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BRADYKININ AND BRADYKININ ANTAGONISTS EFFECTS ON ENDOTHELIAL CELL PHOSPHOINOSITIDE METABOLISM: IMPLICATIONS FOR SEPTIC SHOCK

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INTRODUCTION

The potent vasodilator bradykinin (BK) may be a factor in septic shock (Hirsch et al., 1974), and it is known to act on the endothelium (Vanhoutte and Miller, 1985), although measurement of blood levels may present difficulties (Nielsen et al., 1983). In other tissues, there appear to be two classes of kinin receptors. BK type B1 receptors have high affinity for the agonist des-arg^9-BK and for the antagonist des-arg^9,[leu^8]-BK (Regoli et al., 1986). BK type B2 receptors have high affinity for the agonists BK and Lys-BK (kallidin) and for antagonists such as [D-arg^0, Hyp^3, Thi^{5,8}, D-Phe^7]-kallidin (abbreviated dRRPJGUSdFUR) (Stewart and Vavrek, 1986).

Regoli et al. (1986) have reported that BK type B1 receptors occur in stressed tissues. Rabbits treated with lipopolysaccharide had a hypotensive response to B1 agonists (Regoli et al., 1981). Churchill et al. (1986) demonstrated that B1 receptors may mediate vasorelaxation. BK stimulates phosphoinositide metabolism in bovine and porcine aortic endothelial cells in culture (Derian and Moskowitz, 1986; Lambert et al., 1986), but the nature of BK receptors involved is unclear. In this report, we describe the presence of two subtypes of BK receptors on the same endothelial cell line.

EXPERIMENTAL PROCEDURES

The bovine aortic endothelial cell line GM7372 (EC) from the Institute for Medical Research, Camden, NJ was propagated in Medium 199 with 20% fetal bovine serum. The EC were trypsinized, seeded into 24-well plates and grown to confluence. The day before assessment of phosphoinositide metabolism the medium was changed to Medium 199. The assay consisted of three sequential incubations at 37°C. I. The cells were incubated for 4 hr with 1 uCi [^{3}H]-inositol (Dupont-NEN) in basal medium of Eagle. II. Potential antagonists, as appropriate, and LiCl (10 mM final) were added for 15 min. Each condition was assayed in quadruplicate. III. Agonists were added and the incubation continued for 60 min. The reaction was terminated by aspiration of the incubation solution, rinsing with Dulbecco's PBS (D-PBS) and addition of cold methanol-HCl (200:1). [^{3}H]-inositol phosphate was isolated by solvent partition and ion-exchange chromatography (Roth et al., 1986) and counted in a liquid scintillation counter. For kinin binding assays, [Tyr^1]-kallidin (T1 K), [Tyr^5]-BK (T5 BK) and [Tyr^8]-BK (T8 BK) (Penninsula Laboratories) were iodinated as described by Odya et al. (1980). Prior to kinin binding studies, EC were washed 3 times with D-PBS, trypsinized and washed with 0.1 mM phenyl methyl sulfonyl fluoride in D-PBS. A portion of the suspension was taken for protein analysis and the remainder centrifuged and the pellet frozen. Immediately before the assay the pellet was thawed and resuspended in the following: 0.1 mM bacitracin, 0.01% casein, 1 mM EDTA, 1 mM sodium azide, and 20 mM PIPES, pH 6.8. Kinin binding assays were performed as described by Odya et al. (1980): about 100 ug membrane protein was incubated with 25,000 cpm (25 pg) of labeled kinin in the absence or presence of 5 ug unlabeled BK. Protein assays were performed by the method of Bradford (1976). Several novel peptides, including dRRPJGUSdFUR, were kindly provided by Drs. J. Stewart and R. Vavrek (Univ. of Colorado). Dr. C. Odya (NMRI) generously provided bovine myometrial membranes.

RESULTS AND DISCUSSION

Measurements of inositol phosphate were used to reflect the phosphoinositide metabolism. BK elicited a 27% stimulation in phosphoinositide metabolism at 10^{-8}M

and a 300% stimulation at 10^{-6} M BK (Table I). Des-arg^9-[leu^8]-BK (RPPGFSPF) did not block the stimulation by 10^{-6} M BK, suggesting that this stimulation is not of the B1 type. BK potentiator B (pEGLPPRPKIPP) (an inhibitor of kininase II), and eledoisin (pEPSKDAFIGM) (structurally unrelated) had no effect on the basal activities or on the stimulation by 10^{-6} M or 10^{-8} M BK. These results indicate that there is some degree of specificity in the stimulation by BK, as eledoisin had no effect, and that there is no evidence for degradation of BK by kininase II. Only des-arg^9,[leu^8]-BK blocked the stimulation by 10^{-6} M BK. These results are consistent with the presence of a B1 type receptor responsive to the elevated BK levels.

TABLE 1. EFFECT OF BRADYKININ AND ANALOGS ON PHOSPHOINOSITIDE TURNOVER IN 7372 AORTIC ENDOTHELIAL CELLS

Incubation II[a]	Incubation III[b]	$\bar{x}$ ± SEM	(n)
saline	saline	1.0[d]	(5)
·	10^{-8} M BK	1.27 ± 0.14	(5)
·	10^{-6} M BK	2.96 ± 0.40	(5)
RPPGFSPF[c]	saline	1.19 ± 0.28	(4)
·	10^{-8} M BK	1.25 ± 0.25	(4)
·	10^{-6} M BK	1.51 ± 0.19	(4)
pEGLPPRPKIPP	saline	1.08 ± 0.08	(4)
·	10^{-8} M BK	1.28 ± 0.20	(4)
·	10^{-6} M BK	3.97 ± 1.5	(4)
pEPSKDAFIGM	saline	1.05 ± 0.13	(3)
·	10^{-8} M BK	1.15 ± 0.64	(3)
	10^{-6} M BK	2.81 ± 0.64	(3)

a: Incubation with 10 mM LiCl and 2 x 10^{-3} M agents indicated for 15 min at 37°C. b: Incubation for 60 min at 37°C with the addition of the agents shown. c: The structures are the single letter amino acid codes with pE indicating the L-pyroglutamyl residue. In this notation, BK is RPPGFSPFR, and the other three agents are des-arg^9[leu^8]-BK, potentiator B and eledoisin. d: The average basal was 65 ± 15 cpm.

Stewart and Vavrek (1986) have synthesized a series of analogs of BK which antagonize BK action. The effect of several of these peptides on the stimulation of phosphoinositide metabolism by BK are described in Table 2.

TABLE 2. EFFECT OF BRADYKININ ANALOGS ON PHOSPHOINOSITIDE TURNOVER IN 7372 ENDOTHELIAL CELLS

Incubation II[a]	Incubation III[b]	$\bar{x}$ ± SEM	(n)
saline	saline	1.0[d]	(4)
saline	10^{-8} M BK	1.38 ± 0.15	(4)
dRRPJGUSdFUR[c]	saline	0.90 ± 0.23	(4)
	10^{-8} M BK	0.89 ± 0.15	(4)
RPJGUSdFUR	saline	0.65 ± 0.15	(3)
	10^{-8} M BK	0.87 ± 0.32	(3)
RPPGFSdFFR	10^{-8} M BK	1.37 ± 0.14	(3)
RPPGUSdFUR	10^{-8} M BK	1.31 ± 0.12	(3)
KKRJPGUSdFUR	10^{-8} M BK	1.27 ± 0.05	(3)

a: Incubation with 10 mM LiCl and 2 x 10^{-8}M agents as indicated, for 15 min at 37°C. b: Incubation for 60 min at 37°C with the addition of the agents indicated. c: Single letter amino acid sequence for the agents with the following modifications: J=L-hydroxyproline, U=beta-(2-thienyl)-L-alanine, and d indicates a D-amino acid stereoisomer. d: The mean ± SEM of basal was 25.8 ± 10.8 cpm.

Stimulation by 10^{-8}M BK is blocked by dRRPJGUSdFUR (*B4162) without effect on the basal value. The agent RPJGUSdFUR (*B4146) inhibits the basal level of phosphoinositide metabolism and depresses the level observed with 10^{-8}M. The difference resulting from addition of amino-terminal arginine is in accord with results of Stewart and Vavrek, who find that this change enhances the antagonist properties of the resulting peptide. The other agents tested did not alter the basal level (not shown) and did not block the stimulation by BK (Table 2). These experiments suggest that BK stimulation of phosphoinositide metabolism in these EC has at least two components. One component responds to low levels of BK and is blocked

by a B2 type antagonist. The other component responds to higher levels of BK and is substantially blocked by a B1 type antagonist.

Direct binding measurements of kinins to an EC particulate fraction was undertaken to evaluate the receptors on EC. Binding of three kinin analogs to EC particulate fraction is illustrated in Figure 1. The total binding to EC was about 6% of the label added. Non-saturable binding of ^{125}I-[Tyr^1]-kallidin, ^{125}I-[Tyr^8]-BK and ^{125}I-[Tyr^8]-BK averaged 5, 9 and 8 pg kinin/ug EC protein, respectively. This corresponds to saturable binding of 75%, 38% and 39%, respectively. Saturable binding of these three kinin ligands to EC is shown in Table 3.

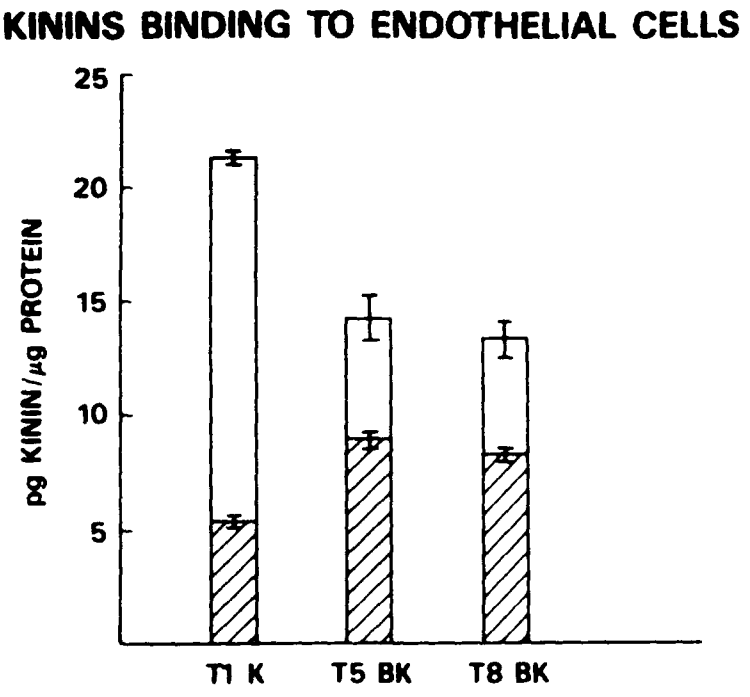

Fig. 1.

TABLE 3. BINDING OF VARIOUS RADIO-LABELED KININS

ligand	saturable binding[a] (pg kinin/mg protein) endothelial cell
^{125}I-[Tyr^1]-kallidin	12.5
^{125}I-[Tyr^5]-bradykinin	4.7
^{125}I-[Tyr^8]-bradykinin	5.8

a: The average of two experiments each performed in triplicate.

Our measured binding of the three ligands to myometrium (not shown) are in accord with the results obtained by Odya et al. (1980). The myometrium has a high density of specific sites for ^{125}I-[Tyr^1]-kallidin, but essentially no specific sites for ^{125}I-[Tyr^5]-BK. Thus modification of the five position of BK by substituting this sterically bulky and hydrophobic group has fundamentally changed the interaction between the ligand and the B2 myometrium receptor. In contrast, while ^{125}I-[Tyr^1]-kallidin binds best to the EC binding site, there is also substantial binding of ^{125}I-[Tyr^5]-BK (Table 3). Thus, the spectrum of binding of three ligands to EC is not the same as the spectrum of binding to a pure B2 system, myometrium. These observations are consistent with (but not proof of) the presence of B2 receptors and another kinin binding component on the ECs. Further characterization of the nature of this binding including the possibility of differential action of lipopolysaccharide is underway. If lipopolysaccharide does indeed induce BK receptors then the vascular response to BK might be sensitized.

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DISCUSSION

Dr. Roth: Is there any knowledge at the present time on the pharmacology of this response, be it a B1 or a B2 response. Do you know?

Dr. Nielsen: My information on that is that it is a B2 response.

Dr. Roth: And is there any evidence yet delimiting which receptor might be operative during endotoxemia?

Dr. Nielsen: The studies of Churchill suggests that there may be a B1 receptor involved, but it is not clear what the overlapping specificity might be.