Vol. 134, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 14, 1986 Pages 299-304

BRADYKININ STIMULATES INCREASED INTRACELLULAR CALCIUM IN PAPILLARY COLLECTING TUBULES OF THE RABBIT

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Received November 26, 1985

The effect of bradykinin on cytoplasmic Ca^{2+} concentration in rabbit papillary collecting tubule cells was determined using the fluorescent indicator Quin 2. Bradykinin stimulated a rapid increase in intracellular Ca^{2+}. The rise in Ca^{2+} was dose dependent, persisted for less than 90 seconds and was independent of extracellular calcium. The ED_{50} for bradykinin induced changes in $[Ca^{2+}]_i$ paralleled that observed previously for hormone-induced PGE_2 formation as well as for inositol trisphosphate labelling. These studies provide additional support for the role of Ca^{2+} as a second messenger for bradykinin in renal papillary collecting tubule cells.

Several investigations have suggested an important role of the kallikrein-kinin system in mediating the action of vasopressin and thus in modulating the excretion of sodium and water by the kidney. In both isolated perfused collecting tubules (1) and toad bladder (2) (a model for the distal nephron) bradykinin appears to antagonize both the hydroosmotic and antinatriuretic effects of vasopressin. The mechanism by which this occurs remains unresolved, in part due to a lack of understanding of how bradykinin transduces its signal, particularly in renal tissue.

We have reported previously rapid changes in phosphatidyl inositol labelling in renal papillary collecting tubule cells stimulated by bradykinin (3). In particular, inositol trisphosphate, a putative mobilizer of

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Abbreviations used in text:
ED_{50}, 50 percent of maximal effective dose; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene-glycol-bis-(B-aminoethyl ether) N,N'-tetraacetic acid, PGE_2, prostaglandin E_2; Quin 2, methoxyquenolone derivative of bis (o-aminophenoxyl) ethane-N,N,N,'N'-tetraacetic acid; Quin 2AM, acetoxymethyl ester of Quin 2.

0006-291X/86 $1.50

intracellular calcium, increases rapidly and reversibly in bradykinin stimulated cells. This apparent rise in inositol trisphosphate occurs over a concentration range of bradykinin and at an ED_{50} comparable to that observed for PGE_2 elaboration. To assess further whether the observed change in inositol trisphosphate is of physiologic significance, we have utilized the intracellular fluorescent probe "Quin 2" to measure intracellular calcium concentrations.

Materials and Methods

Renal papillary collecting tubule cells were isolated and plated as previously described (3,4). In short, female New Zealand white rabbits were anesthetized with pentobarbital (50 mg/kg). The kidneys were resected, placed in phosphate buffered saline, and the papillae were dissected free and minced with a sterile razor blade. The tissue mince was incubated in Krebs solution with collagenase (1 mg/ml) for 90 to 120 minutes. Thereafter, the digestate was filtered through a stainless steel mesh and diluted 1:2, v/v in distilled water. The suspension was pelleted, resuspended in phosphate buffered saline and plated in 25 cm^2 flasks.

Cells were plated in Dulbecco's modified Eagle's media and fetal calf serum (10%). Following 24 hours in culture, the media was changed to a serum-free solution containing a 1:1 v/v mixture of Ham's F12 and DMEM, insulin (5U/ml), hydrocortisone ($5x10^{-8}$M), triiodothyronine ($5x10^{-12}$M), penicillin 50U/ml, streptomycin (50ug/ml), Hepes (10mM) and sodium bicarbonate (1.1 mg/ml). Cells were grown until confluent, usually 3-4 days.

In order to study cells in suspension, adherent cells were dispersed by incubating with 0.02% EDTA for 30 minutes at 37°C. The suspension was washed via centrifugation at 60g for 1 minute and resuspended in fresh medium. Quin 2 acetoxymethylester (10 uM) was added to suspensions of $3-5x10^6$ cells for 60 minutes. The cells were then repelleted and resuspended in a Krebs-Henseleit buffer containing 1.25 mM $CaCl_2$ and 5mM Hepes (pH 7.4).

For fluorescence measurements, intracellular Quin 2 fluorescence changes were measured in 2ml samples of continuously stirred cell suspensions in a 1 cm^2 cuvette at 37° C using a SLM 4880 spectrofluorometer (SLM Instruments, Urbana, IL.) as described by Tsien, et al (5) and as previously employed for renal proximal tubular cells (6). When Quin 2 was excited at 339nM, Ca^{2+} dependent fluorescence was maximal at an emission of 490nM. Slit widths were 8nm and 16nm respectively. The hydrolysis of Quin 2 AM to Quin 2 was followed in each experiment by observing the shift of the maximal fluorescence emission from 432 to 490.

For calcium-free studies, Krebs-Henseleit buffer without $CaCl_2$ was employed. 1.25 mM NaCl was added to maintain osmolality. Intracellular calcium concentration was measured by the ratio of minimal to maximal fluorescence as described by Tsien et al. (5). Briefly, at the end of each experiment, maximal fluorescence was determined by the addition of 50 µl of Triton X; minimal fluorescence was determined by the addition of 50 mM EGTA. Cytosolic calcium concentration was calculated by the formula:

$$[Ca^{2+}]_i = 115 \frac{F-Fmin}{F_{max}-F}$$

where F equals the observed fluorescence and F_{min} and F_{max} are the minimal and maximal fluorescence respectively; and 115 equals the dissociation constant for Ca^{2+}-Quin 2.

The vehicle for Quin 2AM was Me_2SO. Me_2SO concentrations were maintained at 0.1% (v/v). Me_2SO alone had no detectable effect on fluorescence. In each experiment the autofluorescence of unloaded cells was subtracted. Bradykinin had no detectable effect on autofluorescence.

Results and Discussion

Baseline Quin 2 fluorescence at 339 nm emission remained stable throughout the course of the experiments. The extracellular leak of Quin 2 as assessed by the addition of $MnCl_2$ (0.15mM) to the cell suspension was less than 5 percent per hour. This leak was unaffected by the presence of bradykinin at 10^{-7}M. Initial attempts to load the cells with Quin 2 AM in the presence of fully defined media resulted in interfering fluorescence between 410 and 450 nm. This was obviated by loading the cells in Ham's F12:DMEM 1:1 (v/v) with sodium bicarbonate 1.1 mg/ml. The autofluorescence of unloaded cells comprised less that 10 percent of control fluorescence and was unaffected by the presence of bradykinin or EGTA.

The mean intracellular calcium concentration equaled 120 ± 13 nM (n=17). Bradykinin, when added directly to the stirred cell suspension, resulted in a rapid (less than 3 second) increase in Quin 2 fluorescence. This fluorescence peaked by 10 seconds and typically lasted 60 seconds or less (see figure 1). The rapid increase in Quin 2 fluorescence was similar to the time course of bradykinin-induced changes in inositol thirphosphate production previously reported in these cells (3). Further addition of bradykinin to the

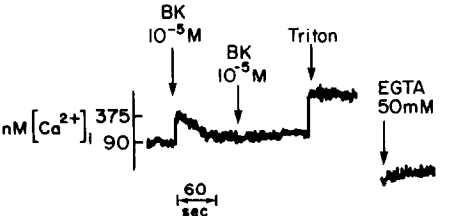
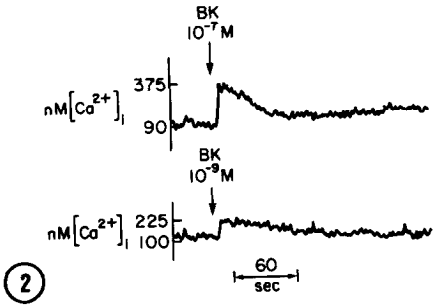


Figure 1: Induced calcium transient in renal papillary collecting tubule cells loaded with 10 μM Quin 2. Characteristically rapid increase in fluorescence with rapid decay to baseline as well as subsequent tachyphylaxis is displayed.



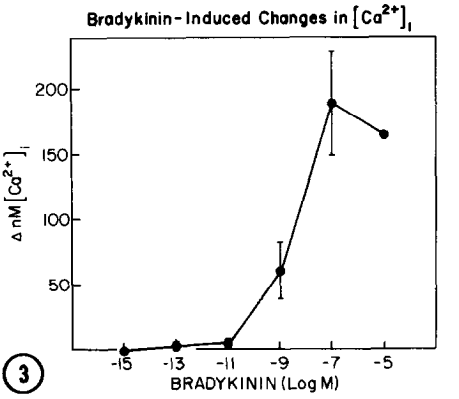


Figure 2: Fluorescence tracings at two doses of bradykinin display dose dependence.

Figure 3: Cumulative data from several cell suspensions in calcium replete Krebs-Henseleit bicarbonate buffer. Each point represents 5 determinations except for 10^{-15} and 10^{-5} molar where only two determinations were made. The ordinate represents the absolute change in calculated intracellular calcium from basal levels at each concentration of bradykinin.

cell suspension was accompanied by the absence of a further calcium transient. A similar tachyphylactic response to bradykinin in prostaglandin E_2 production has been demonstrated in isolated perfused kidneys (7).

Calculated changes in kinin-induced calcium transients demonstrated a dose dependence (Figure 2). Rapidly increasing calcium transients which returned to baseline were observed at all effective doses as was the desensitization to bradykinin. The cumulative data from several experiments assessing kinin-induced changes in Quin 2 fluorescence is displayed in figure 3. The maximal change in intracellular calcium concentration expressed as a function of bradykinin concentration displays an ED_{50} of 5×10^{-8} M. This closely approximates that reported previously for inositol trisphosphate labeling (3).

In order to assess the possible dependency of bradykinin on the presence of extracellular calcium, cells were preincubated with EGTA (1 mM) and subsequently stimulated with bradykinin (10^{-7}M) (Figure 4). The Quin 2 measured intracellular calcium concentration was unaffected by EGTA. The addition of bradykinin, however, did result in an increased, albeit attenuated, intracellular calcium concentration. This attenuation of bradykinin-induced changes in the presence of EGTA may reflect either a true reduction of

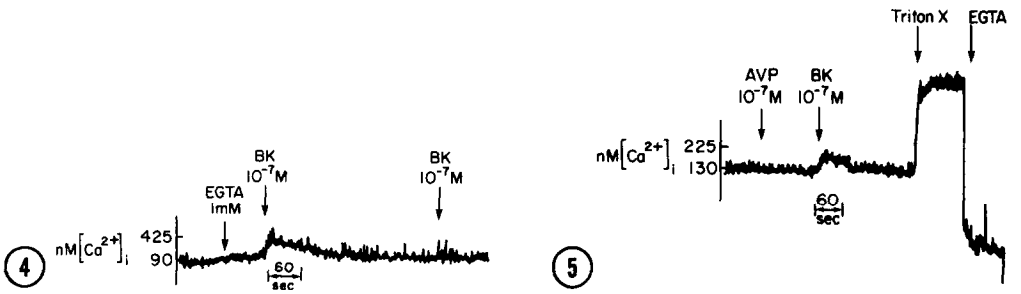


Figure 4: Representative fluorescence tracing of cell suspension incubated in calcium depleted buffer. When EGTA 1mM was added no significant change in fluorescence was observed consistent with a minimal leak of Quin 2.

Figure 5: Failure of arginine vasopressin to induce a change in Quin 2 fluorescence.

putative intracellular calcium stores or a decreased expression of these receptors (8). Bradykinin binding has been demonstrated previously to be a calcium dependent phenomenon (9).

Previous reports have demonstrated a failure of arginine vasopressin to elicit changes in inositol polyphosphate labeling in renal papillary collecting tubule cells (10). In addition, this polypeptide does not induce prostaglandin E_2 formation in these cells. The possible association of arginine vasopressin stimulation with calcium mobilization as detected by Quin 2 was assessed (Figure 5). Vasopressin, at a concentration which stimulates cyclic adenosine monophosphate production, failed to alter Quin 2 fluorescence. When bradykinin was added to the same suspension, however, a persistent but attenuated transient was observed.

Previous observations have demonstrated increased labelling of inositol trisphosphate in response to bradykinin in renal papillary collecting tubule cells. On the basis of an increasing body of data correlating inositol trisphosphate production with the release of ionized calcium from intracellular stores (12,13) one would predict from these observations that bradykinin would increase Quin 2 fluorescence in these cells. The present study confirms this prediction. Bradykinin, a potent agonist of PGE_2 production, increases $[Ca^{2+}]_i$ over a concentration profile and time course observed for both prostaglandin release and inositol trisphosphate

labelling. The ability of bradykinin to mobilize calcium from intracellular stores in the absence of an extracellular pool, provides further support for the view that the transduction of bradykinin signaling in the papillary collecting duct cells of the rabbit is a phosphatidyl inositol mediated event.

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