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Bradykinin B_1 receptors in human umbilical vein: pharmacological evidence of up-regulation, and induction by interleukin-1β

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Abstract

Bradykinin B_1 receptor-mediated responses increase as a function of in vitro incubation in the human umbilical vein. When tissues were continuously treated with the protein synthesis inhibitor, cycloheximide, or with the protein trafficking inhibitor, brefeldin A, pEC_{50} and maximal response to the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, were significantly diminished. The anti-inflammatory steroid, dexamethasone, produced a rightward shift of the concentration-response curve to des-Arg^9-bradykinin, without affecting the maximal response. Furthermore, lipopolysaccharide or recombinant human interleukin-1β potentiate the bradykinin B_1-sensitized responses, showing a leftward shift of the concentration-response curve to des-Arg^9-bradykinin, without modifying the maximal response. On the other hand, bradykinin B_2 receptor-mediated responses were unaffected by continuous exposure to cycloheximide, dexamethasone or lipopolysaccharide. These results provide pharmacological evidence to support the view that the de novo synthesis of bradykinin B_1 receptors is involved in the induction of vascular responses in the human umbilical vein. This up-regulation process seems to be selective for bradykinin B_1 receptors. The inhibitory effect of dexamethasone and the potentiating actions of lipopolysaccharide and exogenous human recombinant interleukin-1β on des-Arg^9-bradykinin-mediated responses, suggest the possible role of interleukin-1β in the bradykinin B_1 receptor up-regulation phenomenon in human umbilical vein.

Keywords: Umbilical vein, human; Bradykinin B_1 receptor; De novo synthesis; Up-regulation; Interleukin-1β

1. Introduction

Bradykinin B_1 receptors were originally described in isolated rabbit tissues after a long in vitro incubation (Regoli and Barabé, 1980). Thereafter, induction of bradykinin B_1 responses was documented in different isolated tissue preparations (for review, see Marceau, 1995). Within human tissues, the increase of bradykinin B_1 receptor-mediated responses in colon, ileum, coronary artery and umbilical vein has been reported (Couture et al., 1981; Zuzack et al., 1996; Drummond and Cocks, 1995; Sardi et al., 1997).

It has been shown that responses to the bradykinin B_1 receptor selective agonist, des-Arg^9-bradykinin, were abolished in tissues permanently exposed to protein synthesis inhibitors, such as cycloheximide or actinomycin D, or a protein trafficking inhibitor, brefeldin A. Therefore, the de novo synthesis of bradykinin B_1 receptors has been hypothesized to account for this sensitization phenomenon (Regoli and Barabé, 1980; Audet et al., 1994).

In vitro studies have demonstrated a link between inflammatory mediators and the expression of bradykinin B_1 receptors. In rabbit isolated tissues, it has been reported that lipopolysaccharide or interleukin-1β treatment increase bradykinin B_1 receptor-mediated responses (Bouthillier et al., 1987; DeBlois et al., 1991). Furthermore, the anti-inflammatory steroid, dexamethasone, has been shown to prevent bradykinin B_1 receptor sensitized responses in the rabbit aorta and human umbilical vein (DeBlois et al., 1988; DeBlois et al., 1991; Sardi et al., 1997). It has been proposed that the inhibition of interleukin-1β synthesis by glucocorticoids may partially account for the inhibitory effect of dexamethasone (DeBlois et al., 1991).

In isolated human umbilical vein obtained from normal deliveries of healthy pregnant subjects, the bradykinin B_1

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receptor selective agonist, des-Arg^9-bradykinin, had no effect at the beginning of the incubation (15 min) (Sardi et al., 1997). Therefore, these receptors seem to lack any physiological significance in normal delivery. On the other hand, in umbilical vessels, there are not any study on bradykinin B_1 receptors under pathophysiologic conditions, such as pre-eclampsia or diabetes. However, the in vitro bradykinin B_1 receptor sensitization in the umbilical vein could represent one interesting experimental model of this up-regulation process based on a human tissue. The present experiments have been undertaken to determine the effects of metabolic inhibitors of protein synthesis or protein trafficking, and of cytokines on des-Arg^9-bradykinin sensitization in human umbilical vein.

2. Materials and methods

2.1. Preparation of the tissues for tension measurements

Approximately 15–35 cm of human umbilical cords ($n = 57$) excised midway between the placenta and infant were obtained from normal full-term deliveries. Immediately, cords were placed in modified Krebs solution at 4°C (of the following mM composition: NaCl 119, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0, EDTA 0.004, D-glucose 11). The veins were dissected out of the cords and cut into rings of approximately 3 mm width. The preparations were suspended in 10 ml organ baths and stretched with an initial tension of 2 g as described previously (Elgoyhen et al., 1993). Changes in tension were measured with Grass isometric transducers (FT 03C, Grass Instrument, Quincy, MA, USA) and displayed on Grass polygraphs (Model 7D). The time from delivery until the tissue was set up in the organ baths was approximately 3 h.

During the incubation period, Krebs solution was maintained at 37°C and at pH 7.4 by constant bubbling with 95% O_2:5% CO_2. The bath solution was replaced every 15 min with fresh bubbled warmed Krebs. Tissues were incubated with captopril (1 μM) 30 or 15 min before any peptide stimulation, in order to avoid peptide degradation by kininase II (angiotensin converting enzyme).

2.2. Kinin receptor stimulation

Cumulative concentration–response curves were obtained for bradykinin or des-Arg^9-bradykinin after a 2 or 5 h incubation period. Only one agonist concentration–response curve was performed on a single ring.

Some human umbilical vein rings were continuously exposed to cycloheximide (70 μM), brefeldin A (18 μM) or dexamethasone (100 μM) for a 5 h equilibration period, before cumulative addition of agonists. Dexamethasone concentration was the same employed by DeBlois et al. (1991) in the rabbit aorta and by Sardi et al. (1997) in the human umbilical vein. Experiments were performed in parallel in rings from the same tissue.

As we have previously found (Sardi et al., 1997) that in the human umbilical vein a marked sensitization behaviour is observed between 15 and 120 min of in vitro incubation, we decided to evaluate the possible potentiating action of lipopolysaccharide on bradykinin B_1 receptor-mediated responses at 2 h. Furthermore, Zhou et al. (1998) have shown a marked increase in bradykinin B_1 binding within 2 h. after stimulation of IMR90 cells with interleukin-1β.

Therefore, human umbilical vein rings were continuously exposed to bacterial lipopolysaccharide (1 μg/ml) for a 2 h incubation period, before constructing the concentration–response curves.

In another series of experiments, some human umbilical vein rings were treated with human recombinant interleukin-1β (50, 100 U/ml) for the initial 75 min. Then, cumulative concentration-effect curves were constructed for the bradykinin B_1 receptor selective agonist, des-Arg^9-bradykinin at 2 or 5 h.

At the end of each experiment, serotonin (10 μM) was applied in order to determine the tissue maximal response.

2.3. Chemicals

All chemicals were purchased from Sigma (St. Louis, MO, USA) except recombinant human interleukin-1β that was a generous gift from Dr. Carlos Gonzalez, Boehringer-Mannheim (Argentina). Lipopolysaccharide was from *Escherichia coli* (serotype 0127:B8) and dexamethasone was dexamethasone 21-phosphate.

2.4. Expression of results and statistical analysis

All data are presented as mean ± S.E.M. Responses are expressed as g of developed contraction. The pEC_{50} values, negative logarithm of the agonist concentration that produces 50% of the maximum effect, and slopes were determined using ALLFIT, a nonlinear curve-fitting computer program (De Lean et al., 1978). Statistical analysis was performed either by means of paired Student's *t*-test or by one-way analysis of variance where appropriate. *P* values lower than 0.05 were taken to indicate significant differences between means.

3. Results

3.1. Effect of the protein synthesis inhibition on bradykinin B_1 receptor sensitization in human umbilical vein

Continuous exposure to the protein translation inhibitor, cycloheximide (70 μM), profoundly inhibited the bradykinin B_1 receptor sensitization in isolated human umbilical vein rings. The pEC_{50} and maximal response to

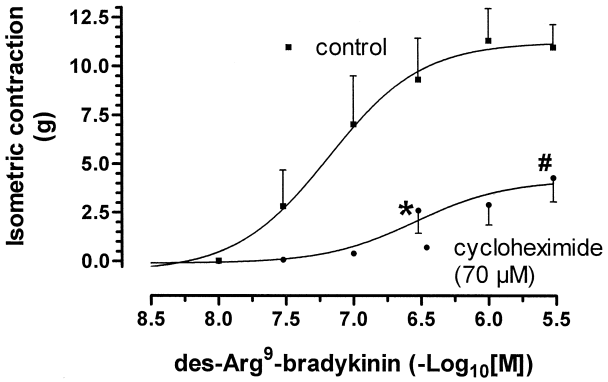

Fig. 1. Concentration–effect curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues permanently exposed to cycloheximide (70 μM, ●). The points represent the mean of 8 determinations made after 5 h equilibration period and the vertical lines show S.E.M. The responses are expressed in g of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Symbols (∗) and (#) represent significant differences ($P < 0.05$) between pEC_{50} and maximal response, respectively.

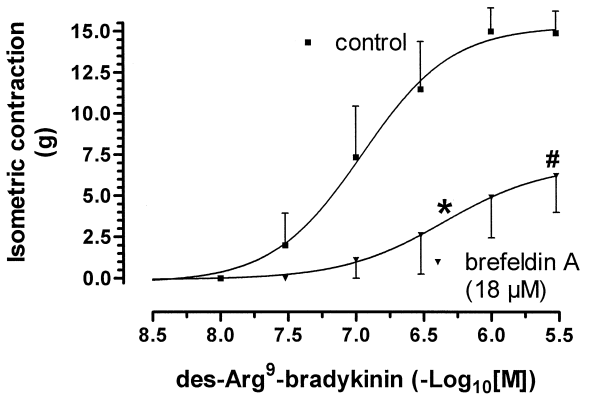

Fig. 2. Concentration–effect curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues permanently exposed to brefeldin A (18 μM, ▼). The points represent the mean of 5 determinations made after 5 h equilibration period and the vertical lines show S.E.M. The responses are expressed in g of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Symbols (∗) and (#) represent significant differences ($P < 0.05$) between pEC_{50} and maximal response, respectively.

des-Arg^9-bradykinin were significantly diminished in 5 h treated tissues (Fig. 1, Table 1). In addition, maximal response to serotonin (10 μM) was not modified by this treatment (control, 13.5 ± 1.5 g; treated, 14.5 ± 2.1, $n = 8$).

On the other hand, continuous exposure of human umbilical vein rings to cycloheximide did not modify the bradykinin concentration–response curve (Table 1).

3.2. Effect of the protein trafficking inhibition on bradykinin B_1 receptor sensitization in human umbilical vein

Some tissues were continuously exposed to brefeldin A, in order to determinate the participation of protein trafficking across the endoplasmic reticulum–Golgi pathway on the bradykinin B_1 receptor sensitization. After 5 h incubation with brefeldin A (18 μM), pEC_{50} and maximal response to the bradykinin B_1 receptor selective agonist, des-Arg^9-bradykinin, were significantly reduced (Fig. 2, Table 1). In addition, maximal response to serotonin (10 μM) was not modified by this treatment (control, 17.4 ± 0.9 g; treated, 16.9 ± 0.7 g, $n = 5$).

3.3. Effect of dexamethasone on bradykinin B_1 receptor sensitization in human umbilical vein

Continuous exposition of human umbilical vein to the anti-inflammatory steroid, dexamethasone (100 μM) caused a rightward shift of the concentration–response

Table 1
Effect of various in vitro treatments on the contractile responses of human umbilical vein to des-Arg^9-bradykinin or bradykinin

	Incubation time (h)	pEC_{50}		Maximal response (g)		n
		Control	Treated	Control	Treated	
Des-Arg^9-bradykinin						
Cycloheximide (70 μM)[a]	5	7.18 ± 0.19	6.54 ± 0.27[c]	11.3 ± 1.6	4.3 ± 1.2[c]	8
Brefeldin A (18 μM)[a]	5	6.94 ± 0.14	6.34 ± 0.38[c]	14.9 ± 1.4	6.2 ± 2.2[c]	5
Dexamethasone (100 μM)[a]	5	7.08 ± 0.11	6.31 ± 0.18[c]	10.7 ± 1.4	9.8 ± 2.1	9
Lipopolysaccharide (1 μg/ml)[a]	2	7.07 ± 0.15	7.52 ± 0.24[c]	8.9 ± 0.8	8.9 ± 1.1	6
Interleukin-1β (50 U/ml)[b]	2	7.01 ± 0.11	6.96 ± 0.10	11.9 ± 1.2	13.2 ± 1.2	7
Interleukin-1β (100 U/ml)[b]	2	6.82 ± 0.11	7.26 ± 0.06[c]	9.1 ± 0.7	10.8 ± 1.4	6
Interleukin-1β (100 U/ml)[b]	5	6.94 ± 0.14	7.56 ± 0.07[c]	14.9 ± 1.4	15.2 ± 1.4	5
Bradykinin						
Cycloheximide (70 μM)[a]	5	8.81 ± 0.07	8.70 ± 0.10	12.2 ± 0.8	11.9 ± 1.2	7
Dexamethasone (100 μM)[a]	5	8.84 ± 0.06	8.73 ± 0.11	12.0 ± 1.4	14.1 ± 0.9	6
Lipopolysaccharide (1 μg/ml)[a]	2	9.19 ± 0.11	9.14 ± 0.15	14.8 ± 1.7	13.1 ± 1.9	7

[a] Tissues were permanently exposed to the drug.
[b] Tissues were exposed to interleukin-1β for the initial 75 min.
[c] Represent significative differences between treated and paired tissues ($P < 0.05$).

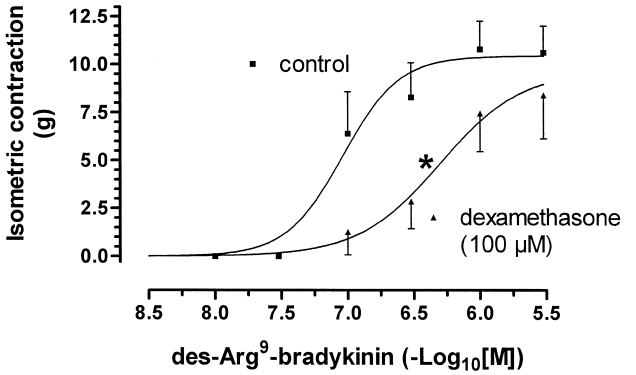

Fig. 3. Concentration–effect curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues permanently exposed to dexamethasone (100 μM, ▲). The points represent the mean of 9 determinations made after 5 h equilibration period and the vertical lines show S.E.M. The responses are expressed in g of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Symbol (∗) represent significant difference ($P < 0.05$) between pEC_{50} values.

curve to des-Arg^9-bradykinin at 5 h (Fig. 3, Table 1). However, the maximal response to the bradykinin B_1 receptor selective agonist was unaffected by this treatment (Table 1). Furthermore, maximal response to serotonin (10 μM) was not modified by this treatment (control, 13.4 ± 1.7 g; treated, 13.5 ± 2.3 g, $n = 9$). Unlike the responses to des-Arg^9-bradykinin, bradykinin-induced contractions after 5 h were unaffected by dexamethasone treatment (Table 1).

3.4. Effect of bacterial lipopolysaccharide on bradykinin B_1 receptor sensitization in human umbilical vein

Some tissues were exposed to lipopolysaccharide (1 μg/ml) during 2 h incubation period, in order to evaluate the possible stimulatory effect of this endotoxin on the sensitization behavior of bradykinin B_1 receptors. Bacterial lipopolysaccharide-treatment produced a significative leftward shift of the concentration–response curve to des-Arg^9-bradykinin (Fig. 4, Table 1). Furthermore, the maxi-

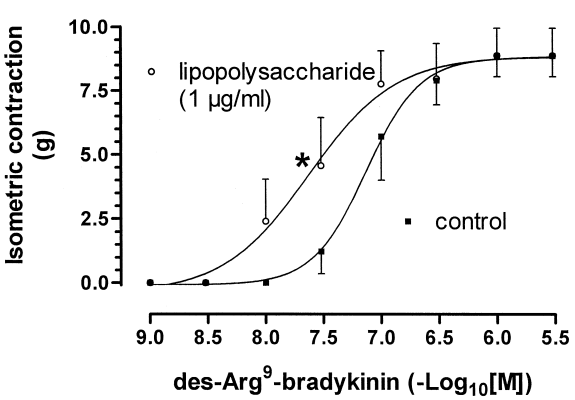

Fig. 4. Concentration–effect curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues permanently exposed to bacterial lipopolysaccharide (1 μg/ml, ○). The points represent the mean of 6 determinations made after 2 h equilibration period and the vertical lines show S.E.M. The responses are expressed in g of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Slopes were not significantly different (control, 1.8 ± 0.4; treated, 1.2 ± 0.3). Symbol (∗) represent significant difference ($P < 0.05$) between pEC_{50} values.

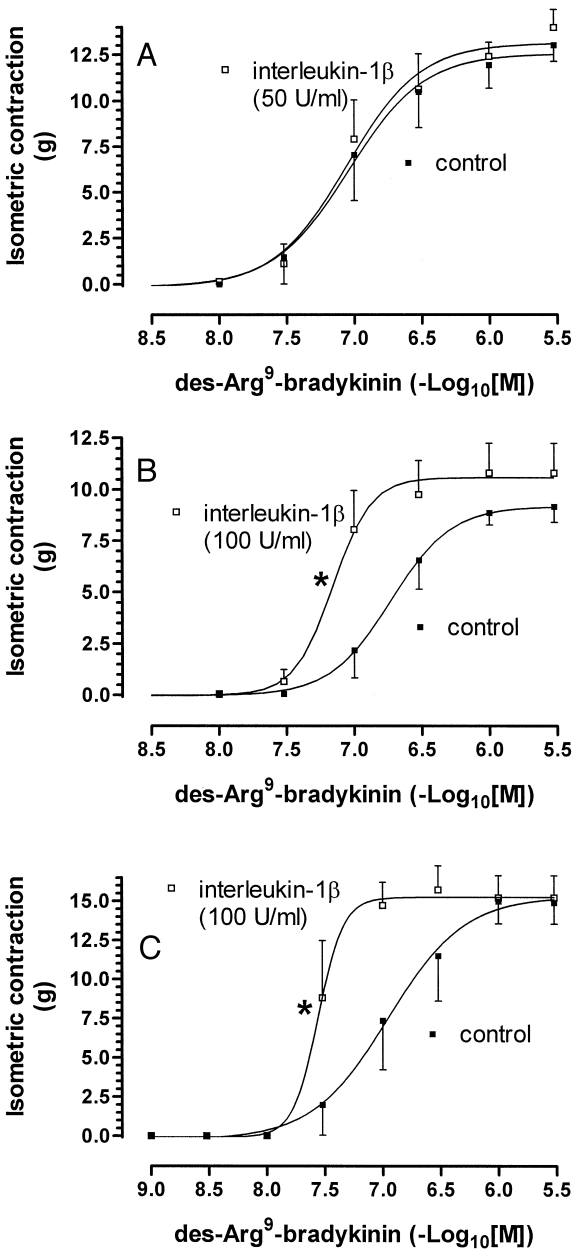

Fig. 5. Concentration–effect curves for des-Arg^9-bradykinin on control human umbilical vein rings (■) and on tissues exposed to interleukin-1β for 75 min (□). Tissues were exposed to (a) 50 U/ml or (b, c) 100 U/ml of recombinant human interleukin-1β. The points represent the mean of 7, 6 or 5 determinations, respectively, made after (a, b) 2 or (c) 5 h equilibration period and the vertical lines show S.E.M. The responses are expressed in g of developed contraction. Abscissa scale: $-\log_{10}$ of molar concentration. Slopes were not significantly different (a: control, 1.5 ± 0.5; treated, 1.3 ± 0.4; b: control, 1.9 ± 0.4; treated, 3.0 ± 0.8; c: control, 1.4 ± 0.5; treated, 3.9 ± 2.5). Symbol (∗) represent significant difference ($P < 0.05$) between pEC_{50} values.

mal response to the bradykinin B_1 receptor selective agonist at 2 h was unaffected by this treatment (Table 1). On the other hand, bradykinin-mediated responses at 2 h were unaffected by this treatment (Table 1).

3.5. Effect of recombinant human interleukin-1β on bradykinin B_1 receptor sensitization in human umbilical vein

Some tissues were exposed to recombinant human interleukin-1β during 75 min in order to evaluate the possible potentiating effect of this cytokine on the bradykinin B_1 receptor sensitization process. When human umbilical vein rings were exposed to 50 U/ml of this cytokine, concentration-response curves to des-Arg^9-bradykinin at 2 h were not modified (Fig. 5a, Table 1). When tissues were treated with interleukin-1β 100 U/ml, contractile responses to the bradykinin B_1 receptor agonist at 2 and 5 h were significantly potentiated (Fig. 5b,c, respectively; Table 1). However, maximal responses to des-Arg^9-bradykinin were unaffected by interleukin-1β 100 U/ml (Table 1).

4. Discussion

The vascular bradykinin B_1 receptors were first described in isolated rabbit anterior mesenteric vein by Regoli et al. (1978) after a long in vitro incubation. These authors have postulated the de novo formation of bradykinin B_1 receptors to account for this phenomenon. In the isolated human umbilical vein, it has been previously demonstrated the presence of kinin B_1 receptors using des-Arg^9-bradykinin and des-Arg^9-[Leu^8]bradykinin, selective bradykinin B_1 receptor agonist and antagonist, respectively. These receptors mediate contraction and exhibit a sensitization phenomenon during in vitro incubation (Sardi et al., 1997). In this tissue and under the same experimental conditions, we have confirmed an increase in bradykinin B_1 receptor-mediated response in a time-dependent manner.

In the present study, the possible process of the bradykinin B_1 receptor induction was addressed by incubating tissues in the presence of cycloheximide, a protein synthesis inhibitor, or brefeldin A, a protein trafficking inhibitor. Previously, we have observed that continuous exposure to cycloheximide (70 μM) throughout 300 min incubation period, decreased the responses to a submaximal concentration of des-Arg^9-bradykinin (Sardi et al., 1997). In the present study, in the same experimental conditions, cycloheximide abolished the concentration–response curves to the bradykinin B_1 receptor selective agonist, but did not modify the maximal response to serotonin, an unrelated agonist. Furthermore, cycloheximide treatment had no effect against bradykinin-mediated contractions. In different tissues, it has been well established that bradykinin B_1 receptor sensitization is completely prevented by protein synthesis inhibitors, such as cycloheximide or actinomycin D (Marceau, 1997).

In rat and pig vessels, the inhibitory effect of brefeldin A on the bradykinin B_1 sensitizated-responses has been described (Audet et al., 1994; Pruneau et al., 1996). This drug is an inhibitor of the migration of vesicles from the endoplasmic reticulum to the Golgi apparatus, blocking protein trafficking and the surface expression of newly synthesized proteins (Helms and Rothman, 1992). In the human umbilical vein, bradykinin B_1-mediated sensitizated responses were inhibited by continuous exposure to brefeldin A. Therefore, the de novo synthesis of a transmembrane protein, such as the bradykinin B_1 receptor, seems to account for the sensitization process.

This sensitization process is a spontaneous phenomenon of unknown mechanism. It has been postulated that bradykinin B_1 receptor sensitization results from trauma during tissue isolation and incubation or from inflammation, due to release of cytokines such as interleukin-1β (DeBlois et al., 1991). Sardi et al. (1997) have previously shown that continuous exposure to the anti-inflammatory steroid, dexamethasone, profoundly inhibited the response to a submaximal concentration of des-Arg^9-bradykinin in the human umbilical vein. The present study demonstrated that dexamethasone caused a rightward shift of the concentration–response curves to the bradykinin B_1 agonist without affecting the maximal response. On the other hand, bradykinin concentration-response curves were unaffected by dexamethasone treatment. It has been proposed that the inhibition of interleukin-1β synthesis by glucocorticoids may partially account for the inhibitory effect of dexamethasone (DeBlois et al., 1991). Recently, Ni et al. (1998) have observed that dexamethasone reduced bradykinin B_1 receptor mRNA in rat vascular smooth muscle cells.

Lipopolysaccharide is known to promote the rapid (within 15 min) synthesis and release of interleukin-1β in human smooth muscle cells (Libby et al., 1986). In the present study, lipopolysaccharide treatment produced a leftward shift of the concentration–response curve to des-Arg^9-bradykinin without modifying the maximal response of human umbilical vein. Furthermore, bradykinin-mediated contractions were unaffected by the endotoxin treatment. When lipopolysaccharide is continuously applied to isolated rabbit aorta, it was found to increase des-Arg^9-bradykinin-mediated responses (Bouthillier et al., 1987). Using the cloned mouse bradykinin B_1 receptor cDNA as a probe, mRNA levels were very low or undetectable in tissues from normal mice, but lipopolysaccharide-treatment caused a dramatic induction of mRNA levels in heart, lung and liver (Pesquero et al., 1996). Moreover, Ni et al. (1998) have recently described the increase in bradykinin B_1 receptor mRNA levels in vascular smooth muscle cells treated with lipopolysaccharide. Therefore, the increase in bradykinin B_1 receptor number could account for the

sensitization phenomenon in the presence of this endotoxin.

In human umbilical vein, interleukin-1β increased, in a concentration-dependent manner, the sensitivity to the selective bradykinin B_1 receptor agonist after 2 or 5 h of incubation, without affecting the maximal response. Interleukin-1β has been hypothesized to be an essential intermediate in the in vivo induction of bradykinin B_1 receptors and has been shown to potentiate the spontaneous induction of this receptors in rabbit isolated vessels (DeBlois et al., 1991; Audet et al., 1994). Ni et al. (1998) have observed the increase in bradykinin B_1 receptor mRNA levels in rat aorta smooth muscle cells treated with interleukin-1β. Furthermore, it has been reported that interleukin-1β increases bradykinin B_1 binding sites in rabbit mesenteric smooth muscle cells (Galizzi et al., 1994). Zhou et al. (1998) have recently shown that in IMR90 cells, bradykinin B_1 binding increased approximately 8-fold within 4 h after stimulation with interleukin-1β.

The concept of spare receptors is used to explain the situation in which not all the receptors have to be occupied by the agonist in order to elicit a maximal response (Ross, 1996). In these conditions, only when the number of these receptors have been reduced further than the spare receptors population, an appreciable fall in the maximal response can be obtained. On the other hand, any treatment that increases the number of these receptors will produce a shift to the left of the concentration–response curve to the agonist without affecting the maximal response. Hence, the lack of modification of the maximal response to des-Arg^9-bradykinin after the treatment with dexamethasone or after either lipopolysaccharide or interleukin-1β treatments, is consistent with the presence of bradykinin B_1 spare receptors in the human umbilical vein after 5 h of incubation. On the other hand, in human umbilical vein, bradykinin-mediated responses were not modified by continuous exposure to cycloheximide, dexamethasone or lipopolysaccharide. According to Campos and Calixto (1994), when both bradykinin receptors are co-expressed in a preparation, the responses mediated by B_2 receptors remain stable and only the bradykinin B_1 responses seem to be gradually increased.

In conclusion, the results reported here with cycloheximide and brefeldin A provide pharmacological evidence to support the view that the de novo synthesis of bradykinin B_1 receptors is involved in the induction of vascular responses in the human umbilical vein. This up-regulation process seems to be selective for bradykinin B_1 receptors. The inhibitory effect of dexamethasone and the potentiating actions of lipopolysaccharide and exogenous human recombinant interleukin-1β on des-Arg^9-bradykinin-mediated responses, suggest the possible role of interleukin-1β in the bradykinin B_1 up-regulation phenomenon in human umbilical vein. Further experiments, inhibiting interleukin-1β synthesis and blocking its activity should be performed to determine if endogenous interleukin-1β is responsible for bradykinin B_1 receptor up-regulation in human umbilical vein.

Acknowledgements

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