Vol. 180, No. 1, 1991
October 15, 1991

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 131-137

A NOVEL VASOPRESSIN RECEPTOR IN RAT EARLY PROXIMAL TUBULE

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Received August 26, 1991

SUMMARY: In order to evaluate the receptor subtypes of arginine vasopressin (AVP) in early proximal tubule (S_1), outer medullary thick ascending limb of Henle's loop (MTAL) and collecting tubule (OMCT), the effect of AVP on intracellular free calcium ($[Ca^{++}]_i$) was determined using the fluorescence indicator Fura-2. Physiological concentration ($\geq 10^{-12}$M) of AVP in MTAL and OMCT mobilized $[Ca^{++}]_i$ in a dose-dependent manner, but relatively high concentration ($\geq 10^{-9}$M) of AVP in S_1 increased $[Ca^{++}]_i$. Moreover, pretreatment with both V_1 and V_2 antagonists in MTAL or OMCT completely inhibited the AVP-induced $[Ca^{++}]_i$ transient, but in S_1 partially blocked it. Using several AVP analogues, a relative distribution of AVP receptor subtypes was tentatively calculated in each nephron segment, indicating that although these nephron segments possess V_1, its density was very low (about 10%). The majority (about 90%) of AVP receptor in MTAL and OMCT was V_2, while that in S_1 was a new subtype (named V_p) which is insensitive to V_1 and V_2 antagonists. To evaluate physiological significance of V_p receptor, AVP-mediated cellular ATP change was measured. Cellular ATP content in S_1 was significantly increased by 10^{-7}M AVP, but in MTAL it was significantly decreased by the same concentration of AVP. This study suggests that a novel AVP receptor exists in isolated rat S_1, and its physiological significance may be the inhibition of ATP-consuming ion transport system.

Vasopressin is suggested as one of the multifactorial regulators of corticotropin release (1). Its receptor in peripheral tissues are classified into two subtypes, V_1 and V_2. V_1 is involved in vasopressor of vascular smooth muscle and glycogenolytic response in the liver, which is mediated by the production of inositol phosphate (2, 3). V_2 is related to the antidiuretic action in the renal cells including glomeruli, Henle's loop and collecting tubule, which is mediated by the activation of the adenylate cyclase (4-7).

0006-291X/91 $1.50

The recording of intracellular free calcium ($[Ca^{++}]_i$) changes is a good parameter for determining receptor-mediated cellular responses (8-10). We observed that dibutyryl cAMP (intact cell) and IP_3 (permeabilized cell) in rat early proximal tubule (S_1), outer medullary thick ascending limb of Henle's loop (MTAL) and outer medullary collecting tubule (OMCT) mobilized $[Ca^{++}]_i$ in a dose-dependent manner, indicating that stimulation of V_1 or V_2 receptor can mobilize $[Ca^{++}]_i$ in distinct nephron segments. From these hypothesis, this study clarified the localization of AVP receptor subtypes in isolated rat nephron segments.

MATERIALS AND METHODS

Defined nephron segments were isolated as previously described (11). In brief, male Sprague-Dawley rats (210-240g) were decapitated, and left kidneys were perfused with cold Hanks' solution containing 10mM CH_3COONa, 2mM pyruvate, 2mM aspartate, 2mM glutamate, 0.1% bovine serum albumin (BSA), 0.07% collagenase (type I, 240U/mg) and 1mM $CaCl_2$ (pH 7.40). The collagenase treatment was carried out at 37°C for 15min with the same solution. The nephron segments were identified as previously reported (11, 12).

$[Ca^{++}]_i$ measurement was carried out by slightly modified method described by us previously (12, 13). In short, A few pieces of isolated defined nephron segments placed on the center of a serological glass were incubated at 25°C for 20min with modified Hanks' solution containing 10uM Fura-2/AM, 10mM CH_3COONa, 2mM pyruvate, 2mM aspartate, 2mM glutamate, 10% fetal calf serum (FCS) and 0.5mM $CaCl_2$ (pH 7.40) on a vibratile stage for histological immunostaining. Fluorescence-loaded cells were washed three times with the same solution, except Fura-2/AM and FCS. The loaded isolated tubules together with 90ul medium were transferred on a cover glass siliconized freshly, and the fluorescence of a single nephron segment was measured at 25°C using a 2 wave-length microscopic fluorometer (CAM 220, JASCO, Japan). During monitoring fluorescence intensity at two wave lengths, agonists or antagonists (10ul) were mixed with the 90ul medium. In each nephron segment, the iris of fluorescence was maximally adjusted to each nephron diameter (12).

The method for cellular ATP content was the same that described by us previously (11, 12, 14). Briefly, medium for ATP measurement was Hanks' solution containing 0.5mM $CaCl_2$ (pH 7.40). After nephron segment was incubated at 25°C for 10min with and without 10^{-7}M AVP, ATP extraction was done by adding 10% TCA/4mM EDTA to each sample. Entire samples including the incubation medium, the extracting agent and the denatured tissues were transferred to disposable polystyrene cuvettes filled with 0.1M Tris/acetate buffer containing 0.5mM EDTA (pH 7.75). The cuvettes were set into the luminometer, and ATP monitoring reagent was added to a sample-containing cuvette by an autodispenser. The light intensity was measured for 10sec by a computer-driven system.

The materials used were purchased as following: Fura-2/AM from Dojin Laboratories (Japan); AVP ([Arg^8-vasopressin), dDAVP ([deamino-Cys^1,D-Arg^8]vasopressin), V_{1a} [$d(CH_2)_5$Tyr(Me)AVP] and V_2 [$d(CH_2)_5$Tyr(Et)AVP] antagonists, collagenase and BSA from Sigma Chemical (St. Louis, MO); oxytocin/vasopressin antagonist [$d(CH_2)_5$,D-Tyr(Et)^{2}Val^{4}Cit^8, VP] from Peninsula Laboratories (Belmont, California); ATP monitoring reagent from LKB (Finland); FCS from Gibco laboratories (N.Y., U.S.A.). All other chemicals were of the highest grade available.

RESULTS AND DISCUSSION

AVP transiently increased $[Ca^{++}]_i$ in S_1, MTAL and OMCT, followed by sustained increase of $[Ca^{++}]_i$ up to 14-17min. AVP concentrations that mobilized $[Ca^{++}]_i$ significantly differed among S_1, MTAL and OMCT (Fig. 1). ED_{50} for $[Ca^{++}]_i$ mobilization in S_1, MTAL and OMCT was $1.5x10^{-7}$, $5x10^{-9}$ and $9x10^{-9}$M, respectively, and their maximal relative increment values were 308.4, 150.7 and 216.8% of the resting levels, respectively. The effect of AVP within the proximal tubule was limited to S_1, but not to S_2 or S_3. Since tissue amounts of isolated nephron

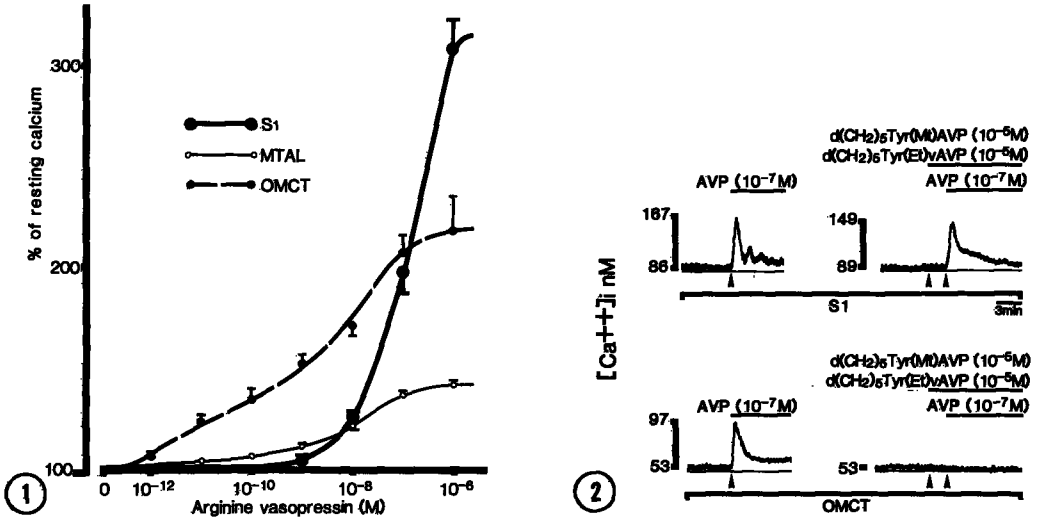

Figure 1. Dose-response curve of AVP on $[Ca^{++}]_i$ transient in rat S_1, MTAL and OMCT. Percent increase of $[Ca^{++}]_i$ transient by AVP was calculated from respective basal values, and plotted. Values are means ± SE of 9 experiments.

Figure 2. Effect of AVP antagonists on AVP-induced $[Ca^{++}]_i$ signal in rat S_1 and OMCT. Both V_1 and V_2 antagonists were applied 2min before adding AVP in each nephron segment.

segments were insufficient to reveal specific ligand bindings, we used renal tubular suspensions. In [^{3}H]AVP-binding study, Kd values for AVP binding in the S_1-contained superficial cortex and MTAL- and OMCT-contained inner stripe of outer medulla were $\geq$5.07nM and 1.2nM, and Bmax values were values $\geq$155 and 100fmol/mg protein, respectively (data not shown). Thus, the kinetic of AVP receptor located in S_1 may be characterized as low affinity and high capacity compared to that existed in MTAL or OMCT.

As shown in Fig. 2, the AVP-induced $[Ca^{++}]_i$ signal in MTAL and OMCT was completely blocked by the pretreatment of these segments with both V_{1a} and V_2 antagonists for 2 min, but that in S_1 was inhibited only partially (about 13%). Vasopressin receptor in adenohypophysis is characterized as a V_{1b}, a subtype different from V_{1a} which exists in liver, blood vessel and cultured mesangial cells (15). To clarify the relationship between V_{1b} receptor and AVP-induced $[Ca^{++}]_i$ transient in S_1, we used both V_{1a} and V_{1b} (desGly$(NH_2)^9$[1-(B-mercapto-B,B-cyclopenta-methylenepropionic acid), 2-D-O-ethyl-tyrosine,4-valine]AVP) antagonists with the same method described in Fig. 2. They both partially (10%) inhibited the AVP-induced $[Ca^{++}]_i$ transient in S_1 (Fig. 3). An AVP analogue, dDAVP, is well known as a highly potent antidiuretic (V_2) agonist and is devoid of V_1 action as well as of oxytocin receptor-mediated responses (15-17). dDAVP (10^{-7}M) transiently increased $[Ca^{++}]_i$ in MTAL and OMCT, but did not affect its basal level in S_1 (Fig. 3). Pretreatment of MTAL and OMCT with V_2 antagonist completely blocked the dDAVP (10^{-7}M)-induced $[Ca^{++}]_i$ signal. The results described in Figs. 2 and 3, therefore, clearly demonstrate the existence of a novel vasopressin receptor (named V_p) in rat S_1, which is different from V_{1a}, V_{1b} and V_2.

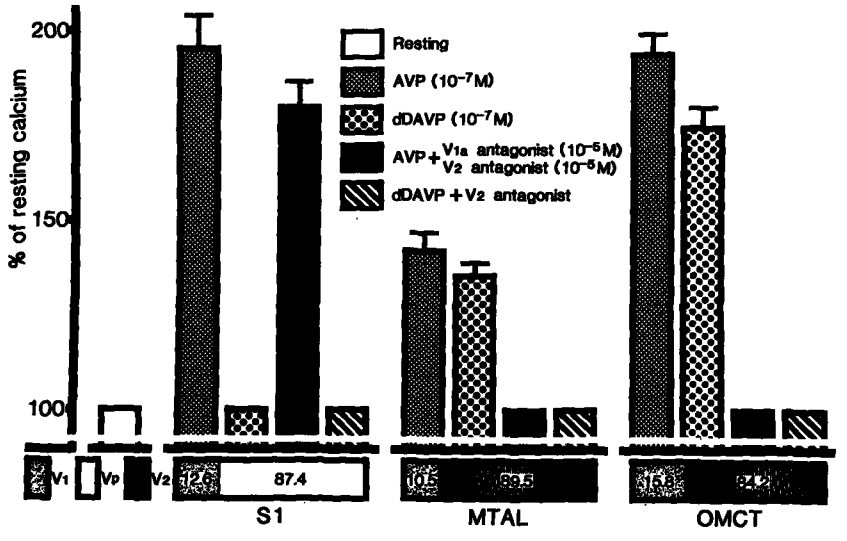

Figure 3. V_1 and/or V_2 antagonists on AVP- or dDAVP-induced $[Ca^{++}]_i$ transient and relative distribution of AVP receptor subtypes in isolated rat S_1, MTAL and OMCT. Experimental methods were the same as that described in Figs. 1 and 2. The relative distributions of receptor subtypes were calculated from the results of combined experiments using well established subtype-specific agonists and/or antagonists.

In contrast, oxytocin shows the diuresis and phosphoinositol formation in renal cells (18), and its receptor showed a high-affinity characteristic (19, 20). Like dDAVP, oxytocin could increase $[Ca^{++}]_i$ in MTAL and OMCT, but not in S_1 (data not shown). The action of oxytocin in MTAL and OMCT was completely blocked by the oxytocin antagonist. Moreover, 10^{-7}M AVP-induced $[Ca^{++}]_i$ increase in S_1 was not affected by 10^{-5}M oxytocin/vasopressin antagonist (20). Accordingly, V_p is not related to the oxytocin receptor. In addition, we tentatively calculated a relative distribution of AVP receptor subtypes in isolated rat S_1, MTAL and OMCT (Fig. 3). Although these nephron segments possess V_1 receptor, its density was very low (about 10%). The majority (about 90%) of AVP receptor in S_1 was V_p, while that in MTAL and OMCT was V_2.

AVP has been known to stimulate Na^+ absorption in MTAL (21, 22). When Na^+ absorption is increased, cellular ATP level should

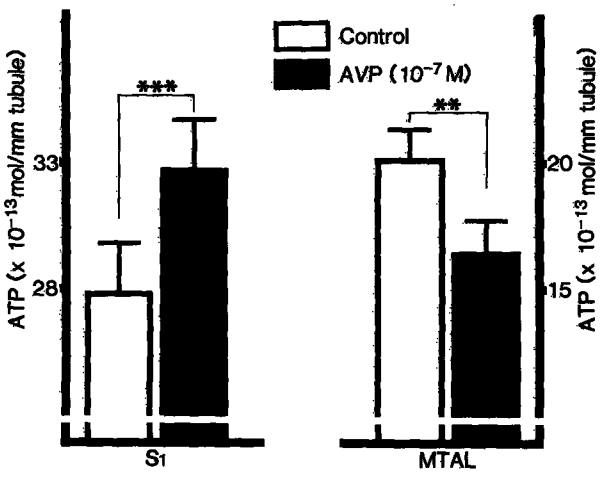

Figure 4. Effect of AVP on intracellular ATP content in isolated rat S_1 and MTAL. Each bar represents means ± SE of 5 experiments. Paired t-test was used for analyzing statistical significance. **p<0.01, ***p<0.005.

decrease without further supply of exogenous substrates (23). Thus, cellular ATP measurement is a useful tool for evaluating Na^+ transport (14). In the absence of substrates, cellular ATP content was significantly decreased by 10^{-7}M AVP in MTAL, whereas in S_1 it significantly increased, indicating that the stimulation of V_p may play an inhibitory role in the Na^+ reabsorption in S_1 (Fig. 4). In previous reports (24, 25), AVP infused into the renal artery at pharmacological concentrations depressed the proximal tubular reabsorption of sodium, phosphate and bicarbonate. This is in good accordance with the present result on V_p existence and its possible function in S_1.

This study suggests the existence of a novel vasopressin receptor, V_p, in rat early proximal tubule. V_p displayed biochemically and pharmacologically distinct properties from already established V_1 or V_2. One V_p action is inhibition of the ATP-consuming transport system in the early proximal tubule.

ACKNOWLEDGMENTS

The authors thank Profs. F. Sakai and M. Endo for their useful advice and encouragement. This study was supported in part

by the Japanese Ministry of Education, Science and Culture, the Japanese Ministry of Health and Welfare, the Hoansya and the Shimabara Science Promotion Foundation.

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