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Effect of Bradykinin on Oxygen Consumption in the Distal Tubule and Cortical Collecting Tubule of Rat

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ABSTRACT

Infusion of bradykinin (BK) into the renal arteries increases sodium excretion. However, it is not clear whether natriuresis results from the renal hemodynamic effects or from the direct effect on renal tubular sodium transport. Therefore, we examined the effects of BK on the transport-dependent oxygen consumption in the distal tubule (DT) and cortical collecting tubule (CCT) of deoxycorticosterone-treated rats. BK inhibited oxygen consumption in a dose-dependent way with a maximal reduction at 0.1 μM BK. The inhibitory effect of BK was not present in the absence of sodium or in the presence of ouabain (1 mM). These data imply that the inhibitory effect of BK is restricted to the sodium transport-dependent oxygen consumption. We also investigated the relationship between the effect of BK on oxygen consumption and arachidonic acid metabolism. Mepacrine (10 μM), an inhibitor of membrane phospholipases, prevented the inhibitory effect of BK, but indomethacin (0.5 mM) didn't. These results suggest that BK decreases the sodium transport-related oxygen consumption in the rat DT and/or CCT, and that it may be mediated by products of enzymes other than cyclooxygenase.

Key Words: Bradykinin, Oxygen consumption, Distal tubule, Cortical collecting tubule

INTRODUCTION

Bradykinin (BK), one of the effective peptides of the kallikrein-kinin system, has been implicated in the control of renal hemodynamics and water and sodium excretion (Scili & Carretero, 1986).

Infusion of BK into the renal arteries increases renal blood flow and the water and sodium excretion rate (Stein *et al.*, 1972; Flamenbaum *et al.*, 1979; Thomas *et al.*, 1982; Granger & Hall, 1986). However, it is not clear whether natriuresis results from the renal hemodynamic effects of BK or from the direct effect on renal tubular sodium transport. Kininogen and kallikrein have been localized to the DT and collecting tubule (Pround *et al.*, 1981, Scicli & Carretero, 1986), and kininases present in the proximal tubule would presumably metabolize any filtered kinins (Carone *et al.*, 1976). Thus, the possible site of action of BK is expected to be the DT and collecting tubule where BK is formed. The inhibitory effect of BK on vasopressin-stimulated water permeability (Schuster *et al.*, 1984) and the high-affinity binding sites for BK in these sites (Tomita & Pisano, 1984) support this possibility. Therefore, in the present experiment we investigated the effect of BK on the transport-dependent oxygen consumption of the DT and CCT.

MATERIALS AND METHODS

Preparation of tubular suspensions

Sprague-Dawley rats weighing 250-300 g were used in all the experiments. The isolated CCT from an untreated rat exhibits little sodium transport (Reif & Schafer, 1984; Reif *et al.*, 1984; Tomita *et al.*, 1985). Thus, to increase sodium transport above a very low basal level, we used deoxycorticosterone-treated rats for all the experiments. The animals were injected with deoxycorticosterone acetate, 0.5 mg

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daily, by intramuscular injection for 10 days before experiments.

The suspension of DT and CCT was prepared from kidneys of male rats according to the method of Vinay *et al.* (1981). Briefly, this entails digesting the cortical tissues with 0.2% collagenase and 0.25% hyaluronidase, and purifying the tubule suspension on a continuous density gradient generated in Percoll medium. After separation on the Percoll gradient the fractions enriched in DT and CCT were rinsed three times in a saline containing 0.6% dextran plus the following (in mM): 115 NaCl, 5 KCl, 25 $NaHCO_3$, 2 NaH_2PO_4, 1 $CaCl_2$, 4 lactate, 1 alanine, 5 glucose. In sodium-free medium, this saline was modified as follows: NaCl was replaced with LiCl, and $NaHCO_3$ with $KHCO_3$.

Oxygen consumption measurements

Tubules were incubated for 10 min at 37°C and aerated with 95% O_2/5% CO_2 prior to measuring oxygen consumption. The suspensions were transferred into a closed chamber to measure oxygen consumption under control conditions or after the addition of BK or other experimental substances. In all the experiments captopril (10 μM) was added to inhibit the degradation of BK by kininase II. Oxygen consumption was measured with a Clark-type polarographic oxygen electrode in a closed 2.8-ml glass chamber that was heated to 37°C with a circulating water bath. When the measurement of oxygen consumption was ended, the renal tubules were homogenized, and proteins were measured on the homogenates by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The results were expressed as nmoles of O_2 utilized per min per mg protein.

BK, indomethacin, mepacrine, ouabain, collagenase (type I), hyaluronidase, and captopril were purchased from Sigma (USA), and Percoll from Pharmacia (Sweden). A statistical analysis was done using Student's *t*-test.

RESULTS

Because in all the experiments captopril (10 μM) was added to inhibit kininase II activity, the effect of captopril alone on oxygen consumption in DT and CCT was tested. Captopril by itself did not affect oxygen consumption. The average values of oxygen consumption were 19.17 ± 1.04 nmoles O_2/min/mg protein (n = 11) prior to addition of captopril, and after the addition of captopril 19.23 ± 1.03 nmoles O_2/min/mg protein (n = 11).

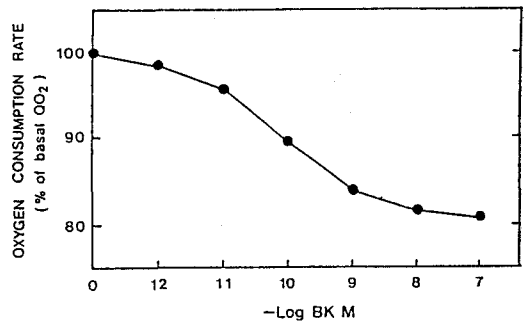

Fig. 1. Effect of bradykinin on the oxygen consumption in renal distal tubule and cortical collecting tubule from deoxycorticosterone-treated rats. Each value represents the mean of three experiments.

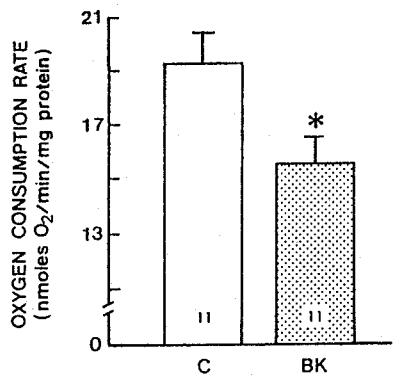

Fig. 2. Effect of bradykinin on the oxygen consumption in renal distal tubule and cortical collecting tubule from deoxycorticosterone-treated rats. Each value represents the mean ± S.E.. The numerics in bars are number of experiments.
BK: 0.1 μM bradykinin, *: $p<0.05$

BK induced a dose-dependent decrease in oxygen consumption rate in DT and CCT (Fig. 1). A significant decline in oxygen consumption rate to 84.4% of control was observed at a dose of 10^{-9} M. Maximum inhibition was seen at a dose of 10^{-7} M or greater and averaged 80.7% of the control (Fig. 2). In all the subsequent experiments BK was used at a concentration of 10^{-7} M.

In order to determine whether the effect of BK was on transport-dependent oxygen consumption, the experiments were done on tubules suspended in a sodium-free medium or pretreated with ouabain. Replacement of sodium by lithium reduced oxygen

Table 1. Effects of bradykinin on oxygen consumption in renal distal tubule and cortical collecting tubule from deoxycorticosterone-treated rats.

Medium	Oxygen consumption			
	Control	n	BK	n
Normal	19.23 ± 1.03	11	15.52 ± 0.98*	11
Na-free	12.17 ± 1.13	9	11.84 ± 1.01	9

Each value represents the mean ± S.E.. Oxygen consumption rate is expressed as nmoles O_2/min/mg protein.
n: Number of experiments, BK: bradykinin 0.1 μM.
*: $p<0.05$

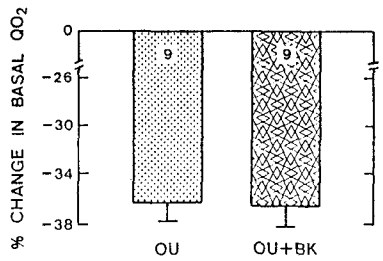

Fig. 3. Effect of pretreatment with ouabain on the bradykinin-induced reduction of oxygen consumption in renal distal tubule and cortical collecting tubules from deoxycorticosterone-treated rats. Each value represents the mean ± S.E.. The numerics in bars are number of experiments.
BK: 0.1 μM bradykinin, OU: 1 mM ouabain
*: $p<0.05$

consumption by 36.7%. BK in the absence of sodium did not reduce oxygen consumption (Table 1). These imply that the inhibitory effect of BK on the oxygen consumption is not due to direct inhibition of oxidative phosphorylation. Ouabain (1 mM), a inhibitor of Na-K-ATPase, inhibited oxygen consumption by 36.2% from an average of 19.23 ± 1.03 nmoles O_2/min/mg protein in control to 12.26 ± 1.21 nmoles O_2/min/mg protein. BK did not induce any further reduction of oxygen consumption after administration of ouabain (Fig. 3). Thus BK reduces oxygen consumption by inhibiting Na^+ entry or by inhibiting the Na-K-ATPase.

Considering that BK leads to arachidonic acid release from phospholipids by stimulation of acylhydrolase (Scicli & Carretero, 1986) and some of the metabolites of arachidonic acid affect the sodium reabsorption by collecting tubules (Stokes & Kokko, 1977; Jacobson *et al.*, 1984), we decide to examine

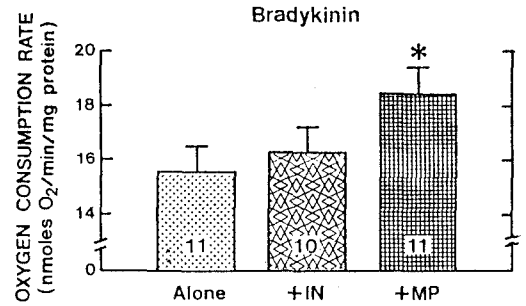

Fig. 4. Effects of indomethacin and mepacrine on the bradykinin-induced reduction of oxygen consumption in renal distal tubule and cortical collecting tubule from deoxycorticosterone-treated rats. Each value represents the mean ± S.E.. the numerics in bars are number of experiments.
IN: 0.5 mM indomethacin, MP: 10 μM mepacrine
*: $p<0.05$

whether BK-induced reduction of oxygen consumption is related to the metabolites of arachidonic acid. It was found that mepacrine (10 μM), an inhibitor of membrane phospholipases, alone had no effect on oxygen consumption, but prevented BK from stimulating oxygen consumption (Fig. 4). It was also clarified that indomethacin (0.5 mM) alone had no effect on oxygen consumption and did not prevent the inhibitory effect of BK.

DISCUSSION

Unlike CCTs from untreated rabbits (Frindt & Burg, 1972; O'Neil & Helman, 1977; Schwartz & Burg, 1978), CCTs from untreated rats exhibit little net sodium and potassium transport (Reif & Schafer, 1984; Reif *et al.*, 1984; Tomita *et al.*, 1985), and it is difficult to evaluate the effect of BK on the transport-dependent oxygen consumption in isolated CCT from untreated rats. Thus, we used deoxycorticosterone-treated rats for all the experiments because chronic administration of deoxycorticosterone to rats increased both sodium absorption and potassium secretion above very low basal levels (Tomita *et al.*, 1985).

Energy utilized in ion transport is mainly supplied from aerobic metabolism (Cohen & Barac-Nieto, 1973), and oxygen consumption in the renal tubules is known to be closely related to the rate of ion transport, particularly sodium transport (Kramer *et al.*, 1969; Chamberlin *et al.*, 1984; Mandel, 1986; Zeidel *et al.*, 1986; Silva *et al.*, 1987). In other words, a large component of epithelial cell oxygen consump-

tion supplies metabolic energy for Na-K-ATPase, which consumes ATP in the active extrusion of sodium from and uptake of potassium into the cell (Balaban *et al.*, 1980; Eveloff *et al.*, 1981; Harris *et al.*, 1981). When Na^+ entry into cells is reduced, Na-K-ATPase activity is reduced, resulting in diminished oxygen consumption, whereas when Na^+ entry is stimulated, Na-K-ATPase-mediated oxygen consumption is augmented. The present experiments show that BK has an inhibitory effect on sodium-dependent and ouabain-sensitive oxygen consumption in the DT and CCT. This result indicates that BK inhibits the transcellular sodium transport in the DT and/or CCT.

BK stimulates the release of arachidonic acid from membrane phospholipids (Scicli & Carretero, 1986) and arachidonic acid is metabolized through several pathways in renal tissue. It is converted by cyclooxygenase into prostaglandins and thromboxanes, by lipoxygenases into hydroxyeicosatetraenoic acids (HETEs) and leukotrienes, and by cytochrome P450-dependent epoxygenase and *w/w*-1 hydroxylases (Yoshimoto *et al.*, 1986) into epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (DHTs) and 19- and 20-HETEs (Schwartzman *et al.*, 1986; 1990). Mepacrine, an inhibitor of membrane phospholipases, attenuated significantly the inhibitory effect of BK. This result indicates that the effect of BK on oxygen consumption is mediated by metabolites of arachidonic acid. It was reported that several pharmacological effects of BK may be related to an increase in intrarenal prostaglandin levels (Levinsky, 1979). Also, prostaglandin E_2 is natriuretic (Tannenbaum *et al.*, 1975) and inhibits the sodium reabsorption by collecting tubules (Stokes & Kokko, 1977). Therefore, it was proposed that increased prostaglandin synthesis contributes to the natriuretic effect of BK (Levinsky, 1979). But, we were unable to conform it in this study. Inhibition of cyclooxygenase with indomethacin did not reduce the effect of BK on oxygen consumption. Thus, it seems that the effect of BK on oxygen consumption is related the products of lipoxygenases and cytochrome P450-dependent enzymes. Some of the products of these enzymes are biologically active in the renal tubules. 5,6-EET is an inhibitor of ion transport in the rabbit collecting tubules (Jacobson *et al.*, 1984). 11,12-EET inhibits AVP-stimulated water transport in the toad bladder (Schlondorff *et al.*, 1987) and its hydrolytic metabolite 11,12-DHT is an inhibitor of Na-K-ATPase (Schwartzman *et al.*, 1985). 19(S)-HETE is a potent stimulator of renal Na-K-ATPase (Escalante *et al.*, 1988).

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＝국문초록＝

흰쥐 원위세뇨관과 피질집합관의 산소소비량에 대한 Bradykinin의 영향

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이 석 용·조 규 철

Kallikrein-kinin계는 신장의 혈류역학과 수분 및 전해질 배설의 조절자로서 역할을 하는 것으로 알려져 있다. Kallikrein-kinin계의 유효한 펩타이드중 하나인 bradykinin(BK)을 신동맥에 주입시 전해질 배설이 증가하는데 이 작용이 신혈류역학적 변동에 기인하는지 또는 신세뇨관의 전해질 운반에 대한 직접적인 작용에 기인하는지 아직 확실치 않다. 따라서 본 연구에서는 원위세뇨관(DT)과 피질집합관(CCT)에서의 전해질운반 의존성 산소소비에 대한 BK의 영향을 관찰하였다. BK(0.1 μM)은 DT과 CCT의 산소소비를 유의하게 감소시켰으며 이 작용은 Na부재시 나타나지 않았고 ouabain전처치에 의해 차단되었다. 또한 이 작용은 mepacrine에 의해 유의하게 차단되었으며 indomethacin에 의하여는 차단되지 않았다.

이상의 결과는 BK이 DT과 CCT에서 Na운반과 관련한 산소소비를 억제시키며 이 작용에는 prostaglandin들이 관여하고 있지 않음을 시사한다.