, Table 1) and at the C-terminus (analogue 5). The four compounds elicited biphasic responses similar to those produced by bradykinin (Boschcov *et al.*, 1984), as is illustrated by the results obtained with Lys-Lys-bradykinin, shown in Figure 3. The four analogues were full agonists for both the relaxant and the contractile components of the response: pD_2 values for the relaxant effect were higher than those for the contractile effect (Table 1). The pD_2 values for the contractile effects showed little variation among the four analogues studied, ranging from 6.26 to 6.48; the efficacies of the analogues were between 22 and 37% relative to that of bradykinin.

A larger range of pD_2 values (from 7.22 to 8.68) was observed for the relaxant component of the responses, [Lys^9]-bradykinin showing the lowest activity (6% of that of bradykinin) whereas Lys-Lys-bradykinin was more potent than bradykinin (170% relative efficacy). The four analogues produced relaxation at concentrations 1–2 orders of magnitude lower than those needed for the contractile effects.

Our attempt to block the relaxant component of the bradykinin responses with the B_2 antagonist [Thi^{5,8},D-Phe^7]-bradykinin was frustrated by the finding that this compound itself behaved as an agonist for producing relaxation. Figure 4 shows that [Thi^{5,8},D-Phe^7]-bradykinin produced a dose-dependent effect in which only the relaxation component of the bradykinin response was observed.

Table 1 pD_2 values of preferential B_2 agonists on the rat isolated duodenum

Agonist	*Relaxant effect*	*Contractile effect*
1 Bradykinin	8.44 ± 0.08 (28)	6.91 ± 0.05 (16)
2 Lys-Lys-bradykinin	8.68 ± 0.07 (4)	6.38 ± 0.07 (10)
3 Tyr-bradykinin	8.04 ± 0.05 (4)	6.48 ± 0.07 (4)
4 Ile-Ser-bradykinin	7.35 ± 0.23 (4)	6.26 ± 0.09 (9)
5 [Lys^9]-bradykinin	7.22 ± 0.19 (4)	6.38 ± 0.08 (4)

pD_2 values were obtained from linear regressions of double reciprocal plots and the s.e.mean are indicated. The number of experiments is indicated in parentheses.

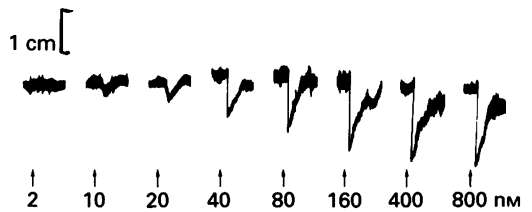

Figure 4 Responses of a rat duodenum preparation to increasing concentrations of [Thi^{5,8},D-Phe^7]-bradykinin, ranging from 2 to 800 nM. Intervals between additions were 10 min, and the preparation was washed 1.5 min after each addition.

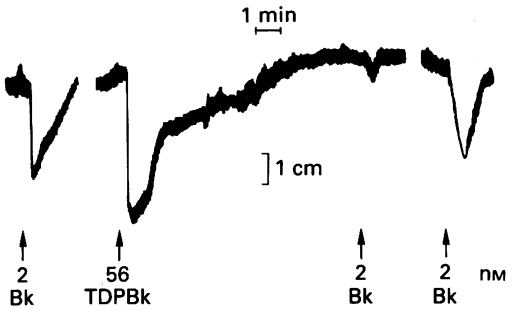

Figure 5 Inhibition of the relaxant response to bradykinin (Bk) in the presence of previously added [Thi^{5,8}, D-Phe^{7}]-bradykinin (TDPBk). The additions of the two compounds, at the concentrations shown, are indicated by the arrows. Interruptions of the tracing indicate washing of the preparation followed by a 30 min resting period.

The analogue behaved as a full agonist with an ED_{50} of 3.2×10^{-8} M ($pD_2 = 7.49 \pm 0.08$, $n = 4$).

Since the relaxant effect of [Thi^{5,8},D-Phe^{7}]-bradykinin is transient, we attempted to determine whether it might possess antagonist activity towards bradykinin by adding bradykinin to the bath after the relaxant effect of the analogue had subsided. Figure 5 shows that the relaxant effect of bradykinin was inhibited in the presence of [Thi^{5,8},D-Phe^{7}]-bradykinin. A study of the effect of different concentrations of this compound on bradykinin dose-response curves by the method of Arunlakshana & Schild (1959), yielded a pA_2 value of 7.50. Interestingly, this value is the same as that for the pD_2 for [Thi^{5,8},D-Phe^{7}]-bradykinin, indicating that the inhibition of the responses to bradykinin shown in Figure 5 may represent true competition between two agonists.

Discussion

The two components of the biphasic response of the rat duodenum to bradykinin have been attributed to different mechanisms. The relaxant effect was shown to be due to a direct action on the smooth muscle (Antonio, 1968; Ufkes & Van der Meer, 1975), and the response appears to be mediated by stimulation of adenylate cyclase (Liebmann *et al.*, 1987). Although two different types of receptors have been postulated for the relaxant and contractile effects (Camargo & Ferreira, 1971; Boschcov *et al.*, 1984), receptor binding studies with rat duodenum membrane preparations revealed a single binding site with an equilibrium dissociation constant of 1.0×10^{-9} M (Liebmann *et al.*, 1987). Our present results, however, clearly indicate that different types of bradykinin receptor are involved in the two components of the response.

The finding that the selective B_1 agonist des-Arg^{9}-bradykinin has a marked ability to produce contraction but not relaxation is strong evidence that the contractile component of the response to bradykinin is due to its interaction with receptors of the B_1 type. The contractile effect of des-Arg^{9}-bradykinin in the rat duodenum is characterized by its persistence after washout of the preparation, which appears to be due to a slow dissociation from the receptors.

Des-Arg^{9}-[Leu^{8}]-bradykinin, which has been shown to be an antagonist in other B_1 receptor systems, did not produce relaxation but possessed a high degree of efficacy for producing contraction of the rat duodenum and was the most potent of the analogues studied in this respect. To our knowledge this is the first description of an agonist action of des-Arg^{9}-[Leu^{8}]-bradykinin, and this observation characterizes the receptor mediating contraction of the rat duodenum as belonging to a subtype of B_1 receptors that, besides binding des-Arg^{9}-[Leu^{8}]-bradykinin, is also activated by this analogue. It must be pointed out, however, that this analogue is not as efficacious as bradykinin in giving rise to a stimulus when it binds to the B_1 receptor, i.e. des-Arg^{9}-[Leu^{8}]-bradykinin is a partial agonist.

With regard to the relaxant component of the bradykinin response, the data presented in Table 1 indicate that it is due to an interaction of the agonists with receptors of the B_2 type. Also in this case, we have found that the analogue [Thi^{5,8},D-Phe^{7}]-bradykinin, which is an antagonist of the B_2 receptor in other tissues, behaves as an agonist in the rat duodenum. Its relaxing effect, like that of bradykinin, is transient, and this permitted the determination of its effect on subsequent responses to bradykinin. The inhibition of responses to bradykinin in the presence of [Thi^{5,8},D-Phe^{7}]-bradykinin (Figure 5) was similar to the inhibition observed in the presence of a previous dose of bradykinin itself. This indicates that, although the relaxant response to either compound had subsided, the receptors may still have been occupied. The inhibition of the response to bradykinin by [Thi^{5,8},D-Phe^{7}]-bradykinin, cannot be attributed to an antagonist action of the latter compound, however, since it may only reflect some degree of refractoriness of the effector system. This finding suggests that it will be important not to neglect a possible transient agonistic effect in studies of supposed antagonists.

Although [Thi^{5,8},D-Phe^{7}]-bradykinin has been shown to be an antagonist of bradykinin in most of

the B_2 receptor systems that have been studied (Regoli *et al.*, 1986), there are published reports of agonist effects of that compound in vas deferens (Llona *et al.*, 1987) and in neuroblastoma cells (Braas *et al.*, 1988). It appears that in these two systems, as well as in the rat duodenum, there are receptors of a B_2 subtype that are characterized by an agonist response to [Thi^{5,8},D-Phe^7]-bradykinin. Indeed the evidence points to the existence of multiple bradykinin receptors in different tissues, as suggested by other studies of structure-activity relationships (Braas *et al.*, 1988).

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