British Journal of Pharmacology (1996) 118, 289-294

Receptors for kinins in the human isolated umbilical vein

F. Gobeil, L.H. Pheng, I. Badini, X.K. Nguyen-Le, A. Pizard, A. Rizzi, *D. Blouin & [1]D. Regoli

Department of Pharmacology, Medical School, Université de Sherbrooke, 3001 12th Avenue North, Sherbrooke (Québec) J1H 5N4, Canada and *Department of Obstetrics and Gynecology, Centre hospitalier universitaire de Sherbrooke, 3001 12th Avenue North, Sherbrooke (Québec) J1H 5N4, Canada

1 The human umbilical vein has been found to contract in response to bradykinin (BK) and desArg^{9}BK.

2 The rank order of potency of agonists, in the presence of the B_1 receptor antagonist Lys[Leu^8]desArg^{9}BK, is as follows: [Hyp^3,Tyr(Me)^8]BK (pD_2 8.88) = [Hyp^3]BK (pD_2 8.86) = LysBK (pD_2 8.81) $\geq$ BK (pD_2 8.60) > > [Aib^7]BK (pD_2 6.38) > > desArg^{9}BK and LysdesArg^{9}BK (inactive).

3 Hoe 140 (pA_2 8.42) inhibits the effects of BK while other B_2 receptor peptide antagonists are very weak and WIN 64338 is practically inactive.

4 Venoconstrictor responses to desArg^{9}BK of fresh tissues increase with time during the *in vitro* incubation and reach a maximum after 4-6 h. The activity of Hoe 140 (pA_2 5.48) is negligible against B_1 receptor agonists.

5 When measured in the presence of the selective B_2 receptor antagonist Hoe 140 (400 nM), the order of potency of kinin related peptides on the B_1 receptor is Lys[desArg^9]BK (pD_2 8.60) > desArg^{9}BK (pD_2 6.69). BK, LysBK, [Hyp^3]BK and other B_2 receptor agonists are inactive.

6 The B_1 receptor antagonist, Lys[Leu^8]desArg^{9}BK (pA_2 7.99), inhibits the response of the human vein to B_1 receptor agonists (LysdesArg^{9}BK or desArg^{9}BK), but do not alter the effect of BK.

7 The results summarized in this paper indicate that the human isolated umbilical vein is a sensitive preparation containing both B_1 and B_2 receptors. The human B_2 receptor shows some similarity with that of the rabbit (at least for agonist potencies) and differs from the B_2 receptor of the guinea-pig. Compared to the rabbit B_1 receptor, the human B_1 receptor shows low sensitivity to peptides that lack the N-terminal Lys.

Keywords: Bradykinin; smooth muscle; human umbilical vein; B_1 and B_2 receptors; agonists; antagonists

Introduction

Bioassays have been instrumental in the identification and characterization of the two receptor types, B_1 and B_2, that subserve the biological effects of bradykinin (BK) and related kinins (Regoli & Barabé, 1980). Recent reports confirm the existence of the two pharmacological entities through the identification of the nucleotide sequences encoding for the rat (McEachern *et al.*, 1991), the human (Eggerickx *et al.*, 1992; Hess *et al.*, 1992) and the mouse (Hess *et al.*, 1994) B_2 receptors. Human and rabbit B_1 receptors have also been cloned and expressed in appropriate cell systems (Menke *et al.*, 1994; McNeil *et al.*, 1995). Cloning of B_2 receptors has revealed that a single genetic sequence is found in each species: however, differences have been found between species, i.e. human/mouse for the B_2 receptor (Hess *et al.*, 1994), human/rabbit for the B_1 receptor (Menke *et al.*, 1994; McNeil *et al.*, 1995). Such species differences have led to the suggestion that classical pharmacological assays of new compounds, particularly antagonists, interacting with kinin receptors, should be carried out, when possible, in human tissues. The presence of B_2 and/or B_1 receptors has been demonstrated in various organs in man, including the colon (Couture *et al.*, 1981), the urinary bladder and the stomach (Gobeil, F., unpublished data) and pulmonary fibroblasts (Goldstein & Wall, 1984). Moreover, experiments in animals over the past twenty years have shown that isolated vessels (both arteries and veins) provide the most sensitive and reliable preparations for studying both B_1 and B_2 receptors (Regoli & Barabé, 1980; Regoli *et al.*, 1993). In the present investigation, the human umbilical vein was selected because it responds to bradykinin and to desArg^{9}BK and therefore should allow characterization of B_1 and B_2 receptors. This preparation was initially described by Altura *et al.* (1972) and more recently used by Marceau *et al.* (1994) and Félétou *et al.* (1995) in kinin pharmacology.

Methods

Pharmacological assays

Human umbilical cords ($n = 95$) from women 23 to 40 years old were collected after spontaneous delivery at term and used immediately or used after storage at 4°C for no longer than 12 h in oxygenated Krebs-Ringer bicarbonate buffer (pH 7.4). In control experiments, some human cords ($n = 7$) kept for 12-48 h in Krebs solution at 4°C were found to be insensitive to desArg^{9}BK. In the same conditions, the maximal effect of BK was markedly attenuated. Therefore, human cords conserved longer than 12 h were discarded. Segments of 25 cm were cut from the cords midway and handled according to Altura *et al.* (1972). The tissues were then cut helically (3-4 mm wide and 1.0-1.5 cm long), and the endothelium was mechanically removed by gently rubbing the internal surface of the strip with a moistened filter paper. The strips were suspended in 10 ml organ baths and stretched with an initial tension of 2 g. Changes in tension were measured with Grass isometric transducers (FT 03C, Grass Instrument Co., Quncy, Mass, U.S.A.) and displayed on Grass polygraphs (Model 7D). Before testing any agent, the preparations were allowed to equilibrate for 60-90 min, during which time fresh physiological medium was applied and the tension readjusted at 15-min intervals. A standard submaximal concentration of BK (5 nM) was tested repeatedly to ensure that the myotropic response to

[1] Author for correspondence.

this B_2 receptor agonist was stable. Similarly, the B_1 receptor agonist, desArg^{9}BK (550 nM), was tested repeatedly during 3–6 h of incubation. The myotropic effects of all agonists were evaluated in the presence of captopril (1 μM) and/or mergetpa (1 μM) (respectively, a kininase II and kininase I inhibitors) preincubated 30 min before measuring full concentration-response curves. In pilot assays, pD_2 values of BK and desArg^{9}BK were determined by consecutive or cumulative concentration-response curves and found to be identical. Cumulative concentration-response curves were then measured for kinin B_2 (BK, LysBK, [Hyp^3]BK, [Hyp^3,Tyr(Me)^8]BK, [Aib^7]BK; (Regoli *et al.*, 1994*b*)) and B_1 (desArg^{9}BK, LysdesArg^{9}BK; (Regoli & Barabé, 1980) receptor agonists in order to determine their apparent affinities in terms of pD_2 (the negative log of the concentration of the agonist that produces 50% of the maximal effect (α^E)). Antagonists were tested against BK for the B_2 receptor and desArg^{9}BK or LysdesArg^{9}BK for the B_1 receptor. Antagonists were applied 10 min before the agonist to estimate pA_2 values according to Schild (1947). All kinin antagonists were applied initially at concentrations of 10 μg ml^{-1} to measure their potential agonistic activities (α^E). In some experiments, at least three concentrations of Hoe 140 and Lys[Leu^8]desArg^{9}BK were used to construct Schild plots and validate the experimental pA_2 values obtained according to the method of Schild (1947).

Drugs

All peptides, except Hoe 140 (Icabitant), were prepared by solid-phase synthesis and purified by high pressure chromatography, as described elsewhere (Drapeau & Regoli, 1988). Abbreviations of non-natural residues used for the peptide synthesis are as follows: Aib: 2-aminoisobutyric acid; Hyp: *trans*-4-hydroxy-L-proline; Thi: β-(2-thienyl)-L-alanine; DTic: D-(1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid); Oic: L-(3aS,7aS)-octahydro-indol-2-carboxylic acid. Hoe 140 (D-Arg [Hyp^3, Thi^5, D-Tic^7, Oic^8]BK (Hock *et al.*, 1991) was made available by Hoechst AG (Frankfurt, Germany), WIN 64338 (phosphonium, [[4-[[2[[*bis*(cyclohexylamino)methylene]amino]-3-(2-naphtalenyl)-1- oxopropyl]amino]phenyl]methyl]tributyl, chloride, monohydrochloride) (Salvino *et al.*, 1993) was supplied by Sterling Winthrop Pharmaceutical Research Division (Collegeville, PA, U.S.A.). Concentrated solutions (1 to 5 mg ml^{-1}) of peptides and other agents were made in twice distilled and deionised water and kept at −20°C. WIN 64338 was solubilized either in water, or in dimethyl sulphoxide (DMSO) (10%) or in ethanol (10%). Captopril ([2S]-1-[3-mercapto-2-methyl-propionyl]-L-proline) was purchased from Squibb Canada and was dissolved in isotonic saline, while Mergetpa (DL-2-mercaptomethyl-3-guanidinoethyl propanoic acid), obtained from Calbiochem-Boehringer (San Diego, CA, U.S.A.), was dissolved in 10% of DMSO.

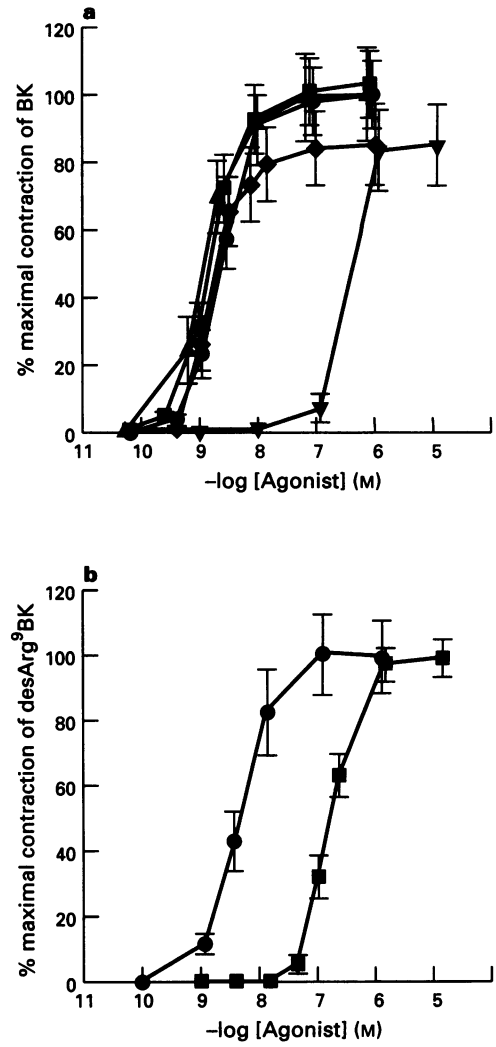

Figure 2 Concentration-response curves of (a) B_2 and (b) B_1 receptor agonists on the human umbilical vein deprived of endothelium. Data are means±s.e.mean of 7 to 20 experiments. B_2 receptor: (◆) [Hyp^3,Tyr(Me)^8]BK; (▼) [Hyp^3]BK; (▲) [Aib^7]BK; (■) KD; (●) BK. B_1 receptor: (●) LysdesArg^{9}BK; (■) desArg^{9}BK.

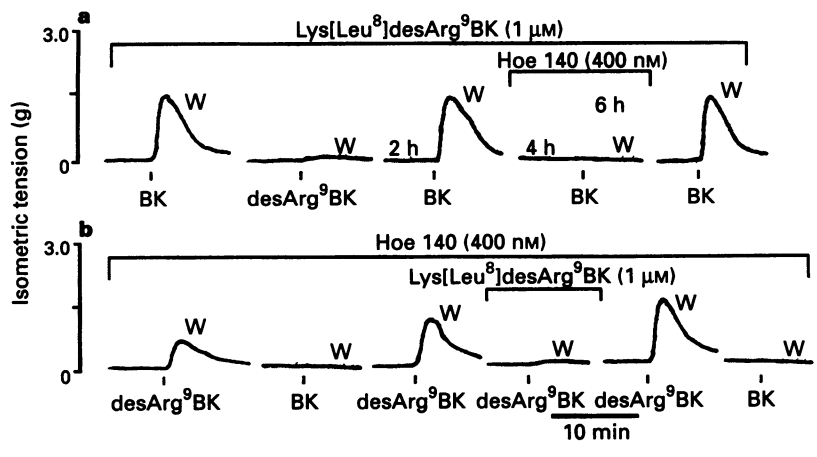

Figure 1 Tracings obtained with kinins on the human isolated umbilical vein without endothelium. (a) Effects of bradykinin (BK), measured in the presence of the B_1 receptor antagonist, Lys[Leu^8]desArg^{9}BK (1 μM): this effect is blocked by Hoe 140 (400 nM). (b) Effects of desArg^{9}BK (DBK) measured in the presence of Hoe 140 (400 nM); this effect is blocked by Lys[Leu^8]desArg^{9}BK (1 μM). Abscissa Scale: time (2, 4, 6 h). Ordinate Scale: isometric tension in grams (g). W: indicates washing out of agents.

Statistics

pD_2 values of agonists and pA_2 values for antagonists are presented as means ± s.e.mean. Data were analyzed statistically with Student's t test for independent samples. P values less than 0.05 were considered to be significant. Schild plot slopes were calculated from the experimental points using the Tallarida & Murray (1987) computer programme.

Results

Control experiments were performed to set up the experimental conditions for studying the myotropic effects of bradykinin (BK), desArg^{9}BK or LysdesArg^{9}BK. The contractions of the human umbilical vein (HUV) to BK or desArg^{9}BK were not modified by pretreatment of the tissues for 30 min with atropine (1 μM) or indomethacin (1 μM) ($n=4$, $P>0.05$, data not shown) confirming the finding by Marceau *et al.* (1994). A mixture of peptidase inhibitors, including bacitracin, bestatin, captopril, mergetpa, chymostatin and thiorphan (all applied at concentrations of 1 μM for 30 min), did not exert any direct effect on tissue baseline nor significantly modify the responses of the vein to BK and desArg^{9}BK ($n=4$, $P>0.05$, results not shown). Captopril (1 μM) and mergetpa (1 μM) were however used in all assays in order to prevent kinin degradation that may occur in some tissues.

Tracings showing the myotropic responses of the HUV to BK or desArg^{9}BK are shown in Figure 1 in order to give qualitative insights of HUV responses to kinins. In the presence of captopril (1 μM), mergetpa (1 μM) and the B_1 receptor antagonist, Lys[Leu^8]desArg^{9}BK (1 μM), the response of HUV to BK (5 nM) is reproducible and stable for 6 to 12 h; desArg^{9}BK (500 nM) is inactive (Figure 1a). The myotropic effect of BK is completely blocked by Hoe 140 (400 nM), given 10 min before the agonist (Figure 1a). On the other hand, the response of the HUV to two consecutive concentrations of desArg^{9}BK (500 nM), in the presence of the B_2 receptor antagonist (Hoe 140, 400 nM), increases during the *in vitro* incubation up to 6 h and this effect is completely blocked by Lys[Leu^8]desArg^{9}BK (1 μM) appled 10 min before; BK (5 nM) is inactive (Figure 1b). These results suggest a time-dependent induction of B_1 receptors on the HUV. The antagonistic effects of Hoe 140 against BK and of Lys[Leu^8]desArg^{9}BK against desArg^{9}BK are reversible, since the contractile effects of the two agonists is fully recovered, 15 min after washing out the antagonists (not shown).

Figure 2 shows concentration-response curves obtained with several B_2 receptor agonists (Figure 2a) and those measured with two B_1 receptor agonists (Figure 2b). The pD_2 values of the agonists as well as their intrinsic activities (α^E) are summarized in Table 1. Four ([Hyp^3,Tyr(Me)^8]BK, [Hyp^3]BK, LysBK and BK) of the five B_2 receptor agonists show very similar affinities, while [Aib^7]BK (pD_2 6.38 ± 0.03) ($n=8$, $P<0.001$) is weaker than the others on the human B_2 receptor. Maximal responses elicited by [Hyp^3,Tyr(Me)^8]BK and [Aib^7]BK, although apparently weaker, are not significantly different from those of the other B_2 receptor agonists (Figure 2). The two B_1 receptor agonists differ only in their affinities, desArg^{9}BK (pD_2 6.69 ± 0.09) being approximatively hundred fold weaker ($n=23$, $P<0.001$) than LysdesArg^{9}BK (pD_2 8.60 ± 0.16) (Figure 2b).

All kinin receptor agonists tested in this study exhibit full agonistic activities and their apparent affinities, expressed in term of pD_2, have been used to establish the order of potency of agonists which, for the B_2 receptor, is: [Hyp^3,Tyr(Me)^8]BK = [Hyp^3]BK = LysBK ≥ BK > > [Aib^7]BK, the B_1 receptor agonists, desArg^{9}BK and LysdesArg^{9}BK, being inactive; and for the B_1 receptor is: LysdesArg^{9}BK > desArg^{9}BK. BK, LysBK and all other B_2 receptor agonists are inactive. When tested on the B_2 receptor against BK, Hoe 140 shows a pA_2 value of 8.42 ± 0.07, D-Arg[Hyp^3, D-Phe^7, Leu^8]BK has a pA_2 of 5.50 ± 0.07, the other three compounds are inactive. Worthy of mention is the finding that WIN 64338 has been found to be inactive ($pA_2 < 5.0$), when dissolved in water, DMSO or ethanol. When tested against the B_1 receptor agonist desArg^{9}BK, Lys[Leu^8]desArg^{9}BK shows a pA_2 value of almost 8.0, [Leu^8]desArg^{9}BK has a pA_2 of only 6.37 ± 0.06, Hoe 140 and D-Arg[Hyp^3, D-Phe^7, Leu^8]BK maintain some antagonistic activity while WIN 64338 is inactive.

Tracings showing cumulative concentration-response curves obtained with BK (Figure 3a) and with desArg^{9}BK (Figure 3b) in the absence (Figure 3, left) and in presence (Figure 3, right) of Hoe 140 or Lys[Leu^8]desArg^{9}BK are presented in Figure 3. The two antagonists have no contractile effects and displace the concentration-response curves of their respective agonist to the right; the maximal responses are recovered by applying high doses of agonists, suggesting that the antagonists act competitively at these concentrations. Schild regression analyses for the two antagonists were then made to define more precisely the type of antagonism. For this purpose, concentration-response curves to BK (Figure 4, a) and Lys-desArg^{9}BK (Figure 4, b) were obtained in the

Table 1 Pharmacological profiles of kinin receptor agonists and antagonists on the endothelium-deprived human umbilical vein

Compound	*B_2 receptor*			*B_1 receptor*		
Agonist	*pD_2*	*RA*	*α^E*	*pD_2*	*RA*	*α^E*
BK	8.60 ± 0.09	100	1.00		Inactive	
[Hyp^3]BK	8.86 ± 0.11	182	1.00		Inactive	
[Aib^7]BK	6.38 ± 0.03	0.6	0.87		Inactive	
[Hyp^3,Tyr(Me)^8]BK	8.88 ± 0.03	191	0.85		Inactive	
Kallidin (LysBK)	8.81 ± 0.13	162	1.03		Inactive	
desArg^{9}BK		Inactive		6.69 ± 0.09	100	1.00
Lys-desArg^{9}BK		Inactive		8.60 ± 0.16	8134	1.00
Antagonists		*pA_2*	*α^E*		*pA_2*	*α^E*
D-Arg[Hyp^3,D-Phe^7,Leu^8]BK		5.50 ± 0.07	0		5.56 ± 0.12	0
WIN 64338		Inactive	0		Inactive	0
Hoe 140		8.42 ± 0.07	0		5.48 ± 0.15	0
[Leu^8]desArg^{9}BK		Inactive	0		6.37 ± 0.06	0
Lys[Leu^8]desArg^{9}BK		Inactive	0		7.99 ± 0.01	0

pD_2 -log of the concentration (M) of agonist that produces 50% of the maximal effect.
pA_2 -log of the concentration (M) of the antagonist that reduces the effect of a double concentration of the agonist to that of a single concentration.
α^E: Maximal effect (intrinsic activity).
RA: relative affinity in percent of bradykinin (BK) for the B_2 receptor and of desArg^{9}BK for the B_1 receptor.
Inactive indicates that the compound has no detectable effect when applied at concentration of 5 μM or less.
Data are means ± s.e.mean of 7 to 23 experiments.

absence or in the presence of increasing concentrations of Hoe 140 (2–800 nM) (Figure 4a) and Lys[Leu^8]desArg^{9}BK (0.1–10 μM) (Figure 4b). The concentration-response curves of BK and LysdesArg^{9}BK show a rightforward and parallel displacement (in the presence of the antagonist) with respect to the control curves and the maximum effects are maintained. From experimental points of the concentration-response curves, Schild plots were drawn, as shown in the right panels of Figure 4. The slopes of the linear Schild regressions are not significantly different from unity ($P>0.05$) (slope 0.99 ± 0.06, correlation 0.9, $n=10$ for Hoe 140) (slope 0.89 ± 0.08, correlation 1.0, $n=12$ for Lys[Leu^8]desArg^{9}BK) and yield extrapolated pA_2 values of 8.49 ± 0.09 for Hoe 140 and 7.82 ± 0.18 for Lys[Leu^8]desArg^{9}BK. These values are very similar to those obtained with the classical Schild's protocol (see Table 1).

Discussion

The results presented above indicate that the human umbilical vein possesses B_1 and B_2 receptors for the kinins. To our knowledge, this is the first study in which the presence of a functional B_1 receptor has been demonstrated in a human vessel. B_1 receptors have been reported to mediate contractile effects of kinins in various human tissues (Regoli & Barabé, 1980), particularly the human colon (Couture *et al.*, 1981). More recently, contractile effects of desArg^{9}BK have been demonstrated in the human stomach and urinary bladder (Gobeil, F., unpublished results). B_1 receptors were also shown to be present in foetal pulmonary fibroblasts by Goldstein & Wall, (1984). Thus, the B_1 receptor may be found in a variety of human tissues, including vessels. The present results conflict with two recent reports by Marceau *et al.* (1994) and Félétou *et al.* (1995) in which the presence of B_1 receptors in the human umbilical vein was not observed. The nature of this discrepancy is not known.

In an attempt to obtain an extensive and precise pharmacological characterization of vascular human B_1 and B_2 receptors; (a) B_1 and B_2 receptor agonists and antagonists were tested in the presence of captopril to prevent their degradation by kininase II; (b) the B_2 receptor agonists were also tested in the presence of mergetpa to avoid their conversion to B_1 receptor agonists; (c) five compounds were used to establish the order of potency of agonists on the B_2 receptor and two compounds for the B_1 receptor, in order to characterize (with agonists) and then compare the human receptors with those of other species; (d) three generations of antagonists, the early peptidic such as D-Arg[Hyp^3, D-Phe^7, Leu^8]BK (Regoli *et al.*, 1990; Roleb *et al.*, 1991), the peptidic Hoe 140 which is long acting and resistant to degradation by peptidases (Hock *et al.*, 1991; Wirth *et al.*, 1991) and the non peptide WIN 64338 (Salvino *et al.*, 1993), were used to characterize the B_2 receptor, to apply the second criterion of receptor classification recommended by Schild (1973) and again compare the human B_2 receptor with those of other species. The human B_1 receptor was characterized with the two most selective and specific antagonists available at present (Regoli *et al.*, 1990; 1994b).

Results obtained with agonists indicate that the human B_2 receptor of the HUV is stimulated by peptides possessing a C-terminal Arg and is insensitive to desArg^9 compounds, similar to the B_2 receptor of other species (rabbit, guinea-pig, etc.) (Regoli & Barabé, 1980) and in accord with recent reports by Marceau *et al.* (1994) and Félétou *et al.* (1995). Affinity of kinin-related peptides for the human B_2 receptor is favoured by the presence of a Hyp residue (which occurs in human bradykinin) in position 3 and by a Lys at the N-terminal, as in Lys BK. [Hyp^3]BK shows higher affinity for the human B_2

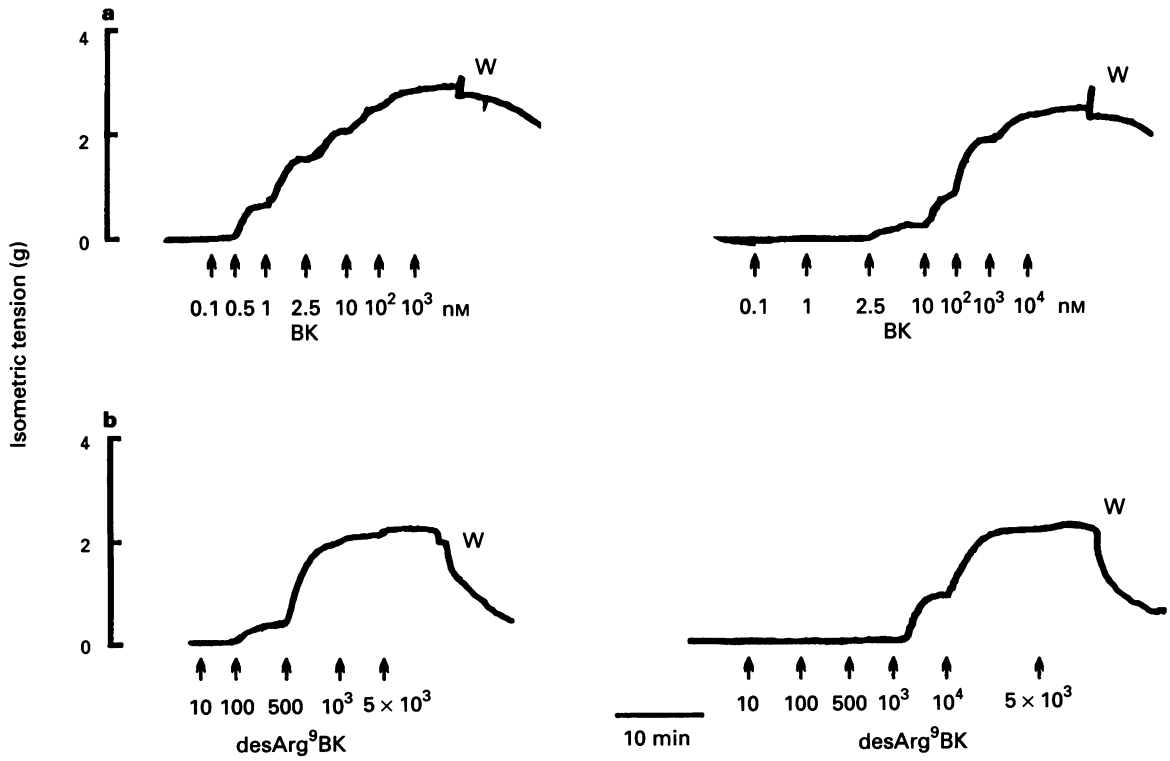

Figure 3 Changes of isometric tension in grams (g) induced by kinins in the human isolated umbilical vein devoid of endothelium. (a) Myotropic effects of increasing concentrations of BK in the absence (on the left) and in presence (on the right) of Hoe 140 (80 nM) preincubated for 10 min. (b) Effects of desArg^{9}BK in the absence (on the left) and in the presence (on the right) of Lys[Leu^8]desArg^{9}BK (0.5 μM). A time-delay of 120 min is left between each concentration-effect curves of BK or desArg^{9}BK. Abscissa Scale: Tension in g. Ordinate Scale: Time in minutes. W: indicates that agents have been washed out.

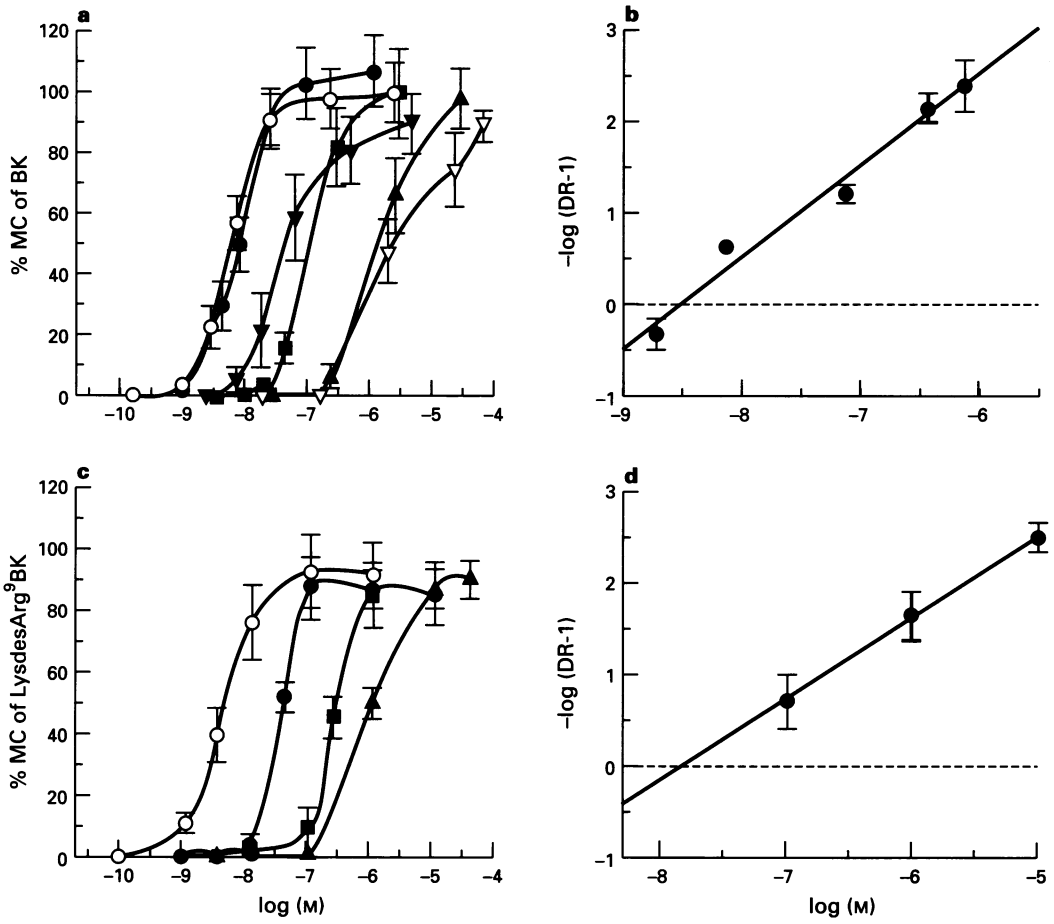

Figure 4 (a) Concentration-response curves of BK obtained in the HUV in the absence and in presence of various concentrations of Hoe 140; (○) BK; (●) Hoe 140 2×10^{-9} M; (▼) Hoe 140 8×10^{-9} M; (■) Hoe 140 7.7×10^{-8} M; (▲) Hoe 140 38.3×10^{-8} M and (▽) Hoe 140 7.7×10^{-7} M. (b) Schild plot of Hoe 140 against BK. (c) Concentration-response curves of LysdesArg^{9}BK obtained in the HUV in the absence and in presence of various concentrations of Lys[Leu^8]desArg^{9}BK; (○) LysdesArg^{9}BK; (●) Lys[Leu^8]desArg^{9}BK 10^{-7} M; (■) Lys[Leu^8]desArg^{9}BK 10^{-6} M; (▲) Lys[Leu^8]desArg^{9}BK 10^{-5} M. (d) Schild plot of Lys[Leu^8]desArg^{9}BK against LysdesArg^{9}BK. Abcissa Scale: In (a) and (c): negative log of the molar concentration of agonist. In (b) and (d): negative log of the molar concentration of the antagonist. Ordinate Scale: In (a) and (c): MC: Maximal contraction. Contractile responses of the HUV expressed in percentage of that of BK (a) or of LysdesArg^{9}BK (c). In (b) and (d): negative log of the dose ratio − 1 (DR − 1) of the agonist, BK (b) and LysdesArg^{9}BK (d). Values are means ± s.e.mean of at least 6 determinations.

receptor than [Aib^7]BK, similar to what has been observed in the B_2 receptor of the rabbit and different from that of the guinea-pig (Rhaleb *et al.*, 1990; Regoli *et al.*, 1993; Gobeil & Regoli, 1994). The pharmacological profile described above is similar to that reported by Hess *et al.* (1992, 1994) for the cloned human B_2 receptor expressed in CHO cells.

The human B_1 receptor of the HUV is stimulated only by peptides devoid of the C-terminal Arg and shows high affinity for the compound containing a Lys residue at the N-terminal, in accord with the findings of Menke *et al.* (1995) on the cloned human B_1 receptor.

Results obtained with antagonists indicate that one of the early peptidic compounds (D-Arg[Hyp^3,DPhe^7,Leu^8]BK) is a very weak antagonist on the human B_2 receptor and it is non selective, since it also blocks the human B_1 receptor. On the other hand, peptidic antagonists of the first generation have been shown to block efficiently (with high affinities) B_2 receptors of various animals, both in classical pharmacological experiments (in the rabbit by Regoli *et al.*, 1990) and incloned receptors (in the mouse by Hess *et al.*, 1994). Such differences may be attributed to the existence of interspecies B_2 receptor isoforms. Hoe 140, although less active than in other species (e.g. the rabbit, Regoli *et al.*, 1990; 1994b) is a fairly potent and selective antagonist of the human B_2 receptor. Hoe 140 exerts a competitive type of antagonism, as demonstrated by the linearity of the Schild plot and in accord with Marceau *et al.* (1994). The affinity of Hoe 140, determined in the present study, is lower than that measured in the cloned human B_2 receptor (pIC_{50} 10.2) by Hess *et al.* (1992). Such difference between classical pharmacological preparations and cloned receptors are not uncommon (see Regoli *et al.*, 1994a, for a similar comparison on neurokinin receptors) and may be (in part) attributed to a lower accessibility of peptidic compounds to receptors in intact tissues than in cell suspensions. WIN 64338 is inactive ($pA_2 < 5.0$). This result was obtained using two batches of WIN 64338, dissolved either in water or in DMSO or in ethanol and incubated for 10 or 30 min with the isolated HUV. We have no explanation for the discrepancy between the present results and those of Salvino *et al.* (1993) and Marceau *et al.* (1994) who found pA_2 values (for WIN 64338) of 6.0 and more for the human B_2 receptor.

The two B_1 receptor antagonists are inactive on the human B_2 receptor and should therefore be considered as selective for the human B_1 receptor. The compound with a N-terminal Lys shows higher affinity (by at least 1.5 log units) than [Leu^8]desArg^{9}BK, confirming the recent findings by Menke *et al.* (1995) on the cloned human B_1 receptor occurring in IMR-90 cells or expressed in COS-7 cells. Lys[Leu^8]desArg^{9}BK exerts a competitive type of antagonism (see linearity of the Schild plot) and should be considered as the most useful antagonist for characterization of the human B_1 receptor. Similar to what has been observed in rabbit tissues (Regoli *et al.*, 1977; Regoli & Barabé, 1980) human tissue responsiveness increases progressively as a function of the *in vitro* incubation time, sup-

porting the hypothesis of a *de novo* formation of B_1 receptors in the human umbilical vein. The nature of this interesting biological phenomenon has been investigated over several years and recently reviewed by Marceau (1995).

Altogether, the results presented in this paper indicate that the human umbilical vein contains contractile B_1 and B_2 receptors which, in our experimental conditions, can be studied and characterized separately. Both receptors show high sensitivity to B_2 (BK: pD_2 8.6) and B_1 (Lysdes-Arg^9BK:pD_2 8.6) receptor agonists. The two antagonists, although less active in human than in other species, (Hoe 140 (pA_2 8.4); Lys[Leu^8]desArg^9BK (pA_2 7.99)) have been found to be strong or selective enough for a precise pharmacological characterization of both human B_1 and B_2 receptors. The human umbilical vein is therefore proposed as a pharmacological preparation useful for the evaluation of emerging new (possibly non peptide) B_1 and B_2 receptor antagonists.

The authors would like to thank H. Morin and L. Dubois for secretarial assistance. We are grateful to the personnel of the Obstetric Clinic of the Centre Hospitalier universitaire de Sherbrooke (C.H.U.S.) for their kind cooperation in collecting the umbilical cords. D.R. is a Career Investigator of the Medical Research Council of Canada (M.R.C.C.). F.G. is a Student of the Natural Sciences and Engineering Research Council of Canada (N.S.E.R.C.) and L.H.P. is a student of the Heart and Stroke Foundation of Canada. Experimental work described in this paper has been supported by grants from the M.R.C.C. and the Heart and Stroke Foundation of Québec.

References

ALTURA, B.M., MALAVIYA, D., REICH, F.C. & ORKIN, L.R. (1972). Effects of vasoactive agents on isolated human umbilical arteries and veins. *Am. J. Physiol.*, **222**, 354–355.

COUTURE, R., MIZRAHI, J., REGOLI, D. & DEVROEDE, G. (1981). Peptides and the human colon: an *in vitro* pharmacological study. *Can. J. Physiol. Pharmacol.*, **59**, 957–964.

DRAPEAU, G. & REGOLI, D. (1988). Synthesis of bradykinin analogs. *Methods Enzymol.*, **163**, 263–272.

EGGERICKX, D., RASPE, E., BERTRAND, D., VASSART, G. & PARMENTIER, M. (1992). Molecular cloning, functional expression and pharmacological characterization of a human bradykinin B_2 receptor gene. *Biochem. Biophys. Res. Commun.*, **187**, 1306–1313.

FÉLÉTOU, M., MARTIN, C.A.E., MOLIMARD, M., NALINE, E., GERMAIN, M., THURIAU, C., FAUCHÈRE, J-L., CANET, E. & ADVENIER, C. (1995). *In vitro* effects of HOE 140 in human bronchial and vascular tissue. *Eur. J. Pharmacol.*, **274**, 57–64.

GOBEIL, F. & REGOLI, D. (1994). Characterization of kinin receptors by bioassays. *Braz. J. Med. Biol. Res.*, **27**, 1781–1791.

GOLDSTEIN, RH. & WALL, M. (1984). Activation of protein formation and cell division by bradykinin and desArg^9-Bradykinin. *J. Biol. Chem.*, **259**, 9263–9268.

HESS, J.F., BORKOWSKI, J.A., MCNEIL, T., STONESIFER, G.Y., FRAHER, J., STRADER, C.D. & RANSOM, R.W. (1994). Differential pharmacology of cloned human and mouse B_2 bradykinin receptors. *Mol. Pharmacol.*, **45**, 1–8.

HESS, J.F., BORKOWSKI, H.A., YOUNG, G.S., STRADER, C.D. & RANSOM, R.W. (1992). Cloning and pharmacological characterization of a human bradykinin (BK-2) receptor. *Biochem. Biophys. Res. Commun.*, **184**, 260–268.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, ST., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140, a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.*, **102**, 769–773.

MARCEAU, F. (1995). Kinin B_1 receptors: a review. *Immunopharmacology*, **30**, 1–26.

MARCEAU, F., LÉVESQUE, L., DRAPEAU, G., RIOUX, F., SALVINO, J.M., WOLFE, H.R., SEOANE, P.R. & SAWUTZ, D.G. (1994). Effects on peptide and nonpeptide antagonists of bradykinin B_2 receptors on the venoconstrictor action of bradykinin. *J. Pharmacol. Exp. Ther.*, **269**, 1136–1143.

MCEACHERN, A.E., SHELTON, E.R., BHAKTA, S., OBERNOLTE, R., BACH, C., ZUPPAN, P., FUJISAKI, J., ALDRICH, R.W. & JARNAGIN, K. (1991). Expression cloning of a rat B_2 bradykinin receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 7724–7728.

MCNEIL, T., BIERILO, K.K., MENKE, J.G. & HESS, J.F. (1995). Cloning and pharmacological characterization of rabbit bradykinin B_1 receptor. *Biochim. Biophys. Acta.*, **1264**, 223–228.

MENKE, J.G., BORKOWSKI, J.A., BIERILO, K.K., MCNEIL, T., DERRICK, A.W., SCHNECK, K.A., RAMSON, R.W., STRADER, C.D., LINESNEYER, D.L. & HESS, J.F. (1994). Expression cloning of a human B-1 bradykinin receptor. *J. Biol. Chem.*, **269**, 21583–21586.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmacol. Rev.*, **32**, 1–46.

REGOLI, D., BARABÉ, J. & PARK, W.K. (1977). Receptors of bradykinin in rabbit aorta. *Can. J. Physiol. Pharmacol.*, **55**, 855–867.

REGOLI, D., BOUDON, A. & FAUCHÈRE, J.L. (1994a). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46**, 551–599.

REGOLI, D., GOBEIL, F., NGUYEN, Q.T., JUKIC, D., SEOANE, P.R., SALVINO, J.M. & SAWUTZ, D.G. (1994b). Bradykinin receptor types and B_2 subtypes. *Life Sci.*, **55**, 735–749.

REGOLI, D., JUKIC, D., GOBEIL, F. & RHALEB, N.-E. (1993). Receptors for bradykinin and related kinins: a critical analysis. *Can. J. Physiol. Pharmacol.*, **71**, 556–567.

REGOLI, D., RHALEB, N.-E., DION, S. & DRAPEAU, G. (1990). New selective bradykinin receptor antagonists and bradykinin B_2 receptor characterization. *Trends Pharmacol. Sci.*, **11**, 156–161.

RHALEB, N.-E., DRAPEAU, G., DION, S., JUKIC, D., ROUISSI, N. & REGOLI, D. (1990). Structure-activity studies on bradykinin and related peptides. Agonists. *Br. J. Pharmacol.*, **99**, 445–448.

RHALEB, N.-E., TÉLÉMAQUE, S., ROUISSI, N., DION, S., JUKIC, D., DRAPEAU, G. & REGOLI, D. (1991). Structure-activity studies of bradykinin and related peptides. B_2 receptor antagonists. *Hypertension*, **17**, 107–115.

SALVINO, J.M., SEOANE, P.R., DOUTY, B.D., AWAD, M., DOLLE, R.E., HOUCK, W.T., FOUNCE, D.M. & SAWUTZ, D.G. (1993). Design of potent non peptide competitive antagonists of the human bradykinin B_2 receptor. *J. Med. Chem.*, **36**, 2583–2584.

SCHILD, H.O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmacol. Chemother.*, **2**, 189–202.

SCHILD, H.O. (1973). Receptor classification with special reference to beta adrenergic receptors. In *Drug Receptors*. pp. 29–36. ed. Rang, H.P. Baltimore, U.S.A.: University Park Press.

TALLARIDA, R.J. & MURRAY, R.B. (1987). *Manual of Pharmacologic Calculations with Computer Programs*. p. 145 (2nd ed.), New York, U.S.A.: Springer Verlag.

WIRTH, K., HOCK, F.J., ALBUS, U., LINZ, W., ALPERMANN, H.G., ANAGNOSTOPULOS, H., HENKE, S., BREIPOHE, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and low acting bradykinin antagonist. *Br. J. Pharmacol.*, **102**, 774–777.

(Received August 11, 1995
Revised January 15, 1996
Accepted January 26, 1996)