ELSEVIER

European Journal of Pharmacology 287 (1995) 101–104

ejp

Short communication

Effect of bradykinin on NaCl transport in the medullary thick ascending limb of the rat

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Received 1 September 1995; accepted 3 October 1995

Abstract

The aim of the present study was to determine whether bradykinin affects NaCl reabsorption in the medullary thick ascending limb of the loop of Henle. At 10^{-8} M, bradykinin significantly inhibited Cl^- transport in the in vitro microperfused rat medullary thick ascending limb by 67% ($P < 0.01$). This inhibitory effect could be totally prevented by preincubating tubules with the bradykinin B_2 receptor antagonist *N*α-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin (10^{-6} M). In contrast, the bradykinin B_1 receptor agonist des-Arg^9 bradykinin (10^{-6} M) had no effect on Cl^- transport. Bradykinin caused transient increases in intracellular Ca^{2+} concentration, which could be blocked by the bradykinin B_2 receptor antagonist, but could not be reproduced with the bradykinin B_1 receptor agonist. These data suggest that the natriuretic and diuretic effect of bradykinin in vivo is due, at least in part, to a bradykinin B_2 receptor-mediated inhibition of NaCl reabsorption in the medullary thick ascending limb of the loop of Henle.

Keywords: Loop of Henle; Bradykinin; Ion transport; Ca^{2+}

1. Introduction

Administration of exogenous bradykinin can induce both a natriuresis and diuresis (Scicli and Carretero, 1986; Lortie et al., 1992). While the contribution of altered renal plasma flow to this response cannot be totally eliminated, the fact that glomerular filtration rate is usually unaffected suggests that bradykinin directly influences renal tubular transport capacity. However, the specific tubular site(s) of bradykinin action have yet to be fully established. Micropuncture studies indicate that bradykinin does not affect solute reabsorption in the proximal tubule (Mertz et al., 1984). In the isolated perfused cortical collecting duct bradykinin decreases both solute transport and transepithelial potential difference (Tomita et al., 1985, 1986), but only in animals maintained on a low salt diet (Rouch et al., 1991).

One recent study using anti-peptide and anti-ligand antibodies has reported the presence of bradykinin B_2 receptors in the thick ascending limb of the loop of Henle in the rat (Figueroa et al., 1995). Given the critical role of this nephron segment in the regulation of both salt and water excretion, the present study utilized in vitro microperfusion to determine whether bradykinin affects NaCl transport in the medullary thick ascending limb of the rat.

2. Materials and methods

2.1. Tubular microdissection

To facilitate tubular microdissection, relatively young (28- to 30-day-old; 35–50 g) male Sprague-Dawley rats were used in all experiments. Under sodium pentobarbital anesthesia (60 mg/kg) the kidneys were perfused in situ via the abdominal aorta with 10 ml of ice-cold bicarbonate-buffered Hanks balanced salt solution, transferred to a Petri dish containing ice-cold Hanks supplemented with 0.1% bovine serum albumin (fraction V) and cut into transverse sections. Medullary thick ascending limb segments (0.5–1.0 mm in length) were microdissected from the inner stripe of the outer medulla under stereomicroscopic observation.

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SSDI 0014-2999(95)00640-0

2.2. In vitro microperfusion

Individual medullary thick ascending limb segments were transferred to a temperature-controlled Lucite bathing chamber attached to the stage of a Nikon Diaphot inverted microscope and microperfused at 8–15 nl/min with albumin-free Hanks utilizing techniques described in detail previously with minor modifications (Kidwell et al., 1994). The bathing medium (Hanks + albumin) was continuously bubbled with 95% O_2/5% CO_2 and exchanged at a rate of 0.5 ml/min throughout the experiment. Tubules were initially allowed to equilibrate for 10–20 min; the perfusate collected during this time was discarded. The standard experimental paradigm then consisted of three 10 min control and three 10 min post-treatment collections. Chloride transport was calculated as the difference in chloride concentration between the perfusion fluid and the collected fluid (determined by electrometric titration as previously described) multiplied by the volume flow rate (Kidwell et al., 1994). Tubule length was determined with a calibrated micrometer to allow all transport data to be expressed per mm tubule length.

2.3. Intracellular Ca^{2+} analysis

Intracellular Ca^{2+} was measured with a fura-2 based fluorescent video imaging system described in detail previously (Meininger et al., 1991). Multiple medullary thick ascending limb segments were transferred to a Lucite bathing chamber in which the glass coverslip floor had been pretreated with poly-L-lysine to ensure immobilization of the tubules. Segments were incubated for 20–30 min at room temperature in Hanks containing 2.5 μM fura-2/AM and subsequently washed extensively with probe-free Hanks. For each experiment, the 340/380 nm fluorescent images were collected at 5 s intervals on optical disc for 15–30 s pre- and 300 s post-treatment.

2.4. Statistical analysis

Data were analysed statistically by analysis of variance. Specific differences were obtained using post-hoc analysis by the Newman-Keuls multiple range test. Significance was considered to be $P < 0.05$.

3. Results

Previous studies from this and other laboratories have indicated that Cl^- transport in in vitro microperfused medullary thick ascending limb segments gradually decreases with time. In the present studies we have modified the preparative procedure to include a period of in situ perfusion of the kidney with ice-cold Hanks.

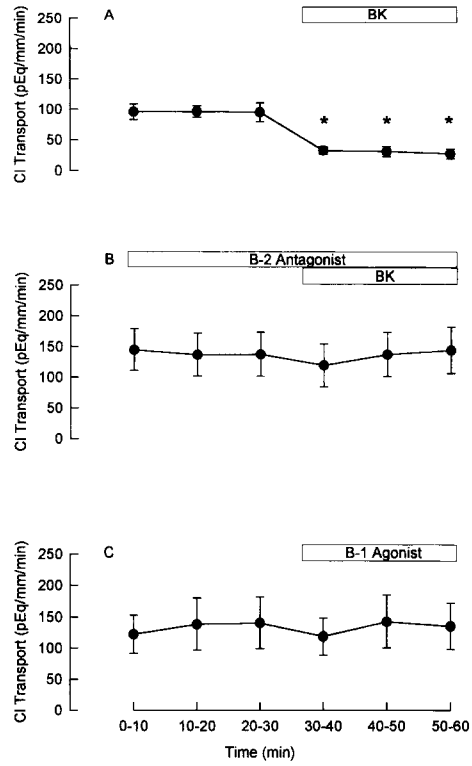

Fig. 1. Effect of (i) bradykinin alone (10^{-8} M; top panel A), (ii) 10^{-8} M bradykinin in the presence of the bradykinin B_2 receptor antagonist $N\alpha$-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin (10^{-6} M; middle panel B), and (iii) the bradykinin B_1 receptor agonist des-Arg^9 bradykinin (10^{-6} M; lower panel C) on Cl^- transport in the in vitro microperfused medullary thick ascending limb of the rat. Each experiment consisted of 3 pre- and 3 post-treatment 10 min fluid collections. Each data point represents mean ± S.E.M. of 3–4 separate experiments. $^*P < 0.05$ or less compared to corresponding control values.

Under these conditions, initial Cl^- transport rates can be sustained for at least 60 min of perfusion (119.9 ± 23.3 vs. 116.7 ± 25.3 pEq/mm/min; $n = 6$).

As presented in Fig. 1 (top panel), addition of 10^{-8} M bradykinin to the bathing medium significantly decreased Cl^- transport by 67% in the medullary thick ascending limb (94.6 ± 11.6 to 30.6 ± 6.7 pEq/mm/min; $P < 0.01$; $n = 4$). Addition of a higher concentration of bradykinin (10^{-6} M) also significantly decreased Cl^- transport (Δ − 42%; 93.1 ± 8.6 to 54.1 ± 9.4 pEq/mm/min; $P < 0.005$; $n = 6$). Although tending to be on average lower, the degree of inhibition occurring with 10^{-6} M bradykinin was not significantly different from that seen with 10^{-8} M bradykinin. This effect is likely mediated via bradykinin B_2 receptors since (i) preincubation with the bradykinin B_2 receptor antagonist $N\alpha$-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin (10^{-6} M) completely blocked the effect of bradykinin (Fig. 1; middle panel), while (ii) the bradykinin B_1 receptor agonist des-Arg^9 bradykinin (10^{-6} M) had no effect on solute transport (Fig. 1; lower panel).

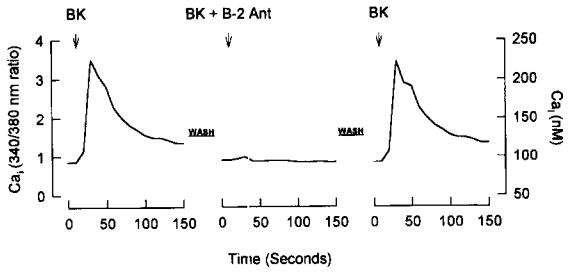

Fig. 2. Effect of bradykinin (10^{-8} M) on intracellular Ca^{2+} concentration in the medullary thick ascending limb of the rat. Three successive 100 s exposures to bradykinin were separated by two 10 min recovery (Wash) periods. Prior to the second stimulation, the bradykinin B_2 receptor antagonist *Nα*-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin was added to the bathing medium, and then removed prior to the third stimulation. Intracellular Ca^{2+} is expressed as both a 340/380 ratio (left axis) and as an absolute (nM) concentration (right axis).

As might be expected with a ligand typically linked to phospholipase C, bradykinin stimulated transient increases in intracellular Ca^{2+} concentration in microdissected medullary thick ascending limb segments. Detectable increases in intracellular Ca^{2+} could be elicited with 10^{-10} M bradykinin, and were maximal at 10^{-7} M peptide. Consistent with the transport responses, the effect of bradykinin on cytosolic Ca^{2+} could be prevented by preincubating with bradykinin B_2 receptor antagonist (Fig. 2), while addition of the bradykinin B_1 receptor agonist had no effect on intracellular Ca^{2+} (pre: 1.19 ± 0.09 vs. post: 1.37 ± 0.12 (340/380 nm ratio); $n = 11$).

4. Discussion

It is well established that intrarenal infusions of bradykinin induce prompt increases in salt and water excretion in the absence of major changes in glomerular filtration rate, suggesting that this response involves direct effects on tubular transport (Lortie et al., 1992). To date, however, the specific tubular site(s) of bradykinin action has not been conclusively established. The results of the present study indicate that the medullary thick ascending limb of Henle's loop may be a primary site of action for this peptide, at least in the rat. Specifically, we have demonstrated that bradykinin significantly inhibits Cl^- transport in the in vitro microperfused medullary thick ascending limb (Fig. 1). This response is likely mediated via bradykinin B_2 receptors, since the inhibitory effect of bradykinin could be completely blocked by preincubation with the bradykinin B_2 receptor antagonist *Nα*-adamantaneacetyl-D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]bradykinin, while in contrast, the bradykinin B_1 receptor agonist des-Arg^9 bradykinin had no effect on Cl^- transport (Fig. 1).

The complete intracellular mechanism of bradykinin action in the medullary thick ascending limb remains to be determined. However, using a fura-2 based fluorescence video-imaging system, we have confirmed that bradykinin elicits transient increases in intracellular Ca^{2+} concentration in the microdissected medullary thick ascending limb, consistent with the well-established coupling of bradykinin receptors to phospholipase C and the subsequent hydrolysis of phosphatidylinositol to inositol trisphosphate and diacylglycerol (Portilla and Morrison, 1986). In keeping with the transport data, bradykinin-mediated Ca^{2+} transients could also be totally eliminated by preincubation with the bradykinin B_2 receptor antagonist (Fig. 2), while bradykinin B_1 receptor agonist had no effect on intracellular Ca^{2+}. It remains to be determined whether the bradykinin-dependent inhibition of transport results directly from this rise in cytosolic Ca^{2+}. Consistent with a recent report from Neant et al. (1994), however, we have established that pharmacologic elevation of intracellular Ca^{2+} with the ionophore A23187 can inhibit Cl^- reabsorption in the rat medullary thick ascending limb (unpublished observation). Several alternative mechanisms do however exist. For example, diacylglycerol (and Ca^{2+})-dependent increases in protein kinase C activity may affect transport. However, at least one report has indicated that pharmacologic activation of protein kinase C using phorbol esters has no effect on transport in the medullary thick ascending limb (Neant et al., 1994). Additionally, bradykinin has been associated with enhanced arachidonic acid metabolism and increased cyclic GMP levels, and both of these second messenger systems have been implicated in the regulation of loop solute transport (Culpepper and Andreoli, 1983; Neant et al., 1994).

The kidney is capable of both synthesis as well as degradation of bradykinin. Consequently, it has been proposed that this peptide acts in a paracrine fashion to modulate renal function (Siragy, 1993). In this context therefore, it is quite conceivable that in contrast to many renally active endocrine factors (for example, antidiuretic hormone), local bradykinin concentrations may well rise to the relatively high levels tested in the present study (10^{-8} M or greater). Recent studies have indicated that intrarenal bradykinin levels increase in response to a low sodium diet (Siragy et al., 1994). Under these conditions, bradykinin receptor antagonists result in significant reductions in urine volume and sodium excretion, which is consistent with an inhibitory effect of the elevated bradykinin levels on tubular transport, while the antagonist was without effect in animals maintained on a 'normal' sodium diet (Siragy, 1993). Interestingly, available evidence indicates that consistent inhibitory effects of bradykinin on solute transport in in vitro microperfused collecting tubules can only be detected in tubules microdissected

from animals maintained on a low sodium diet (Tomita et al., 1985, 1986; Rouch et al., 1991). Whether the response to bradykinin in the medullary thick ascending limb will differ in animals maintained on a low sodium diet remains to be determined.

The concept of tubuloglomerular feedback would predict that a bradykinin-mediated inhibition of NaCl reabsorption in the medullary thick ascending limb should result in a compensatory reduction in glomerular filtration rate, yet this is typically not observed in vivo. One plausible explanation for this apparent inconsistency is that bradykinin may also affect solute transport by the cells of the macula densa, thereby suppressing the effects of an increased delivery of NaCl to this sensing element. In support of this hypothesis, it is well established that furosemide is a potent inhibitor of loop solute transport, yet it typically has no effect on glomerular filtration rate (Braam et al., 1993).

In summary, the present studies have demonstrated that bradykinin, acting via bradykinin B_2 receptors, elevates intracellular Ca^{2+} and inhibits NaCl transport in the medullary thick ascending limb of the rat. The intracellular mechanism(s) involved in this response are currently under investigation.

Acknowledgements

These studies were supported by grants from the American Heart Association, Kentucky Affiliate (B.A.J.), NIH grant HL 53818 (J.C.F.), and NASA EPSCoR 522611 (C.E.O.).

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