ELSEVIER

European Journal of Pharmacology 313 (1996) 115–118

ejp

Short communication

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

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Received 11 April 1996; revised 30 July 1996; accepted 2 August 1996

Abstract

The aim of the present study was to determine whether vasopressin affects NaCl reabsorption in the medullary thick ascending limb of the loop of Henle when administered selectively to the luminal membrane. At 5×10^{-6} M and 10^{-8} M, luminal [Arg^8]vasopressin significantly inhibited Cl^- transport in the in vitro microperfused rat medullary thick ascending limb by $46.4 \pm 5.9\%$ ($P < 0.01$) and $32.4 \pm 2.0\%$ ($P < 0.05$) respectively. The response to 10^{-8} M luminal [Arg^8]vasopressin was completely blocked by the vasopressin V_1 receptor antagonist [β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (10^{-6} M), and was mimicked by the vasopressin V_1 receptor agonist [Phe^1,Ile^5,Orn^8]vasopressin (10^{-8} M; $\Delta - 35.0 \pm 4.5\%$; $P < 0.05$). Luminal administration of the vasopressin V_2 receptor agonist [deamino-Cys^1,D-Arg^8]vasopressin (5×10^{-6} M) had no effect on transport. These data suggest that luminal vasopressin can inhibit NaCl transport in the medullary thick ascending limb of the rat via vasopressin V_1 receptors.

Keywords: Loop of Henle; [Arg^8]Vasopressin; Microperfusion, in vitro; Ion transport

1. Introduction

The traditional concept that renal tubular transport is modulated by hormones which act via receptors located exclusively on the basolateral membrane appears to be no longer valid. In the proximal tubule for example, angiotensin II can modulate solute transport via receptors located on the luminal as well as the basolateral membrane (Douglas and Hopfer, 1994). Similarly, vasopressin receptors have also been identified on the luminal membrane of the collecting tubule. In the rabbit cortical collecting tubule, the stimulatory effects of basolateral vasopressin on both osmotic water permeability and on NaCl reabsorption can be antagonized by luminal vasopressin (Ando et al., 1991; Ando and Asano, 1993; Naruse et al., 1995). While these responses may be mediated by luminal vasopressin V_1 receptors (Ando and Asano, 1993), Nonoguchi et al. (1995a) have recently reported that vasopressin V_2 as well as V_1 receptors are present on the luminal membrane of the rat inner medullary collecting duct.

It is well established that vasopressin, acting via basolateral membrane vasopressin V_2 receptors can also stimulate NaCl transport in the thick ascending limb of the loop of Henle (Culpepper and Andreoli, 1983). Recent studies by Burgess et al. (1994) suggest that luminal vasopressin receptors also exist, since sustained increases in intracellular Ca^{2+} could be elicited by luminal exposure to vasopressin in the in vitro microperfused medullary thick ascending limb of the rat. The aim of the present study therefore was to determine whether selective luminal administration of vasopressin can modulate NaCl transport in this nephron segment.

2. Materials and methods

2.1. Tubular microdissection

Male Sprague-Dawley rats (35–50 g) were anesthetized with sodium pentobarbital (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 containing (in mM): 140 NaCl; 1.4 $CaCl_2$; 0.8 $MgSO_4$; 5.4 KCl; 0.44 KH_2PO_4; 0.33 Na_2HPO_4; 4 $NaHCO_3$ and 100 mg% D-glucose (pH 7.4). The left kidney was removed, 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 microdis-

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PII S0014-2999(96)00620-6

sected from the inner stripe of the outer medulla under stereomicroscopic observation.

2.2. In vitro microperfusion

Individual 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 previously (Grider et al., 1995). The bathing medium (Hanks + albumin) was continuously bubbled with 95% O_2 : 5% CO_2, maintained at 37°C, 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 consisted of three 10 min control collections 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. 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 in in vitro microperfused thick ascending limb segments using a fura-2 based fluorescent video imaging system described previously (Grider et al., 1995). Segments were incubated for 20–30 min at room temperature in Hanks containing 2.5 μM fura-2 acetoxymethyl (AM) ester, 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 30 s prior to and 300 s after treatment. Following each procedure, R_{max} and R_{min} values were obtained using the Ca^{2+} ionophore bromo-A23187 and the calcium chelator ethyleneglycol-bis-(β-aminoethyl ether) N,N'-tetraacetic acid (EGTA) respectively. These values were subsequently used to determine changes in absolute Ca^{2+} concentration for each individual tubule (Grider et al., 1995).

2.4. Statistical analysis

Data were analyzed 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

For these studies, the standard experimental paradigm requires a complete exchange of the control luminal perfusate for perfusate containing selected agonists/antagonists after three initial 10 min control collections. The first series of experiments confirmed that this exchange procedure per se had no effect on transport (Fig. 1, top panel). As presented in Fig. 1 (middle panel), addition of 5×10^{-6} M [Arg^8]vasopressin to the luminal perfusion solution caused a significant sustained inhibition of Cl^- transport in the medullary thick ascending limb (Δ − 46.4 ± 5.9%; $P < 0.01$). In contrast, addition of the vasopressin V_2 receptor specific agonist [deamino-Cys^1,D-Arg^8]vasopressin (5×10^{-6} M) to the luminal perfusate had no effect on Cl^- transport (Fig. 1, bottom panel). Two series of experiments suggested that this vasopressin-dependent inhibition of Cl^- transport is mediated via luminal vasopressin V_1 receptors. Firstly, the reduction in Cl^- transport caused by luminal perfusion with 10^{-8} M [Arg^8]vasopressin (Δ − 32.4 ± 2.0%; $P < 0.05$; Fig. 2, top panel) could be abolished by preincubation with the vasopressin V_1 receptor antagonist [β-mercapto-β,β-cyclopenta-methylenepropionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (10^{-6} M; Fig. 2, middle panel). Secondly, luminal perfusion with the

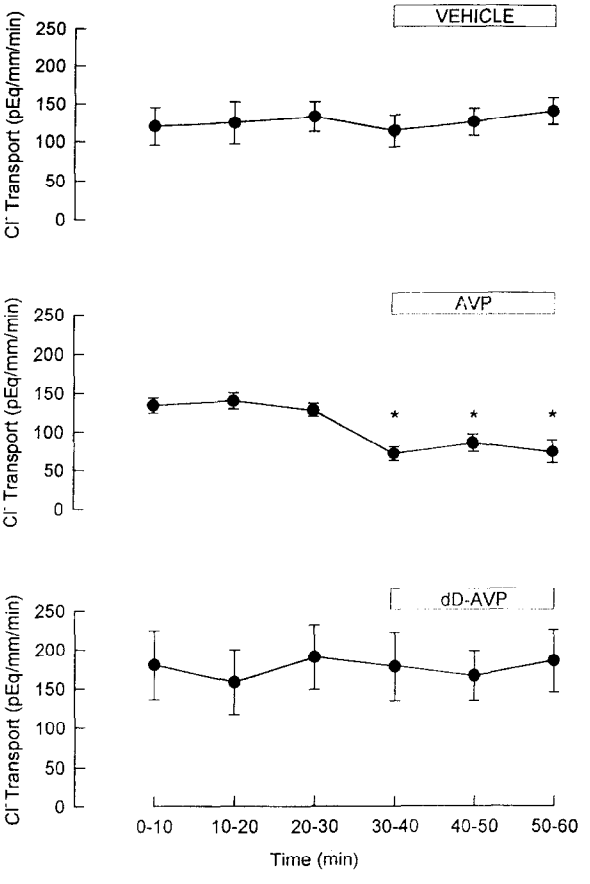

Fig. 1. Effect of (i) exchange of luminal perfusate alone (top panel), (ii) luminal [Arg^8]vasopressin (5×10^{-6} M; middle panel), and (iii) luminal [deamino-Cys^1,D-Arg^8]vasopressin (5×10^{-6} M; lower panel) on Cl^- transport in the in vitro microperfused medullary thick ascending limb of the rat. Each experiment consisted of three control and three post-treatment 10 min fluid collections. Each data point represents the mean ± S.E.M. of at least five separate experiments. * $P < 0.05$ or less compared to controls.

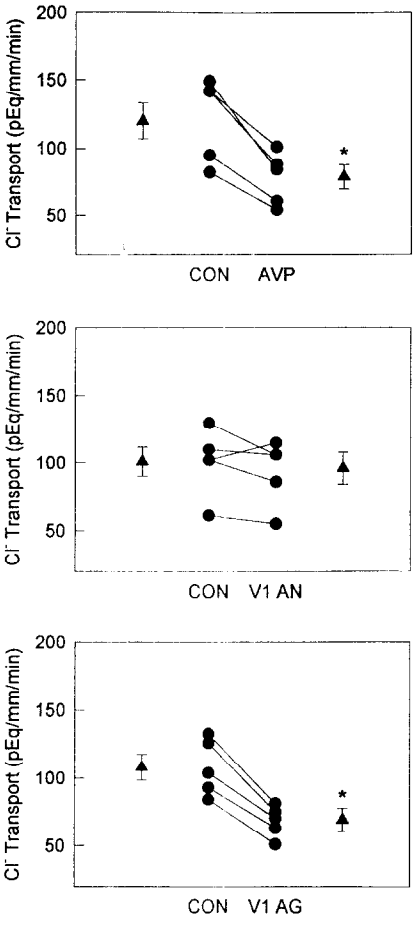

Fig. 2. Effect of (i) luminal [Arg^8]vasopressin (AVP; 10^{-8} M; top panel), (ii) luminal [Arg^8]vasopressin (10^{-8} M) in the presence of the vasopressin V_1 receptor antagonist [β-mercapto-β,β-cyclopenta-methyleneprop ionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (V1 AN; 10^{-6} M; middle panel), and (iii) luminal vasopressin V_1 receptor agonist [Phe^1,Ile^5,Orn^8]vasopressin (VI AG; 10^{-8} M; lower panel), on Cl^- transport in the medullary thick ascending limb of the rat. Each experiment consisted of three control (CON) and three post-treatment 10 min fluid collections. Data points connected by lines represent the mean of the three control and the three post-treatment Cl^- transport values. Closed triangles represent the mean ± S.E.M. for all control and treatment values. * $P < 0.05$ or less compared to corresponding controls.

vasopressin V_1 receptor agonist [Phe^1,Ile^5,Orn^8]vasopressin (10^{-8} M) reduced Cl^- transport to a similar extent to that seen with 10^{-8} M [Arg^8]vasopressin alone ($\Delta - 35.0 \pm 4.5\%$; $P < 0.05$; Fig. 2, bottom panel).

Typically, vasopressin V_1 receptors are coupled to phosphatidylinositol metabolism and the consequent inositol trisphosphate-dependent release of Ca^{2+} from intracellular stores (Michell et al., 1979). However, in the present studies, neither luminal nor basolateral administration of [Arg^8]vasopressin (5×10^{-6} M) had any effect on intracellular Ca^{2+} in the in vitro microperfused thick ascending limb (data not shown). In contrast, as reported previously (Grider et al., 1995), basolateral administration of bradykinin (10^{-6} M) consistently increased intracellular Ca^{2+} in these tubular preparations.

4. Discussion

It is well established that functionally distinct vasopressin receptors exist on the luminal as well as the basolateral membrane of collecting tubule cells (Ando et al., 1991; Ando and Asano, 1993; Naruse et al., 1995; Nonoguchi et al., 1995a). The present studies substantiate a recent report that the rat medullary thick ascending limb of Henle's loop also contains luminal vasopressin receptors (Burgess et al., 1994), and demonstrate for the first time that selective luminal administration of [Arg^8]-vasopressin causes an inhibition of Cl^- reabsorption. A series of experiments indicate that this luminal receptor is vasopressin V_1-like since (i) luminal administration of the vasopressin V_2 receptor specific agonist [deamino-Cys^1,D-Arg^8]vasopressin had no effect on Cl^- transport (Fig. 1, lower panel); (ii) the inhibitory effect of [Arg^8]vasopressin could be completely blocked by preincubation with the vasopressin V_1 receptor antagonist [β-mercapto-β,β-cyclopenta-methyleneprop ionyl^1,O-Me-Tyr^2,Arg^8]vasopressin (Fig. 2, middle panel); and (iii) the vasopressin V_1 receptor agonist [Phe^1,Ile^5,Orn^8]vasopressin decreased Cl^- transport to a similar extent to that seen with [Arg^8]-vasopressin (Fig. 2, lower panel).

The fura-2 fluorescence data (not shown) however are not fully consistent with this conclusion. Typically, vasopressin V_1 receptors are associated with increases in intracellular Ca^{2+}, resulting from enhanced phosphatidylinositol metabolism, and the subsequent generation of inositol trisphosphate (Michell et al., 1979). In the present studies, however, neither luminal nor basolateral [Arg^8]vasopressin had any effect on intracellular Ca^{2+} in the rat thick ascending limb. These data conflict with the recent report from Burgess et al. (1994) who demonstrated that vasopressin, but not a vasopressin V_2 specific agonist, significantly increased cytosolic Ca^{2+} in the rat thick ascending limb. However, it should be noted that even these data are not totally consistent with a classic vasopressin V_1 receptor-mediated (inositol trisphosphate-dependent) response. Firstly, vasopressin caused a sustained elevation of Ca^{2+} which was preceded by a slow onset phase. Secondly, the vasopressin response could be blocked by eliminating Ca^{2+} from the extracellular medium. Interestingly, the molecular analysis of vasopressin V_1 receptor localization is similarly inconsistent. For example, Firsov et al. (1994) reported that mRNA for the vasopressin V_{1a} receptor is absent from the rat thick ascending limb, while a preliminary study by Nonoguchi et al. (1995b) has reported that vasopressin V_{1a} receptor mRNA is restricted to the medullary thick ascending limb of short-looped nephrons under control conditions, but is also detectable in the thick ascending limb of long-looped nephrons after dehydration.

Further studies will be required to establish the intracellular mechanism of action of luminal vasopressin. Our recent studies have demonstrated that the inhibitory effect

of basolateral bradykinin on Cl^- reabsorption in the thick ascending limb is mediated by cytochrome P-450-dependent metabolites of arachidonic acid (Grider et al., submitted for publication). Since early studies by Schwartzman et al. (1985) demonstrated that vasopressin stimulated P-450-arachidonic acid metabolism in rabbit thick ascending limb cells, it is tempting to speculate that these metabolites also mediate the inhibitory effects of vasopressin on Cl^- transport. In contrast to vasopressin, bradykinin elicited transient increases in intracellular Ca^{2+} in the thick ascending limb, suggesting that an early step in the intracellular signaling cascade involves the activation of a Ca^{2+}-dependent phospholipase A_2 leading to the release of arachidonic acid. Clearly, a similar mechanism cannot be proposed for vasopressin, given the lack of effect of this peptide on intracellular Ca^{2+}. However, at least in aortic smooth muscle cells, it has been established that vasopressin-dependent release of arachidonic acid is predominantly mediated by a Ca^{2+}-independent phospholipase A_2 (Lehman et al., 1993).

To this point we have not assessed the effects of simultaneous exposure to basolateral and luminal vasopressin on NaCl reabsorption in the thick ascending limb. In our hands, basolateral vasopressin causes a modest but significant increase in Cl^- transport ($\Delta + 34.6 \pm 15.7\%$; $P < 0.05$). Consequently, based on the present results and on the antagonistic effects of simultaneous exposure to vasopressin in the collecting tubule (Ando et al., 1991; Ando and Asano, 1993; Naruse et al., 1995; Nonoguchi et al., 1995a), we would anticipate that luminal vasopressin will attenuate or eliminate this stimulatory response to basolateral peptide. Clearly, the physiological significance of this interaction will require further study. The urinary concentration of vasopressin (10^{-11} to 10^{-9} M) is 10–100 times higher than that of plasma (10^{-12} to 10^{-10} M; Nonoguchi et al., 1995b). Whether the luminal concentration of vasopressin at the level of the thick ascending limb is high enough to affect solute transport in vivo remains to be determined.

In summary, the present studies have demonstrated for the first time that selective luminal administration of vasopressin inhibits Cl^- absorption in the in vitro microperfused medullary thick ascending limb of the rat. Agonist-antagonist studies suggest that this response is mediated by a vasopressin V_1-like receptor that is not coupled to phosphatidylinositol metabolism.

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 WKU 522611 (C.E.O.).

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