Bradykinin Stimulation of Oxidative Metabolism in Renal Cortical Tubules from Rabbit

Possible Role of Arachidonic Acid

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Abstract

Vasoactive peptides may have direct effects on both renal vasculature and renal tubules. In this study, we examined the direct and immediate effects of bradykinin on oxygen consumption by suspensions of cortical tubules from rabbit kidney. Bradykinin (10^{-11} to 10^{-7} M) stimulated oxygen consumption rates (QO_2) in a dose-dependent manner with a maximal increase of $+0.80\pm0.13$ nmol · mg protein^{-1} · min^{-1}. This stimulation was prevented by calcium-free media or by the addition of inhibitors of calcium transport, calcium-calmodulin complex formation, Na,K-ATPase activity, mitochondrial respiration, and phospholipase activity. Addition of bradykinin increased the ADP and AMP contents of cortical tubules without changing the ATP content. These data indicate that bradykinin stimulates ATP use and Na,K-ATPase activity.

We also examined the effects of exogenous arachidonic acid on QO_2 in cortical tubules. Acute additions of arachidonic acid stimulated QO_2 at low concentrations (10^{-8} to 10^{-6} M) and uncoupled mitochondrial respiration at high concentrations (10^{-5} M). The effect of arachidonic acid on adenosine nucleotide content was dose-dependent and indicated increased use of ATP. Bradykinin increased QO_2 in the presence of low concentrations of arachidonic acid (10^{-11} to 10^{-9} M), but had no further effect on QO_2 in the presence of higher concentrations of arachidonic acid (10^{-8} to 10^{-6} M). Bradykinin stimulation of QO_2 was not prevented by inhibition of cyclooxygenase activity with indomethacin but was prevented by inhibition of lipoxygenase-like activity with nordihydroguariaretic acid. These results suggest that the bradykinin effect on QO_2 may be mediated by arachidonic acid release and subsequent metabolism.

Introduction

The renal kallikrein-kinin system is the least defined of the renal endocrine systems that regulate vascular tone and sodium and water excretion (1). Bradykinin is one of the effector peptides of the kallikrein-kinin system. Infusion of bradykinin into renal arteries of dogs increases renal blood flow, sodium excretion, and renal production of other hormones, such as renin and eicosanoids (2–5). Similarly, infusion of bradykinin into ex vivo perfused hydronephrotic kidneys from rabbits stimulates release of arachidonic acid and synthesis of prostaglandin E_2 and thromboxane (6–8). The renal effects of bradykinin are usually attributed to its action on renal hemodynamics. However, bradykinin also reacts with specific binding sites on intestinal (9, 10) and renal epithelia (9, 11). In intestinal epithelia, addition of bradykinin to the serosal media stimulates short-circuit current, chloride secretion, and prostaglandin E_2 production (9, 10, 12, 13). These effects are also produced by addition of exogenous arachidonic acid (13). The effects of bradykinin on renal epithelia are less defined. Therefore, in the present studies we investigated the direct effects of bradykinin and exogenous arachidonic acid on oxidative metabolism by cortical tubules from rabbit kidney. In this system, oxygen consumption rates appear to be a sensitive index of changes in epithelial functions, including both transport and metabolism (14–16).

Methods

We prepared suspensions of separated tubules from the cortex of rabbit kidney according to the methods of Balaban et al. (17) as modified by Harris et al. (18). Briefly, we flushed kidneys of New Zealand White rabbits in situ for 15 min with a solution containing 80 mg/dl collagenase (Worthington Chemical Co., Freehold, NJ), followed by infusion of basic media (see below). We excised the kidneys, dissected the cortex, and dispersed the cortical tubules. We washed the suspension three times in cold basic medium (4°C) and then separated nonvital cells and cellular debris by centrifugation on a cushion of Ficoll (400,000 mol wt [Pharmacia Fine Chemicals AB, Uppsala, Sweden]). After removal from the Ficoll cushion, tubules were washed three more times in the experimental medium and then resuspended to give a concentration of 3–5 mg tubule protein/ml. Three different media were used in these studies. The basic medium for control conditions contained in millimolar concentrations: sodium chloride, 105; sodium bicarbonate, 25; potassium chloride, 5; monosodium phosphate, 2; magnesium sulfate, 1; calcium chloride, 1.5; sodium lactate, 10; L-alanine, 1; D-glucose, 5; and 0.6 g/dl dialyzed dextran (40,000 mol wt [Pharmacia Fine Chemicals]). In calcium-free medium, the basic medium was modified by substitution of sodium chloride for calcium chloride. In sodium-free media, basic medium was modified as follows: potassium chloride (120 mM) replaced sodium chloride; 10 mM K-Hepes replaced sodium bicarbonate; and potassium salts of phosphate and organic substrates were used. Tubules (which were studied in calcium-free or sodium-free media) were washed in the appropriate medium three times before incubation.

We incubated suspensions of cortical tubules in a shaker bath for 15 min at 37°C and aerated them with 95% O_2 to maintain oxygenation and with 5% CO_2 to maintain the pH between 7.35 and 7.45. After this preliminary incubation, we transferred the suspensions directly into a closed chamber to measure oxygen consumption rates (QO_2)[1] during control conditions and after the addition of bradykinin or other experimental substances.

We measure oxygen tension polarographically in a thermostated (37°C) closed chamber (1.7 ml vol) using a Clark-type electrode (Yellow

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1. *Abbreviations used in this paper:* NDGA, nordihydroguariaretic acid; QO_2, oxygen consumption rate.

Spring Instrument Co., Yellow Springs, OH). The oxygen tension in the suspension is recorded as a function of time, and the slope reflects the rate of oxygen consumption. We studied each experimental condition with tubules prepared on several different days. QO_2 were normalized per milligram protein as determined by the Bio-Rad method (Bio-Rad Laboratories, Palo Alto, CA) using bovine serum albumin standards. Data are expressed as means±SE, and we used *t* test for statistical comparisons.

The adenosine nucleotide content of tubule cells was determined by high performance liquid chromatography (19). Aliquots of tubule suspension were added to test tubes containing a 2:1 mixture of dibutyldioctyl phthalate. The tubules were separated from the media by centrifugation through the phthalate layer. The tubules were treated with 10% perchloric acid. The supernatant of the acid-treated tubules was removed, neutralized with potassium hydroxide, and injected (15 μl) into a Waters high performance liquid chromatography system (Waters Associates, Milford, MA). We used a stepwise gradient with methanol for separation on a C_{18} Bondapak column (Waters Associates) with 5-μm beads. The buffer was 0.1 M ammonium phosphate (pH 5.5). We detected peaks with an ultraviolet absorbance monitor at 254 nm. Peak location and quantitation were determined by running known standards under identical conditions. Nucleotide content of the tubules was normalized per milligram protein.

Some of our suspensions were centrifuged and fixed for examination by light microscopy. This analysis demonstrated open tubule lumens and the presence of proximal tubules and cortical collecting tubules. Glomeruli accounted for <5% of the total cell volume. To evaluate their potential contribution, we examined the effect of bradykinin on the QO_2 of a preparation of pure glomeruli that were isolated from collagenase-digested tissue by passage through a series of stainless-steel screens (20). After three washes, the suspension of glomeruli was preincubated at 37°C in control media and the QO_2 was measured as described above. Control QO_2 averaged 10.71±0.97 nmol O_2 · mg protein^{-1} · min^{-1} and did not change after addition of 50 ng/ml bradykinin (10.27±0.87 nmol · mg protein^{-1} · min^{-1}, n = 9). Therefore, the few glomeruli present in suspensions of cortical tubules should have had no effect on the bradykinin-induced changes reported in this paper.

We purchased bradykinin, ouabain, arachidonic acid, nordihydroguariaretic acid, and metabolic substrates from Sigma Chemical Co. (St. Louis, MO). Bradykinin was stored in lyophilyzed aliquots at −70°C and a fresh solution in experimental media was prepared daily. Captopril, SQ14225, was obtained as a powder from E. R. Squibb and Sons, Inc. (Princeton, NJ). Verapamil was provided by Ms. Jean Ehinger of Knoll Pharmaceutical Co. (Whippany, NJ); diltiazem was provided by Dr. Ronald Brown of Marion Laboratories, Inc. (Kansas City, MO); trifluoperazine was provided by Mr. Stephen Vitelli of Smith, Kline and French Co. (Philadelphia, PA); and promethazine was provided by Mr. Peter Russell of Wyeth Laboratories (Philadelphia, PA). We prepared stock solutions of these substances in experimental media every day. Rotenone, antimycin A, and oligomycin were obtained from Calbiochem-Behring Corp. (La Jolla, CA) and were dissolved in 95% ethanol. A 95% solution of ethanol added at concentrations up to 1% of final volume, had no effect on QO_2 measurements.

Results

Addition of bradykinin to suspensions of cortical tubules from rabbit kidney had a direct and immediate effect on QO_2. Bradykinin at a concentration of 2×10^{-8} M caused an immediate increase in QO_2 from an average value of 20.47±0.38 to 21.27±0.41 nmol · mg protein^{-1} · min^{-1}, with a mean paired difference of +0.80±0.13 ($P < 0.01$; n = 31). This effect was transient, lasting 2–3 min in basic medium, but was sustained for up to 6 min by addition of captopril (0.1 mM) to basic media. Captopril inhibits kininase II, an enzyme in proximal tubules that binds and degrades bradykinin (11). Time controls for measurement of oxygen consumption rates with this procedure, including sham additions of media, were 100.4±0.5% of initial QO_2 values (n = 30). Fig. 1 shows the concentration dependence of bradykinin-induced stimulation of QO_2 in suspensions of cortical tubules. We observed no effect at 2×10^{-11} M and the bradykinin effect appeared to saturate at concentrations between 10^{-8} and 10^{-7} M.

Because many peptide hormones exert their effects via transcellular calcium movement, we examined the role of calcium in bradykinin stimulation of QO_2. When we suspended tubules in calcium-free media (three washes in this media reduced calcium concentration to 25–50 μM), the value of control QO_2 was similar to that of cortical tubules in calcium-containing media (21.87±0.56 vs. 21.62±0.79 nmol · mg protein^{-1} · min^{-1}). However, incubation of cortical tubules in calcium-free media prevented the response to bradykinin (see Table I), with a mean paired difference of +0.13±0.21 nmol · mg protein^{-1} · min^{-1} ($P > 70.5$). We also evaluated the role of calcium by adding pharmacologic agents that affect calcium transport by several different mechanisms. In these studies, we measured control values of QO_2, added the experimental compound, and determined the new value of QO_2, and then added bradykinin (50 ng/ml). The data are shown in Table I. Addition of lanthanum to tubule suspensions in basic media significantly reduced control QO_2 (mean paired difference of −0.82±0.12 nmol · mg protein^{-1} · min^{-1}; $P < 0.01$; n = 18) and bradykinin had no effect on QO_2 in the presence of lanthanum and calcium. Verapamil did not affect control QO_2 but altered the response of tubules to bradykinin. Addition of bradykinin to suspensions containing verapamil resulted in a significant fall in QO_2 with a mean paired difference of −0.70±0.12 nmol · mg protein^{-1} · min^{-1} ($P < 0.01$; n = 32). Diltiazem increased QO_2 in calcium-containing media (mean paired difference of +0.46±0.16 nmol · mg protein^{-1} · min^{-1}; $P < 0.01$; n = 24) and prevented any further increase in QO_2 with addition of bradykinin. We also studied the effects of calcium-calmodulin antagonists on bradykinin stimulation of QO_2. Addition of trifluoperizine (10^{-6} M) to suspensions of cortical tubules reduced QO_2 by −0.28±0.07 nmol · mg protein^{-1} · min^{-1} ($P < 0.01$; n = 16) and subsequent addition of bradykinin caused a further reduction in QO_2 (see Table I). Addition of promethazine (10^{-6} M), a phenothiazine that is a less potent antagonist of calmodulin, reduced QO_2 by −0.26±0.08

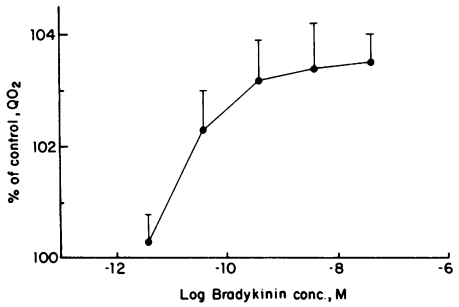

Figure 1. Effect of bradykinin on QO_2 in rabbit cortical tubules. QO_2 for cortical tubules in the presence of bradykinin are expressed as the percent of control values measured in these tubules before addition of bradykinin. The symbols represent the mean±SE for 8–14 observations at each concentration (conc.) of bradykinin. The time controls for QO_2 in this preparation were 100.4±0.5% of initial values (n = 30).

Table I. Effect of Calcium Availability on Bradykinin Stimulation of QO_2

Experimental condition	QO_2			
	Control	Experiment	+BK	(BK-Experiment)
	nmol · mg protein^{-1} · min^{-1}	*nmol · mg protein^{-1} · min^{-1}*	*nmol · mg protein^{-1} · min^{-1}*	*nmol · mg protein^{-1} · min^{-1}*
Calcium-free media (n = 21)	21.62±0.79	21.87±0.56	22.00±0.66	+0.13±0.21
+Lanthanum (0.1 mM) (n = 18)	21.46±0.65	20.64±0.67*	20.62±0.66	−0.02±0.09
+Verapamil (150 nM) (n = 32)	21.42±0.37	21.26±0.36	20.56±0.32‡	−0.70±0.12
+Diltiazem (150 nM) (n = 24)	23.00±0.91	23.46±0.95*	23.50±0.97	+0.04±0.07
+Trifluoperazine (1 M) (n = 16)	17.41±0.56	17.13±0.59*	16.76±0.57‡	−0.37±0.12
+Promethazine (1 M) (n = 16)	17.25±0.61	16.98±0.62*	16.89±0.60	−0.09±0.11

Values represent mean±SE for cortical tubules studied first under control condition, then in the experimental condition, and again, after addition of 50 ng/ml bradykinin (BK). (Bk-Experiment) represents the mean paired difference between BK and experiment values. * Values that are statistically different from control by paired t test ($P < 0.05$). ‡ Values of +BK that are statistically different from experiment by paired t test ($P < 0.01$).

nmol · mg protein^{-1} · min^{-1} ($P < 0.01$; n = 16) and subsequent addition of bradykinin had no further effect on QO_2.

Bradykinin may increase oxygen consumption by increasing mitochondrial respiration, i.e., oxidative phosphorylation, or by stimulating some pathway of cytosolic oxidation. We used inhibitors of oxidative phosphorylation to determine if the bradykinin effect on QO_2 occurred via changes in mitochondrial respiration (see Table II). First, we used rotenone, an inhibitor of mitochondrial NADH dehydrogenase. Addition of rotenone at a maximal dose (5×10^{-6} M) reduced QO_2 to 21% of control values. Subsequent addition of bradykinin had no stimulatory effect on QO_2. Addition of antimycin A (5×10^{-6} M, a maximal dose), an inhibitor of electron transport at the cytochrome b-C_1 complex, reduced QO_2 in cortical tubules to 24% of control values and prevented the bradykinin-induced stimulation of respiration. Addition of oligomycin (5×10^{-6} M), an inhibitor of the mitochondrial ATPase, reduced QO_2 to 40% of control values. The presence of oligomycin prevented bradykinin from stimulating QO_2. Together, these data indicate that bradykinin stimulates mitochondrial respiration and oxidative phosphorylation.

Approximately 50% of oxygen consumption by suspensions of cortical tubules is related to the activity of the Na,K-ATPase (17). For this reason, we investigated whether bradykinin-induced changes in QO_2 require continued Na,K-ATPase activity. We inhibited Na,K-ATPase activity either by addition of ouabain (10^{-4} M) or by resuspending tubules in sodium-free media (sodium <0.2 meq/liter by measurement with a flame spectrophotometer). Both maneuvers markedly reduced QO_2 and blocked the bradykinin effect on QO_2 (see Table III).

In other systems, bradykinin appears to induce the specific release of arachidonic acid from endogenous phospholipids (7, 8). Therefore, arachidonic acid metabolism may mediate the change in oxidative metabolism in our preparation. We added arachidonic acid to suspensions of rabbit cortical tubules and measured the acute change in QO_2. The dose-response curve for arachidonic acid (data shown in Fig. 2) indicated no significant change in QO_2 at concentrations between 10^{-11} and 10^{-9} M, a modest increase in QO_2 at concentrations of 10^{-8} and 10^{-6} M, and a marked increase in QO_2 at 10^{-5} M. This stimulation of QO_2 occurred within 1 min after addition of arachidonic acid and persisted for up to 5 min (the duration of the experiment). The stimulation of QO_2, which resulted from adding 10^{-6} M arachidonic acid, was prevented by the presence of rotenone (4.93±0.20 vs. 4.89±0.18 nmol · mg protein^{-1} · min^{-1}; n = 16), antimycin A (4.43±0.18 vs. 4.30±0.24 nmol · mg protein^{-1} · min^{-1}; n = 14), oligomycin (7.45±0.21 vs. 7.44±0.20 nmol · mg protein^{-1} · min^{-1}; n = 8) or 10^{-4} M ouabain (11.27±0.62 vs. 11.65±0.61 nmol · mg protein^{-1} · min^{-1}; n = 16). However, in the presence of lanthanum (10^{-4} M) arachidonic

Table II. Effect of Mitochondrial Inhibitors on Bradykinin Stimulation of QO_2

Inhibitor	QO_2		
	Control	+BK	Δ
	nmol · mg protein^{-1} · min^{-1}		
Rotenone, (5×10^{-6} M) (n = 26)	4.42±0.17	4.48±0.16	+0.06±0.05
Antimycin A, (5×10^{-6} M) (n = 18)	4.88±0.77	4.86±0.25	−0.02±0.15
Oligomycin, (5×10^{-6} M) (n = 16)	7.97±0.40	8.01±0.39	+0.04±0.09

Values represent mean±SE for cortical tubules studied first in the presence of an inhibitor and again after addition of 50 ng/ml bradykinin (+BK). Δ represents the mean paired differences. No values were statistically different by a paired t test ($P < 0.05$).

Table III. Effect of Na,K-ATPase Activity on Bradykinin Stimulation of QO_2

Condition	QO_2		
	Control	+BK	Δ
	nmol · mg protein^{-1} · min^{-1}		
Ouabain, (0.1 mM) (n = 12)	13.17±0.39	13.01±0.40	−0.16±0.10
KCl media, "Na-free" (n = 16)	8.05±0.51	8.01±0.47	−0.04±0.10

Values represent mean±SE for cortical tubules studied before (control) and after (+BK) addition of 30 ng/ml bradykinin. Δ represents the mean paired differences. No +BK values were significantly different by paired t test.

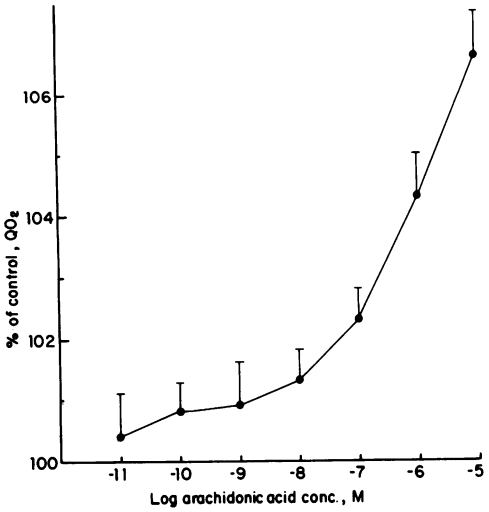

Figure 2. Effect of arachidonic acid on QO_2 in rabbit cortical tubules. QO_2 for cortical tubules in the presence of arachidonic acid are expressed as the percent of control values measured in these tubules before addition of arachidonic acid. The symbols represent the mean±SE for 8–16 observations at each concentration (conc.) of arachidonic acid.

acid still stimulated QO_2 (16.39±0.38 vs. 15.49±0.44; $P < 0.01$; $n = 8$).

Additions of higher concentrations of arachidonic acid (10^{-5} M) had a different effect on QO_2 in the presence of these inhibitors. The presence of rotenone did prevent this concentration of arachidonic acid from stimulating QO_2 (4.62±0.06 vs. 4.62±0.14 nmol · mg protein^{-1} · min^{-1}; $n = 6$), but the presence of oligomycin did not (8.68±0.45 vs. 7.39±0.29 nmol · mg protein^{-1} · min^{-1}; $P < 0.05$; $n = 6$). Similarly, 10^{-5} M arachidonic acid stimulated QO_2 in the presence of ouabain (12.96±0.45 vs. 12.52±0.32 nmol · mg protein^{-1} · min^{-1}; $P < 0.05$; $n = 8$). These data indicate that stimulation of QO_2 by 10^{-5} M arachidonic acid occurs by a different mechanism than stimulation of QO_2 by 10^{-6} M arachidonic acid. The QO_2 data with 10^{-5} M arachidonic acid are consistent with an uncoupling of mitochondrial oxidative phosphorylation.

Next, we examined the effect of bradykinin on oxidative metabolism in the presence of arachidonic acid. After determining the control QO_2, we added arachidonic acid to final concentrations of 10^{-11} to 10^{-6} M. We waited 2 min and then added bradykinin (50 ng/ml). In these suspensions of cortical tubules, addition of bradykinin in the absence of arachidonic acid increased QO_2 by an average of 0.55±0.09 nmol · mg protein^{-1} · min^{-1}. Control QO_2 averaged 18.45±0.80 nmol · mg protein^{-1} · min^{-1}. Addition of arachidonic acid at concentrations of 10^{-11} to 10^{-9} M caused an insignificant increase in QO_2 (+0.11±0.09 nmol · mg protein^{-1} · min^{-1}), but the subsequent addition of bradykinin significantly increased QO_2 by an average of 0.30±0.06 nmol · mg protein^{-1} · min^{-1} ($P < 0.01$; $n = 22$). At higher concentrations of arachidonic acid (10^{-8} to 10^{-6} M), in which control QO_2 was already stimulated, addition of bradykinin caused no further increase in QO_2 (change in QO_2 averaged −0.10±0.06 nmol · mg protein^{-1} · min^{-1}; $n = 20$).

To clarify the mechanism by which addition of bradykinin or arachidonic acid increased QO_2, we examined the adenosine nucleotide content of tubules shortly after addition of these substances. The measured contents of ATP, ADP, and AMP are shown on Table IV. As compared to value from control tubules, addition of bradykinin increased the tubule content ADP and AMP without changing ATP. Addition of 10^{-7} M arachidonic acid increased only the ADP content of tubules, whereas addition of 10^{-6} M arachidonic acid increased all measured nucleotides. Arachidonic acid at a concentration of 10^{-5} M increased the content of ADP and AMP without altering the content of ATP.

If bradykinin stimulates QO_2 by releasing arachidonic acid from endogenous phospholipids, then we predict that an inhibitor of phospholipase activity would block the effect of bradykinin on QO_2. To examine this possibility, we added mepacrine (10^{-5} M), an inhibitor of membrane phospholipases (21), to suspensions of cortical tubules. Mepacrine alone had no effect on QO_2 (mean paired differences averaged +0.17±0.14 nmol · mg protein^{-1} · min^{-1}; $n = 18$). The presence of mepacrine, however, prevented bradykinin from stimulating QO_2 (see Fig. 3). In a similar protocol, we examined whether mepacrine had an effect on arachidonic acid stimulation of QO_2. In the presence of mepacrine, addition of arachidonic acid (10^{-6} M) stimulated QO_2 to the same degree as it did in the absence of mepacrine (data shown in Fig. 3).

Bradykinin stimulation of oxygen consumption may be related to the metabolism of arachidonic acid by cyclooxygenase, lipoxygenase, or cytochrome P-450–dependent monooxidase enzyme systems (22). To examine these possibilities, we evaluated the effect of indomethacin or nordihydroguariaretic acid (NDGA) on bradykinin stimulation of QO_2. Suspensions of cortical tubules were incubated in the absence or presence of indomethacin (0.5 mM) for 15 min before measurement of QO_2. At this dose, indomethacin significantly reduces prostaglandin E_2 production by cortical tubule suspensions (unpublished data). After determination of the control QO_2, we added either bradykinin (50 ng/ml) or arachidonic acid (10^{-6} M). The data, shown in Fig. 4, indicate that the presence of indomethacin had no effect on the stimulation of QO_2 by either bradykinin or arachidonic acid. In similar studies, addition of NDGA (0.1 mM) blocked the bradykinin stimulation of QO_2. Oxygen consumption rates with NDGA averaged 19.07±0.52 nmol · mg protein^{-1} · min^{-1} and after addition of bradykinin QO_2 averaged 18.84±0.48 (mean paired difference was −0.23±0.11; $n = 12$). Thus, the bradykinin effect on QO_2 is not blocked by indo-

Table IV. Effects of Bradykinin or Arachidonic Acid Additions on Adenosine Nucleotide Content of Rabbit Cortical Tubules

Addition	ATP	ADP	AMP
	nmol/mg protein		
Control	8.38±0.36	1.53±0.17	0.72±0.06
Bradykinin	8.44±0.44	1.95±0.09*	0.95±0.07*
Arachidonic acid			
10^{-7} M	7.80±0.48	1.92±0.21*	0.78±0.17
10^{-6} M	9.38±0.56*	2.01±0.23*	1.02±0.15*
10^{-5} M	8.49±0.54	1.96±0.18*	0.95±0.08*

Values represent mean±SE for six cortical tubule suspensions. Suspensions were preincubated for 15 min at 37°C and samples were taken 2 min after addition of blank (control) or experimental substance (bradykinin or arachidonic acid). *Values statistically different from control by paired t test ($P < 0.05$).

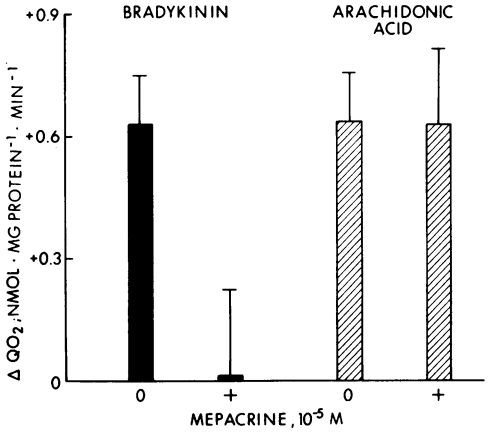

Figure 3. Effect of bradykinin or arachidonic acid on QO_2 in presence of mepacrine. QO_2 of cortical tubules were measured under control conditions in the absence or presence of mepacrine (10 μM) and again after addition of 50 ng/ml bradykinin (solid bars) or 1 μM arachidonic acid (hatched bars). Data are presented as mean paired difference±SE in QO_2 between bradykinin or arachidonic acid and control conditions. Each bar represents 10–18 observations. Addition of mepacrine alone had no effect on QO_2.

methacin, an inhibitor of the cyclooxygenase pathway, but is blocked by NDGA, an inhibitor of lipoxygenase and cytochrome P-450–dependent monooxidase pathways (23, 24).

Discussion

Our studies indicate that bradykinin has a direct effect on cortical tubules from rabbit kidney and that this effect is to increase rates of oxidative metabolism by increasing use of ATP. Previous studies in other systems have shown a direct interaction between bradykinin and epithelia as demonstrated by the presence of specific binding sites for bradykinin on guinea pig ileum (9, 10), guinea pig kidney (9), and cortical collecting tubules from rabbit kidney (11). In intestinal epithelia, bradykinin binding is associated with a stimulation of short-circuit current and chloride secretion (10, 12, 13). In cortical collecting tubules from rabbit kidney (25), addition of lysyl bradykinin blunts the effect of antidiuretic hormone. Thus, the effects of bradykinin in vivo may be due to direct interactions with epithelia in addition to alterations in local hemodynamics. Therefore, we need to know more about the mechanisms involved in bradykinin's direct interaction with epithelia to understand its action on whole organs.

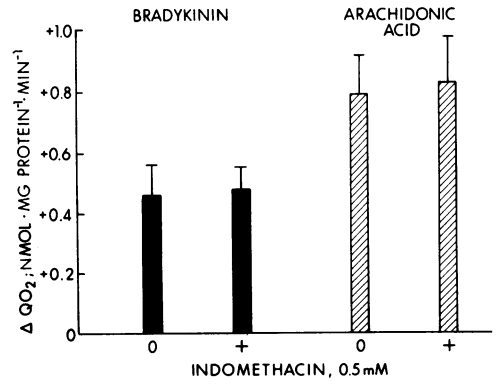

Figure 4. Effect of bradykinin or arachidonic acid on QO_2 in presence of indomethacin. QO_2 of cortical tubules were measured under control conditions in absence or presence of indomethacin (0.5 mM) and again after addition of 50 ng/ml bradykinin (solid bars) or 1 μM arachidonic acid (hatched bars). Data are presented as mean paired differences±SE in QO_2 between bradykinin or arachidonic acid and control conditions. Each bar represents 8–22 observations. Incubation of cortical tubules with indomethacin reduced control QO_2 from 20.37±0.54 to 16.48±1.08 nmol · mg protein^{-1} · min^{-1} ($P < 0.05$; $n = 20$).

In our study with rabbit cortical renal tubules, bradykinin stimulated QO_2 at a minimum concentration of $\sim 10^{-11}$ M and the effect saturated at $\sim 10^{-8}$ M (see Fig. 1). Half maximal stimulation of QO_2 occurred at a bradykinin concentration of $\sim 10^{-10}$ M, which is slightly lower than that reported for half maximal binding of radio-labeled bradykinin to guinea pig ileum (9) and rabbit cortical collecting tubules (11). Maximal stimulation of QO_2 by bradykinin averaged 0.80 nmol · mg protein^{-1} · min^{-1}, which is readily detectable but quantitatively small. The meaning of a response of this magnitude is somewhat difficult to interpret. A bradykinin-induced increase of 3.5% in control QO_2 is equivalent to a 10% increase in ouabain-sensitive QO_2. Furthermore, if bradykinin receptors are not distributed equally among all the tubule segments present in our preparation, an assumption supported by data of Tomita and Pisano (11), then the percent change in QO_2 may be much greater in a subset of tubule segments. Our interest, however, was not in the magnitude of this response but in the mechanisms involved.

The bradykinin effect on QO_2 in cortical tubules appeared to require calcium. We blocked the bradykinin-induced increase in QO_2 by removal of calcium from the extracellular media. In other experiments we used pharmacologic manipulations to alter calcium transport and calcium-calmodulin complex formation (see Table I). These pharmacologic compounds probably have multiple, undefined effects of renal tubules, but they all blocked the bradykinin-induced increase in QO_2. Thus, it seems reasonable to conclude that some aspect of calcium availability is required for the bradykinin effect. A similar calcium dependence occurs in other systems. Bradykinin's stimulation of chloride secretion by guinea pig ileum (10) is markedly inhibited by addition of verapamil. With this result, Manning et al. (10) suggested that bradykinin acts to alter calcium availability to the cell via an interaction with a calcium channel. Although our data are consistent with this hypothesis, the increase in QO_2 may be several steps removed from calcium entry.

The present studies characterized three features of the relationship between bradykinin-induced increase in oxygen consumption and cellular metabolism. First, inhibitors of mitochondrial electron transport (see Table II) completely blocked the effect of bradykinin on QO_2. Thus, the major part of bradykinin-induced QO_2 is via mitochondrial respiration. Second, the effect of bradykinin on QO_2 depends on Na,K-ATPase activity (see Table III). This relationship implies that the hydrolysis of ATP by the Na,K-ATPase is the stimulus for increased oxygen consumption. Third, the addition of bradykinin is associated with an increase in the tubule content of ADP and AMP without a change in ATP (see Table IV). These findings suggest that bradykinin stimulates the hydrolysis of ATP to ADP and AMP and that the ATP content is maintained by increased mitochondrial oxidative phosphorylation. Because the bradykinin effect requires Na,K-ATPase activity, the increased use of ATP may be secondary to increased sodium transport. Therefore, bradykinin may stimulate QO_2 by increasing sodium entry into

the cell. One possible mechanism of increased sodium entry is that of sodium-calcium exchange across the basolateral membrane (26). Our data, however, do not exclude the possibility of bradykinin effects on other transport mechanisms or on other pathways of ATP hydrolysis.

In intestinal and renal epithelia, bradykinin appears to release arachidonic acid from plasma membranes and the effects of bradykinin may be mediated by metabolites of arachidonic acid. For example, in intestinal epithelia addition of exogenous arachidonic acid stimulates chloride secretion in a manner similar to bradykinin (13). Furthermore, inhibition of endogenous arachidonic acid release with mepacrine, an inhibitor of membrane bound phospholipases (21), blocks bradykinin-induced secretion of chloride (13). In studies with isolated perfused hydronephrotic kidneys from rabbits, Schwartzman and Raz (7) and Schwartzman et al. (8) showed that bradykinin infusion stimulated lipolysis and selective release of arachidonic acid. Some of the arachidonic acid was coupled to production of prostaglandins. The rest of the arachidonic acid was available for reacylation into phospholipids or metabolism by other pathways. In our study, we examined the metabolic response of cortical renal tubules to additions of arachidonic acid. At very low concentrations (10^{-11} to 10^{-9} M), arachidonic acid had no effect on control QO_2 and did not block the bradykinin-induced stimulation of QO_2. At slightly higher concentrations (10^{-8} to 10^{-6} M), arachidonic acid stimulated control QO_2 (Fig. 2) and prevented expression of bradykinin's effect on QO_2. These concentrations of arachidonic acid were associated with an increase in tubule ADP content and appeared to increase ATP hydrolysis just as additions of bradykinin did. Still higher concentrations of arachidonic acid (10^{-5} M) further stimulated QO_2 but appeared to uncouple mitochondrial oxidative phosphorylation. Previous studies have reported this phenomenon with high concentrations of long chain fatty acids, includng arachidonic acid (27, 28). Thus, these data show that addition of arachidonic acid in a certain concentration range can mimic the effects of bradykinin on renal QO_2. Furthermore, addition of mepacrine blocked the effect of bradykinin on QO_2 but did not alter the effect of exogenous arachidonic acid (Fig. 3). Together, these observations suggest that arachidonic acid may mediate the effect of bradykinin on QO_2 in rabbit cortical renal tubules. However, these data do not exclude the possibility that other metabolic intermediates are also involved in this effect.

Metabolism of arachidonic acid by renal tissue involves several pathways. The cyclooxygenase enzyme system converts arachidonic acid into cyclic endoperoxides, from which prostaglandins, thromboxanes, and prostacyclin are derived. Arachidonic acid may also be metabolized by the lipoxygenase pathway to form hydroxyeicosatetraenoic acids and leukotrienes (23). Recent studies indicate that renal tissue metabolizes arachidonic acid by a cytochrome P-450–dependent mixed function oxidase system to form leukotriene-like nonpolar products (29, 30). Inhibition of the cyclooxygenase pathway with indomethacin causes a small reduction in the effect of bradykinin on chloride secretion by intestinal epithelia (13), but did not reduce the effect of bradykinin on QO_2 in our system (Fig. 4). However, addition of NDGA, which inhibits formation of both leukotrienes and products of the cytochrome P-450–dependent monoxygenase system (23, 24), completely blocks the bradykinin effect on short-circuit current in intestinal epithelia (13) as well as bradykinin stimulation of QO_2 in renal cortical tubules. Thus, these data suggest that some of the effects of bradykinin on epithelial function may be mediated by products of arachidonic acid metabolism via the cyclooxygenase pathway, whereas other effects may be mediated by products of the lipoxygenase or cytochrome P-450–dependent monooxygenase systems.

In summary, the present studies show that bradykinin induces a direct and immediate stimulation of oxygen consumption by cortical tubules isolated from rabbit kidney. Although several cellular mechanisms may be involved in this hormone-induced phenomenon, we propose the following events as being consistent with our data: Bradykinin interacts with the plasma membranes to increase the availability of calcium within the cell. Calcium-calmodulin complexes are formed and activate membrane bound phospholipases. These enzymes release arachidonic acid and diacyglycerides from membrane phospholipids. These products or their metabolites alter membrane permeability to calcium and/or sodium and stimulate use of ATP by enzymes such as the Na,K-ATPase. The newly formed ADP stimulates mitochondrial respiration. While the mechanisms outlined above are speculative and only indirectly supported by our data, these studies demonstrate that the vasoactive hormone bradykinin has direct effects on metabolism of renal tubules. These effects on epithelial metabolism may be responsible in part for the observed actions of bradykinin on the intact kidney.

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