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Bradykinin B_1 receptor in isolated human umbilical vein: an experimental model of the *in vitro* up-regulation process

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KEY WORDS Bradykinin receptors; umbilical veins; up-regulation (physiology); NF-kappa B

ABSTRACT

Bradykinin (BK) B_1 receptors are not normally expressed in physiological conditions but could be induced in immunopathological states. Molecular approaches have confirmed that BK B_1 receptor gene is transcriptionally induced in injured tissues. In these situations, the cytokine network and other proinflammatory mediators are close linked to BK B_1 receptor expression.

In this article, we describe the functional characterization of the BK B_1 receptor up-regulation process in the isolated human umbilical vein and the pharmacological tools employed to demonstrate the *de novo* synthesis of these receptors. BK B_1 receptors are up-regulated in a time- and protein synthesis-dependent process. Furthermore, in this tissue we have demonstrated the close link between the BK B_1 receptor sensitization and proinflammatory cytokines, such as interleukin-1beta and tumor necrosis factor-alpha. We also discuss the possible relationship between nuclear factor-kappa B and BK B_1 receptor induction in human umbilical vein.

INTRODUCTION

Kinins are biologically active peptides that are formed locally after tissue damage and inflammatory stimuli from precursor kininogens by limited proteolysis. Kinins exert a broad spectrum of physiological and pathological effects, including smooth muscle contraction, vasodilatation, increased vascular permeability and nociception. In 1977, two mammalian bradykinin (BK) receptor subtypes were proposed, B_1 and B_2[1]. The endogenous agonists for BK B_1 and B_2 receptors are the nonapeptide bradykinin and the decapeptide Lys-bradykinin (kallidin). BK and kallidin are equipotent agonists at the BK B_2 receptor. Kallidin is also active at BK B_1 receptors[2]; in contrast, BK is a weak agonist of these receptors[3]. Degradation of BK and kallidin by a carboxypeptidase produces the selective BK B_1 receptor agonists, des-Arg^9-BK and des-Arg^{10}-kallidin. Furthermore, the pharmacological characterization of the BK receptors was made possible by the development of specific antagonists for both BK receptors. Molecular cloning has revealed the primary structures of BK B_2 and B_1 receptors, and identified them as members of the G-protein coupled receptor family characterized by seven membrane-spanning alpha-helices[4,5].

BK B_1 receptors are in some way atypical, in as much as their cell surface expression is often inducible rather than being constitutive, while BK B_2 receptor is thought to be expressed constitutive and ubiquitously[6]. The vascular BK B_1 receptors were first described in isolated rabbit anterior mesenteric vein after a long *in vitro* incubation[7]. These authors postulated the *de novo* formation of BK B_1 receptors to account for this phenomenon. Afterwards, the induction of BK B_1 responses was documented in different isolated tissue preparations[6]. Within human tissues, the induction of BK B_1 receptor mediated responses has been reported in colon, ileum, coronary artery and umbilical vein[8-11].

On the other hand, BK B_1 receptor expression is induced by exposure to lipopolysaccharide (LPS) or inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)[6].

BRADYKININ RECEPTORS IN ISOLATED HUMAN UMBILICAL VEIN

In the isolated human umbilical vein BK promotes a

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potent and effective vasoconstrictor response[12]. It has been demonstrated that this pharmacological action depends on BK B_2 receptor stimulus[11,13-15].

On this preparation, we have demonstrated that the contractile effect to des-Arg^9-BK develops from an initial null level and increases in magnitude as a function of the *in vitro* incubation time[11]. The maximal response was (0.6±0.2) g at 15 min, (4.5±0.6) g at 120 min and (9.1±0.9) g at 300 min. The approximated maximal response to the BK B_1 receptor selective agonist at 5 h was 80 % of the maximal response to serotonin. Moreover, BK B_2 and 5-HT receptors are present in a preformed and stable manner, and mediate a maximal vasoconstriction in this tissue. On the other hand, des-Arg^9-BK mediated responses were competitively antagonized by the selective BK B_1 receptor antagonist des-Arg^9-[Leu^8]-BK confirming that BK B_1 receptors are present in this tissue[11].

BRADYKININ B_1 RECEPTOR *DE NOVO* SYNTHESIS IN ISOLATED HUMAN UMBILICAL VEIN

The *de novo* synthesis of a transmembrane protein requires the activation of one or more genes, transcription of mRNA (s) of low basal abundance, and translation at the level of ribosomes. For a receptor protein which is predicted to possess a signal peptide or glycosylation sites, the processing through the endoplasmic reticulum and the Golgi apparatus is necessary to finally locate in the cytoplasmic membrane. All these metabolic processes are sensitive to pharmacological inhibition (Fig 1).

In different tissues, it has been well established that BK B_1 receptor responses are abolished by transcription inhibitors[6]. The antibiotic actinomycin D, produced by *Streptomyces species*, binds to double-helical DNA blocking the RNA polymerase movement, thus preventing RNA synthesis[16].

In isolated human umbilical vein in our experimental conditions, sensitized responses to the BK B_1 receptor agonist des-Arg^9-BK are selectively inhibited by continuous exposure to actinomycin D[17].

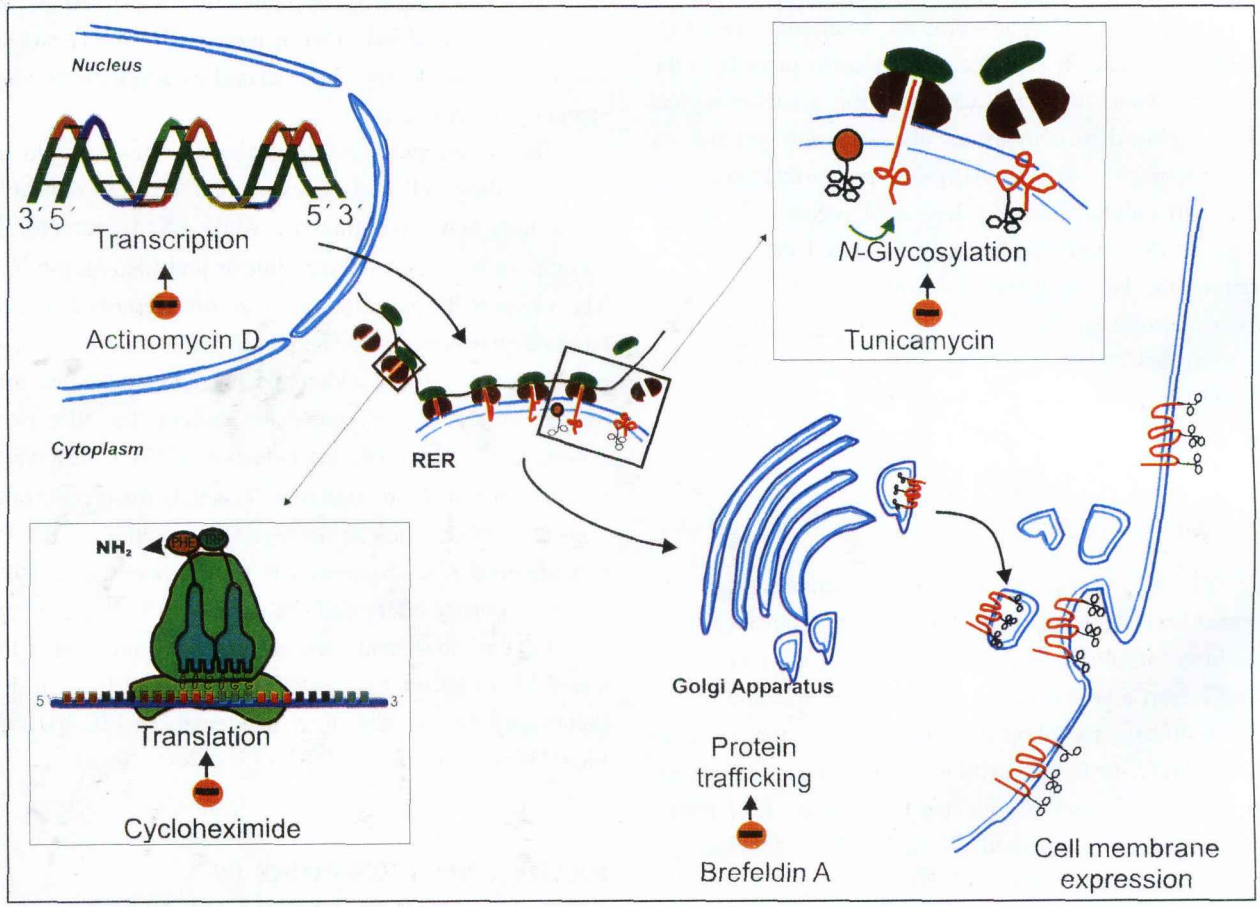

Fig 1. Bradykinin B_1 receptor *de novo* synthesis. Metabolic sites sensitive to pharmacological inhibition. RER = rough endoplasmic reticulum. Other references in the text.

In accord with previous results in rabbit vascular tissues[7,18,19] we have observed that continuous exposure for 5 h to cycloheximide, a protein synthesis inhibitor that blocks the translocation reaction on ribosomes, profoundly inhibits the BK B_1 receptor sensitization in isolated human umbilical vein rings[20]. Furthermore, in this human tissue, when cycloheximide is applied only for the first 4 h, a complete reversion of its inhibitory effect is obtained[17]. Similar results were reported by DeBlois *et al* (1991) in the rabbit aortic preparation[19]. These results suggest that the temporal sequence of the process involving BK B_1 receptor synthesis from its mRNA, trafficking and functional membrane expression is relatively rapid, less than 1 h.

Brefeldin A is an inhibitor of the migration of vesicles from the endoplasmic reticulum to the Golgi apparatus, blocking protein trafficking and in consequence the surface expression of newly synthesized proteins[21]. In the human umbilical vein, we have reported that BK B_1 mediated sensitizated responses are inhibited by continuous exposure to this trafficking inhibitor[20]. Previously, the inhibitory effect of brefeldin A on the BK B_1 sensitizated responses has been described in rat and pig vessels[22,23].

Many G-protein coupled receptors contain potential N-linked glycosylation sites (defined by Asparagine-X-Serine/Threonine, where X represents any amino acid)[24]. These sites may confer varying functional roles depending on the receptor. For example, they could be important for efficient G-protein coupling, ligand binding or membrane expression[25,26]. According to Menke *et al* (1994), human BK B_1 receptor contains three potential N-linked glycosylation sites, which may be of importance for receptor assembly, stability or function[5]. The nucleoside antibiotic tunicamycin, produced by *Streptomyces lysosuperificus*, is a highly selective direct inhibitor of *N*-linked glycosylation of various proteins. Apparently, its mechanism of action is blocking the enzymatic transfer of *N*-acetyl-glucosamine-1-phosphate to dolichol-mono-phosphate[27,28]. In our experimental conditions, when human umbilical vein rings are exposed to tunicamycin for 5 h, BK B_1 receptor mediated contractions are almost completely abolished[17]. In isolated rabbit aorta, permanent exposure to tunicamycin has partially inhibited the development of des-Arg^9-BK mediated responses[22].

Taken together, the inhibition of the des-Arg^9-BK mediated responses after a 5 h treatment with actinomycin D, cycloheximide, brefeldin A or tunicamycin, provide pharmacological evidence to support the view that the *de novo* synthesis of a transmembrane protein such as the BK B_1 receptor, is involved in the induction of this vascular responses in the human umbilical vein. On the other hand, we have shown that not one of these treatments modify the responses mediated by BK or the unrelated agonist, 5-HT[17,20]. Thus, the BK B_1 receptor sensitization in the isolated human umbilical vein represents an interesting experimental model of the *in vitro* up-regulation process on a human tissue.

BRADYKININ B_1 RECEPTOR UP-REGULATION: RELATIONSHIP BETWEEN CYTOKINE AND NUCLEAR FACTOR-KAPPA B NETWORK

BK B_1 receptors are not normally expressed under physiological conditions but are induced in situations of stress, such as septic shock and inflammation. In these immunopathological situations the cytokine network and other proinflammatory mediators are close linked to BK B_1 receptor expression[6,19]. Infection by Gram-negative bacteria provides LPS, a complex polymer usually in the form of high molecular weight aggregates, capable of recruiting numerous host effector systems and modifying gene expression in various cell types. Notably, LPS is an inducer of cytokine synthesis in leukocytes and other cell types[29].

Whole cardiovascular system of the rabbit and other animals receiving LPS intravenous injections can be senzitized to BK B_1 receptor agonists after 5 h while BK B_2 responses remain unaffected[30,31]. Intravenously injected human recombinant IL-1β produces similar effects[19].

In vitro studies have reported that LPS or IL-1β treatment increase BK B_1 receptor mediated responses in rabbit vascular tissues[18,19]. In IMR90 cells, Zhou *et al* (1998) have shown that after stimulation with IL-1β, BK B_1 receptor binding increased approximately 8-fold[32]. Furthermore, Ni *et al* (1998) have observed the increase in BK B_1 receptor mRNA levels in rat aorta smooth muscle cells treated with LPS, IL-1β or TNF-α[33]. In the human umbilical vein, we have shown that exposure to LPS or human recombinant IL-1β significantly increases des-Arg^9-BK mediated responses, showing a leftward shift of the concentration-response curve without modifying the maximal response to the same stimulus[20]. Furthermore, we have recently observed that human re-

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combinant TNF-α treatment also potentiates the BK B_1 receptor mediated response of the isolated human umbilical vein[17].

After the cloning of the BK B_1 receptor, several groups have begun to determine the transduction mechanisms involved in its induction. The 5′-flanking region of the human BK B_1 receptor gene bears putative nuclear factor-κB (NF-κB) as well as numerous activator protein-1 binding motifs, a promoter organization consistent with an activation by cytokines, such as IL-1β or TNF-α[34]. In resting cells, NF-κB is held inactive in the cytosol by association with inhibitory proteins of the I-κB family. NF-κB pathway is activated by agents such as LPS, IL-1β and TNF-α that initiate a phosphorylation dependent proteolytic degradation of I-κB, allowing NF-κB to translocate into the nucleus (Fig 2). Traenckner *et al* (1994) have reported that pyrrolidine-dithiocarbamate (PDTC), an antioxidant inhibitor of NF-κB, prevents *de novo* phosphorylation of I-κBα as well as its subsequent degradation[35]. Recently, we have reported that continuous incubation with PDTC inhibits, in a concentration-dependent manner, the contractile response to the selective BK B_1 receptor agonist, in human umbilical vein[17].

At present it is well known that glucocorticoids (GCs) reduce the inflammation and may reflect an inhibitory effect on the expression of cytokines and other cellular mediators. Most of the genes involved are not directly controlled by an interaction with activated GC receptors because the promoter sequence of these genes have not a GC response element (Fig 2), their expression is rather regulated by a direct interaction between GC receptors and transcription factors[36]. Although potentially acting at multiple levels, GC can directly inhibit activated NF-κB via a protein-protein interaction between GC receptors and NF-κB. GC can also increase the transcription and expression of I-κBα in some cells. Furthermore, GC can reduce phosphorylation and degradation of I-κBα in cultured rat vascular smooth muscle cells, thus providing an additional mechanism for control of NF-κB activation[37]. In consequence, GC could inhibit the

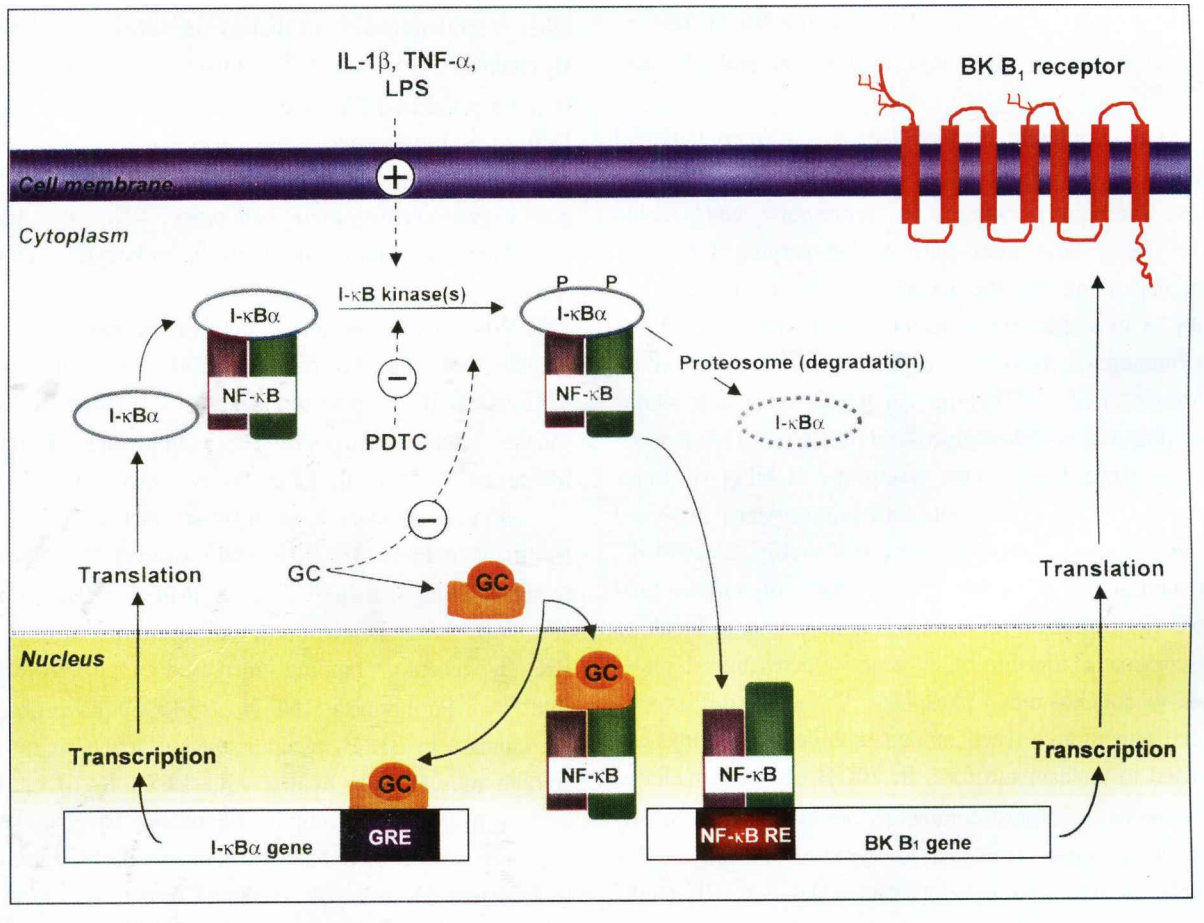

Fig 2. Bradykinin B_1 receptor expression: relationship between cytokine and nuclear factor-kappaB. GRE: glucocorticoid response element, NF-κB RE: nuclear factor-κB response element. Other references in the text.

expression of BK B_1 receptors and this is likely to be mediated by inhibition of NF-κB mediated pathway.

The anti-inflammatory steroid, dexamethasone, has been shown to prevent BK B_1 receptor sensitizated responses in rabbit isolated tissues[19,38]. Ni *et al* (1998) have shown that dexamethasone decreases the expression of the BK B_1 receptor mRNA in rat vascular smooth muscle cells[33]. In human umbilical vein, we have also observed the selective inhibition of BK B_1 receptor mediated responses by this anti-inflammatory steroid, showing a rightward shift of the concentration-response curve to des-Arg^9-BK, without affecting its maximal response[11,20].

In summary, functional studies of BK B_1 receptor up-regulation in the isolated human umbilical vein confirm that inflammatory cytokines and LPS play an important role in induction of the BK B_1 receptor in accord with molecular and functional reported results in other tissues. The inhibitory action observed with PDTC suggests that NF-κB plays a crucial role in the functional expression of BK B_1 receptor. Furthermore, the inhibitory effects of dexamethasone on BK B_1 receptor mediated response agree with a receptor expression regulated by cytokines, and provide additional evidence that NF-κB pathway could be involved in its induction.

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REFERENCES

1 Regoli D, Barabe J, Park WK. Receptors for bradykinin in rabbit aortae. Can J Physiol Pharmacol 1977; 55: 855 – 67.
2 Regoli D, Barabe J. Pharmacology of bradykinin and related kinins. Pharmacol Rev 1980; 32: 1 – 46.
3 Butt SK, Dawson LG, Hall JM. Bradykinin B_1 receptors in the rabbit urinary bladder: induction of responses, smooth muscle contraction, and phosphatidylinositol hydrolisys. Br J Pharmacol 1995; 114: 612 – 7.
4 McEachern AE, Shelton ER, Bhakta S, Obernolte R, Bach C, Zuppan P, *et al*. Expression cloning of a rat B_2 bradykinin receptor. Proc Natl Acad Sci USA 1991; 88: 7724 – 8.
5 Menke JG, Borkowski JA, Bierilo KK, Macneil T, Derrick AW, Schneck KA, *et al*. Expression cloning of a human B_1 bradykinin receptor. J Biol Chem 1994; 269: 21583 – 6.
6 Marceau F, Hess JF, Bachvarov DR. The B_1 receptors for kinins. Pharmacol Rev 1998; 50: 357 – 86.
7 Regoli D, Marceau F, Barabe J. *De novo* formation of vascular receptors for bradykinin. Can J Physiol Pharmacol 1978; 56: 674 – 7.
8 Couture R, Mizrahi J, Regoli D, Devroede G. Peptides and the human colon: an in vitro pharmacological study. Can J Physiol Pharmacol 1981; 59: 957 – 64.
9 Zuzack JS, Burkard MR, Cuadrado DK, Greer RA, Selig WM, Whalley ET. Evidence of a bradykinin B_1 receptor in human ileum: pharmacological comparison to the rabbit aorta B_1 receptor. J Exp Ther 1996; 277: 1337 – 43.
10 Drummond GR, Cocks TM. Endothelium-dependent relaxation to the B_1 kinin receptor agonist Des-Arg^9-bradykinin in human coronary arteries. Br J Pharmacol 1995; 116: 3083 – 5.
11 Sardi SP, Pérez H, Antúnez P, Rothlin RP. Bradykinin B_1 receptors in human umbilical vein. Eur J Pharmacol 1997; 321: 33 – 8.
12 Altura BM, Malaviya D, Reich CF, Orkin LR. Effects of vasoactive agents on isolated human umbilical arteries and veins. Am J Physiol 1972; 222: 345 – 55.
13 Marceau F, Levesque L, Drapeau G, Rioux F, Salvino JM, Wolfe HR, *et al*. Effects of peptide and nonpeptide antagonists of bradykinin B_2 receptors on the venoconstrictor action of bradykinin. J Pharmacol Exp Ther 1994; 269: 1136 – 43.
14 Feletou M, Martin CAE, Molimard M, Naline E, Germain M, Thurieau C, *et al*. *In vitro* effects of Hoe 140 in human bronchial and vascular tissue. Eur J Pharmacol 1995; 274: 57 – 64.
15 Gobeil F, Pheng LH, Badini I, Nguyen XK, Pizard A, Rizzi A, *et al*. Receptors for kinins in the human isolated umbilical vein. Br J Pharmacol 1996; 118: 289 – 94.
16 Chabner BA, Allegra CJ, Curt GA, Calabresi P. Antineoplastic agents, in goodman & gilmans: the pharmacological basis of therapeutics. In: Hardman J, Limbird L, Molinoff P, Ruddon R, Goodman Gilman A, editors. McGraw- Hill, International Edition. 1996. p 1233 – 87.
17 Sardi SP, Daray FM, Errasti AE, Pelorosso FG, Pujol Lereis VA, Rey Ares V, *et al*. Further pharmacological characterization of bradykinin B_1 receptor up-regulation in human umbilical vein. J Pharmacol Exp Ther 1999; 290: 1019 – 25.
18 Bouthillier J, DeBlois D, Marceau F. Studies on the induction of pharmacological responses to Des-Arg^9-bradykinin *in vitro* and *in vivo*. Br J Pharmacol 1987; 92: 257 – 64.
19 DeBlois D, Bouthillier J, Marceau F. Pulse exposure to protein synthesis inhibitors enhances vascular responses to Des-Arg^9-braykinin: possible role of interleukin-1. Br J Pharmacol 1991; 103: 1057 – 66.
20 Sardi SP, Rey Ares V, Errasti AE, Rothlin RP. Bradykinin B_1 receptors in human umbilical vein: pharmacological evidence of up-regulation, and induction by interleukin-1 beta. Eur J Pharmacol 1998; 358: 221 – 7.
21 Helms JB, Rothman JE. Inhibition by brefeldin a of golgi membrane enzyme that catalyses changes of guanine nucleotides bound to ARF. Nature 1992; 360: 352 – 4.
22 Audet R, Petitclerc E, Drapeau G, Rioux F, Marceau F. Further analysis of the up-regulation of bradykinin B_1 receptors in isolated rabbit aorta by using metabolic inhibitors. Eur J Pharmacol 1994; 271: 551 – 5.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Feb; 21 (2)
E-mail aps@server.shcnc.ac.cn Phn/Fax 86-21-6474-2629

23 Pruneau D, Luccarini JM, Defrene E, Paquet JL, Belichard P. Characterisation of bradykinin receptors from juvenile pig coronary artery. Eur J Pharmacol 1996; 297: 53 – 60.
24 Marshall RD. Glycoproteins. Annu Rev Biochem 1972; 41: 673 – 702.
25 Rands E, Candelore MR, Cheung AH, Hill WS, Strader CD, Dixon RA. Mutational analysis of beta-adrenergic receptor glycosylation. J Biol Chem 1990; 265: 10759 – 64.
26 Walsh MT, Foley JF, Kinsella BT. Characterization of the role of N-linked glycosylation on the cell signaling and expression of the human thromboxane A^2 receptor alpha and beta isoforms. J Pharmacol Exp Ther 1998; 286: 1026 – 36.
27 Tkacz JS, Lampen O. Tunicamycin inhibition of polyisoprenyl N-acetylglucosaminyl pyrophosphate formation in calf-liver microsomes. Biochem Biophys Res Commun 1975; 65: 248 – 57.
28 Elbein AD. Inhibitors of the biosynthesis and processing of N-Linked oligosaccharide chains. Annu Rev Biochem 1987; 56: 497 – 534.
29 Libby P, Ordovas JM, Birinyi LK, Auger KR, Dinarello CA. Inducible interleukin-1 gene expression in smooth muscle cells. J Clin Invest 1986; 78: 1432 – 8.
30 Regoli DC, Marceau F, Lavigne J. Induction Of B_1-receptors for kinins in the rabbit by a bacterial lipopolysaccharide. Eur J Pharmacol 1981; 71: 105 – 15.
31 Drapeau G, DeBlois D, Marceau F. Hypotensive effects of Lys-Des-Arg^9-bradykinin and metabolically protected agonist of B_1 receptors for kinins. J Pharmacol Exp Ther 1991; 259: 997 – 1003.
32 Zhou X, Polgar P, Taylor L. Roles for interleukin-1β, phorbol ester and a post-transcriptional regulator in the control of bradykinin B_1 receptor gene expression. Biochem J 1998; 330: 361 – 6.
33 Ni A, Chao L, Chao J. Transcription factor nuclear factor κB regulates the inducible expression of the human B_1 receptor gene in inflammation. J Biol Chemistry 1998; 273: 2784 – 91.
34 Bachvarov DR, Hess JF, Menke JG, Larrive JF, Marceau F. Structure and genomic organization of the human B_1 gene for kinins. Genomics 1996; 33: 374 – 81.
35 Traenckner EB, Wilk S, Baeuerle PA. A proteasome inhibitor prevents activation of NF-kappa B and stabilizes a newly phosphorylated form of I-kappa B-alpha that is still bound to NF-Kappa B. EMBO J 1994; 13: 5433 – 41.
36 Barnes PJ, Adcock IM. Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol Sci 1993; 14: 436 – 41.
37 Katsuyama K, Shichiri M, Kato H, Imai T, Marumo F, Hirata Y. Differential inhibitory actions by glucocorticoid and aspirin on cytokine-induced nitric oxide production in vascular smooth muscle cells. Endocrinology 1999; 140: 2183 – 90.
38 DeBlois D, Bouthillier J, Marceau F. Effect of glucocorticoids, monokines and growth factors on the spontaneously developing responses of the rabbit isolated aorta to Des-Arg^9-bradykinin. Br J Pharmacol 1988; 93: 969 – 77.

离体人类脐静脉中的缓激肽 B_1 受体：体外调控过程的实验模型

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关键词 缓激肽；脐静脉；上调(生理学)；NF-κB

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