Effect of Atrial Natriuretic Factor on Vasopressin-Stimulated Cyclic AMP Accumulation in Papillary Collecting Tubule

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Dear Sir,

A number of studies indicate that atrial natriuretic factor (ANF) acts to impair arginine vasopressin (AVP)-induced water transport. In the perfused rabbit cortical collecting tubule, ANF inhibited the hydraulic conductivity response to AVP, but not to forskolin or cyclic AMP (cAMP) [1]. It was concluded that ANF acted proximally to the cAMP generation step. In perfused rat inner medullary collecting ducts, ANF inhibited AVP-induced osmotic water permeability, and also exogenous cAMP-stimulated osmotic water permeability [2]. It was felt that ANF acted distally to cAMP generation. In another study in perfused rat inner medullary collecting ducts, ANF inhibited AVP-stimulated hydraulic conductivity, but had no effect on the exogenous cAMP-induced hydroosmotic effect, suggesting that the effect occurs before cAMP formation [3]. Finally, in toad bladder, ANF inhibited vaso-tocin-induced water reabsorption [4].

These studies indicate that ANF does appear to impair AVP-induced water transport. However, there is some disagreement as to the exact mechanism. We therefore chose to study the effect of ANF on cAMP metabolism. Renal papillary collecting tubule (RPCT) cells were isolated from the papillae of 125- to 150-gram male Sprague-Dawley rats [5]. Intact RPCT cells were suspended in serum-free, fully defined medium which favors epithelial cell growth. Medium was changed daily until the cells approached confluence at 3-4 days. Experiments were performed on the primary cultures. Triplicate determinations of cAMP were made under each experimental condition in each of 6

As shown in table 1, AVP caused a dose-dependent increase in intracellular cAMP accumulation in cultured RPCT cells. ANF at concentrations from 10^{-9} to 10^{-7} M had no effect on basal cAMP accumulation. In addition, ANF at concentrations from 10^{-9} to 10^{-7} M did not change the dose-response effect of AVP on intracellular cAMP accumulation (table 1).

A number of other studies have looked at the effect of ANF on cAMP metabolism in a variety of tissues. In microdissected rat cortical and medullary collecting tubules, ANF had

no effect on basal or AVP-stimulated cAMP generation [7]. In isolated rat inner medullary collecting duct segments, ANF had no effect on basal or AVP-stimulated separate primary cultures. Incubations were carried out in room air at 37 ° C in the presence of 0.1 mM 3-isobutyl-1-methylxanthine. Dose-response data for the effect of synthetic ANF and AVP were generated by the addition of the agonist at time zero, followed by a 5-min incubation that was ended by the removal of the experimental medium, followed by the addition of 0.1 N HCl which extracts intracellular cyclic nucleotides. Intracellular cAMP was measured by radioimmunoassay after acetylation. Cellular protein was measured in each culture dish by the Lowry method [6]. Results are expressed as mean $\pm$ SEM. Statistical significance was determined by use of Student's t test for unpaired data.

Table 1. Effect of ANF on AVP-stimulated cAMP accumulation

Values represent intracellular cAMP (fmol/ μ g protein/5 min). Values are mean $\pm$ SEM from 6 separate primary cultures, each condition studied in triplicate.

cAMP generation [8]. In cultured rabbit cortical collecting tubule cells, ANF had no effect on basal or AVP-stimulated cAMP generation [9]. In rabbit outer and inner medullary tubule suspensions, and in rat microdissected inner medullary collecting ducts, ANF had no effect on AVP-stimulated cAMP generation [10]. In homogenates of dog medullary collecting ducts, ANF inhibited basal adenylate cyclase activity, but had no effect on AVP-stimulated adenylate cyclase activity [11]. Finally, in rat cultured papillary collecting tubule cells, ANF had no effect on basal cAMP accumulation [12]. ANF did inhibit AVP and forskolin-stimulated cAMP accumulation; however, most studies required at least 10^{-7} to 10^{-6} M ANF to see an effect.

In summary, it appears that ANF does not significantly impair AVP-induced cAMP accumulation in vasopressin-sensitive epithelia. The reduction of vasopressin-induced water transport caused by ANF most likely does not result from impaired adenylate cyclase activity.

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