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# COMMENTARY

# VASOPRESSIN $V_1$ RECEPTORS AND INTER-RECEPTOR REGULATION IN VASCULAR SMOOTH MUSCLE CELLS

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The management of blood pressure is a complex process involving a large number of transmitters and receptors. The transmitters and their receptors regulate the three significant factors determining blood pressure: cardiac output, vascular tone and blood volume. These interactions involve elements of the autonomic nervous system, circulating hormones and transmitters, endothelial and smooth muscle cells, the kidney and blood cells. To manage blood pressure in real time (second to second), the effects of each of the effectors and processes must be coordinately regulated, and numerous interactions have been identified that regulate these processes [1].

Despite recent advances in the receptor biology of vasoactive hormones and transmitters, relatively little attention has been directed to one potentially important mechanism to manage vascular tone and perhaps other factors that determine blood pressure: receptor–receptor interactions that may result in inter-receptor regulation of vascular tone. The potential utility of such inter-receptor regulation is that it may be an effective method to rapidly adjust intracellular concentrations of key second messengers, and, thus, the degree of contraction of smooth muscle cells.

Vasopressin is a hormone that plays a key role in the regulation of vascular tone and blood volume [2, 3]. Via interactions with vasopressin receptors ($V_2$) in the collecting ducts of the kidney, vasopressin induces reabsorption of water. To effect increased water reabsorption, vasopressin $V_2$ receptors induce activation of adenylate cyclase and an increase in cAMP [4]. Adenylate cyclase and $V_2$ receptors are coupled by the stimulatory guanine nucleotide binding protein, $G_s$. Increased vascular tone is mediated by $V_1$ receptors. Recent data from our laboratory and others have shown that activation of $V_1$ receptor induces an increase in inositol-trisphosphate and calcium mobilization [5–7]. These receptors are also coupled through a guanine nucleotide binding protein since the addition of guanine nucleotide to the membranes decreased the affinity of the agonist for the receptors [8, 9].

Because of the pivotal role vasopressin plays in the regulation of vascular tone and blood volume, its effects both on cAMP and inositol phosphate turnover, and the fact that it causes a release of arachidonic acid [10], we reasoned that it is likely that activation of $V_1$ receptors may directly alter the activities of other vasoactive receptors and have reported recently the results of a number of our initial studies in this regard [11, 12]. We believe that our studies demonstrate inter-receptor regulation effected by $V_1$ receptors and that this may play a key role in the management of blood pressure. Thus, studies addressing inter-receptor regulatory processes, in general, may provide insights into normal and pathophysiologic behaviors. The purpose of this commentary is to summarize our findings, propose a model to explain $V_1$ receptor-mediated effects on other vasoactive receptors, and identify some of the critical questions that remain to be answered. We would also hope to stimulate research in this general area.

To study potential interactions between receptors and signal transduction processes, we concluded that it was essential to use a clonally derived smooth muscle cell line, maintained in continuous tissue culture, that displays a number of receptors for vasoactive agents. Screening of a number of cell lines identified the vascular smooth muscle cell line A-10 (ATCC CRL 1476) as being ideally suited for such studies. These cells display a high density of vasopressin receptors and atrial natriuretic factor (ANF†) receptors, and low density of $\beta$-adrenergic receptors. Based on competitive binding studies, these vasopressin receptors are classified as $V_1$ receptors [13].

### *Beta-adrenergic agonist-stimulated cAMP accumulation in A-10 cells*

The $\beta$-adrenergic receptor agonist, isoproterenol, when added to A-10 cells, induces a dose- and time-dependent increase in cAMP which was blocked by

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† Abbreviations: ANF, atrial natriuretic factor or atriopeptin; AVP, arginine vasopressin; PDBu, phorbol-12,13-dibutyrate; 4$\alpha$-PDD, 4$\alpha$-phorbol-12,13-didecanoate; NEM, *N*-ethylmaleimide; PI-PLC, phosphoinositide specific phospholipase, C; PC-PLC, phosphatidylcholine specific phospholipase C; $PLA_2$, phospholipase $A_2$; and PKC, protein kinase C.

the β-adrenergic antagonist, propranolol. These β-adrenergic receptors belonged to the $\beta_2$ subtype since epinephrine was much more potent than norepinephrine in inducing cAMP production. In addition to beta adrenergic agonists, forskolin also stimulated cAMP accumulation in these cells [11].

*ANF-stimulated cGMP accumulation in A-10 cells*

ANF I, II, and III induced a concentration-dependent increase in cGMP in A-10 cells [12]. An increase in cGMP was measurable within 1 min of addition of ANF and continued for at least 60 min. The half-maximal concentration for the three ANF analogs was between 200 and 300 nM or about 50 times the dissociation constant ($K_d$) for ANF binding to these cellular receptors. At present we have no explanation for this discrepancy. Since the A-10 cells displayed no soluble guanylate cyclase, and since neither nitroprusside nor sodium azide, stimulants of soluble guanylate cyclase, had any stimulatory effect on cGMP accumulation, we conclude that the stimulatory effects of ANF are mediated by a particulate guanylate cyclase [12].

*Vasopressin-induced inositol trisphosphate accumulation and calcium mobilization*

Vasopressin induced a rapid increase in inositol mono-, di- and trisphosphate (within 15 sec) accumulation in A-10 cells [5]. The concentration of vasopressin required to increase these inositol phosphates correlated well with the $K_d$ of vasopressin in these cells [13]. In addition, calcium efflux was observed also within 15 sec of treatment with vasopressin. These effects were mediated by $V_1$ receptors. In competitive binding studies using the recently developed radiolabeled vasopressin antagonist, [$^3$H]SK&F-101926, we have demonstrated that these $V_1$ receptors are coupled to a guanine nucleotide binding protein [8, 9]. The involvement of $G_s$, or $G_i/G_o$ [14] was tested by pretreating the cells with cholera toxin and pertussin toxin. While cholera toxin had no effect on vasopressin-induced inositol phosphate accumulation, pertussin toxin marginally inhibited (15%) this response. On the other hand, *N*-ethylmaleimide (NEM), which is known to inhibit G proteins [15], induced a concentration-dependent inhibition of the vasopressin-mediated increase in inositol phosphate accumulation and calcium efflux [16]. NEM appeared to affect the G protein since the binding of [$^3$H]AVP to inhibit cells was unaffected by NEM. At the same time, NEM uncoupled the $V_1$ receptors from the G protein as indicated by the competition binding experiments with [$^3$H]SK&F-101926 and vasopressin and by the inhibition of vasopressin-stimulated GTPase activity. The basal levels of inositol phosphate accumulation were unaffected by NEM, indicating that phospholipase C was probably not affected.

*Vasopressin-induced arachidonic acid release*

In A-10 cells, vasopressin induced the release of arachidonic acid [10]. Arachidonic acid release is mediated by $V_1$ receptors, is vasopressin concentration-dependent, is rapid, and does not require protein synthesis. Vasopressin-induced arachidonic acid release was partially blocked by dexamethasone, a $PLA_2$ inhibitor, and by manoalide, an inhibitor of PI-PLC and $PLA_2$, but the combination of the two was not additive. The 50% inhibition of arachidonic acid release induced by manoalide occurred at concentrations that completely inhibit PI-specific PLC-induced inositol phosphate metabolism, and even higher concentrations of manoalide that inhibit both PI-PLC and $PLA_2$ [17, 18] failed to inhibit arachidonic acid release by more than 50% [10].

*Effects of vasopressin on isoproterenol-induced cAMP and ANF-induced cGMP accumulation*

Vasopressin induced a concentration-dependent inhibition of isoproterenol-stimulated cAMP accumulation [11]. A maximal inhibition of 30–40% was observed, and the half-maximal effective concentration was 0.8 nM vasopressin. Vasopressin also inhibited by 25–30% forskolin-induced cAMP increases, and both these effects were clearly mediated by $V_1$ receptors. In contrast to studies in platelets [19], at no concentration studied did vasopressin affect the basal level of cAMP. Pertussis toxin had no effect on the vasopressin-induced inhibition of cAMP accumulation.

Vasopressin inhibited the increases in cGMP induced by ANF. In a manner similar to that observed in studies on its effects on isoproterenol-induced cAMP accumulation, vasopressin inhibited approximately 50% of the cGMP accumulation induced by ANF. The half-maximal concentration for vasopressin inhibition of ANF-stimulated accumulation of cGMP was 0.4 nM, corresponding well with the $K_d$ for $V_1$ receptors, and the effects of vasopressin were blocked by $V_1$ antagonists. Thus, these effects are also mediated by $V_1$ receptors [12]. Again, vasopressin had no significant effect on basal cGMP levels.

*Role of protein kinase C (PKC)*

Protein kinase C is a ubiquitous enzyme with broad substrate specificity that has been found in most mammalian cells [20]. One of the products of phosphatidylinositol turnover, diacylglycerol, stimulates PKC by increasing the affinity of the enzyme for calcium. Of great significance is the finding that this enzyme can be activated by synthetic diacylglycerols and also by tumor promoting phorbol esters. As a result, these phorbol esters have been widely used to assess the role of PKC in cellular regulation by various hormones. Pretreatment of A-10 cells with phorbol-12,13-dibutyrate (PDBu) had no effect on the binding of [$^3$H]AVP to $V_1$ receptors or [$^{125}$I] ANF to ANF receptors in intact cells, nor did PDBu affect the basal levels of cAMP, cGMP, or inositol phosphate accumulation. However, PDBu significantly inhibited AVP-induced inositol phosphate accumulation and calcium fluxes. This inhibition was dose and time dependent and was observed as early as 2 min after preincubation with PDBu. Higher concentrations of AVP did not overcome the inhibitory effect of PDBu, suggesting that the effect may not be competitive. The mechanism by which PDBu inhibits AVP-mediated inositol phosphate accumulation appears to be through activation of PKC since 4α-phorbol-12,13-didecanoate (4α-PDD), a phorbol ester which does not activate PKC, did not inhibit

AVP-mediated inositol phosphate accumulation. Activation of PKC appears to uncouple $V_1$ receptors from guanine nucleotide binding protein as assessed by competitive binding experiments with the radiolabeled vasopressin antagonist [$^3$H]SK&F-101926 [9].

Similarly, pretreatment of the cells with PDBu inhibited isoproterenol-stimulated cAMP accumulation. This inhibition was dose-dependent. The maximal inhibition was 48%, and as much as 23% inhibition was observed at a PDBu concentration as low as 1 nM. Addition of vasopressin to PDBu-pretreated cells did not result in an additive inhibitory effect, suggesting that both vasopressin and PDBu may be affecting the same site [9].

In addition to the inhibition of beta-adrenergic agonist-stimulated cAMP accumulation, pretreatment with PDBu also resulted in inhibition of ANF-stimulated cGMP accumulation in these cells. Similar to the effect on cAMP, PDBu inhibition of ANF-stimulated cGMP accumulation was time and dose dependent and addition of vasopressin to PDBu-pretreated cells did not produce an additive effect [21].

The above data suggest that vasopressin mediates its inhibitory effects on the formation of cyclic nucleotides secondary to its stimulatory effect on PLC activity (by the formation of diacylglycerol which activates PKC). If this were the case, the formation of diacylglycerol in these cells should lead to inhibition of the formation of cyclic nucleotides. We tested this hypothesis by studying the effects of sodium fluoride which has been shown to stimulate inositol phosphate accumulation in a number of cells [22]. Pretreatment of these cells with sodium fluoride resulted in a concentration- and time-dependent increase in inositol phosphate accumulation. Addition of vasopressin to sodium fluoride pretreated cells resulted in only a marginal stimulatory effect. In addition to the stimulation of inositol phosphate accumulation, sodium fluoride pretreatment resulted in the inhibition of beta-adrenergic agonist- and ANF-stimulated cAMP and cGMP accumulation respectively. The concentration of sodium fluoride and the time-dependence of treatment to mediate these inhibitory effects were very similar to those observed for stimulation of inositol phosphate accumulation. Addition of vasopressin to fluoride-pretreated cells did not result in additive inhibition of cAMP and cGMP accumulation. These data also suggest that vasopressin-induced inhibition of cyclic nucleotides in these cells may be secondary to its effects on PLC stimulation.

*Discussion*

Figure 1 presents our current model to explain the inter-receptor regulatory processes identified in vascular smooth muscle cells. The clonally derived A-10 cells display beta-adrenergic, ANF and vasopressin receptors. Beta-adrenergic receptors are coupled to adenylate cyclase through $G_s$, whereas ANF receptors mediate cGMP accumulation through stimulation of membrane guanylate cyclase. The mechanism by which ANF stimulates guanylate cyclase is not known. Vasopressin receptors are coupled to PLC through a G protein which is sensitive to NEM but not a substrate for pertussis toxin. These vasopressin receptors also mediate inhibition of beta-adrenergic and forskolin-stimulated cAMP and ANF-stimulated cGMP accumulation respectively.

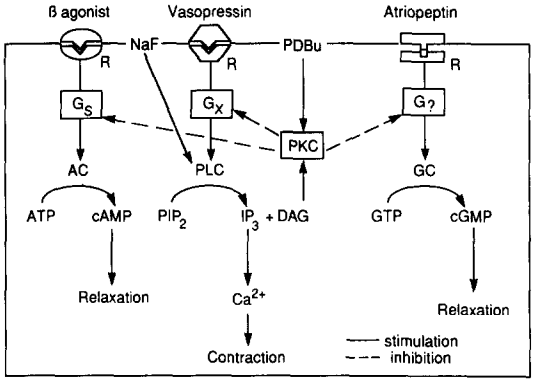


Fig. 1. Proposed molecular mechanisms of vasopressin action in A-10 cells. Abbreviations: PDBu, phorbol-12,13-dibutyrate; PLC, phospholipase C; PKC, protein kinase C; $PIP_2$, phosphatidyl inositol bisphosphate; $IP_3$, inositol trisphosphate, $G_2$, stimulatory guanine nucleotide binding protein; DAG. diacylglycerol. AC, adenylate cyclase; GC, guanylate cyclase; and R, receptor.

Phorbol ester tumor promoters, activators of PKC, modulate the activity of each of the vasoactive hormones and mimic the effect of vasopressin on the activities of beta-adrenergic agonist and ANF. The most likely explanation for these observations is that PKC phosphorylates some key regulatory protein(s) involved in each of these pathways. The lack of additive effects of vasopressin and phorbol esters suggests that both may affect the same site and that vasopressin-mediated inhibition of the formation of cyclic nucleotides may be secondary to its activation of inositol phosphate and diacylglycerol formation. Studies with sodium fluoride are consistent with the above hypothesis that diacylglycerol production was sufficient to inhibit cyclic nucleotide accumulation.

Vasopressin also induces the release of arachidonic acid [10]. Our data suggest that this activity is effected via several routes. Arachidonic acid release may be associated with metabolism of diacylglycerol released by PI-specific PLC. It also is released via the activity of $PLA_2$ that is activated by vasopressin via $V_1$ receptors by a mechanism that does not require protein or RNA synthesis. More recent evidence indicates that vasopressin activates a PC-specific PLC [10] as well as a PI-specific PLC and that this is another mechanism involved in the liberation of arachidonic acid. The role of arachidonic acid released by vasopressin is unclear. That it may be important is suggested by the fact that there are general independent mechanisms induced by the interactions of vasopressin with $V_1$ receptors that liberate arachidonic acid. One possibility is that, in the A-10 cells, arachidonic acid may be metabolized primarily to relaxant prostanoids such as prostacyclin, providing, as it were, a brake on vasopressin-induced contractile activities.

Clearly these data demonstrate that the signal transduction systems of vasoactive receptors are

interactive in A-10 cells. Vasopressin is an ideal vasoconstrictor as it can induce contraction directly via at least one signal transduction process (PI-specific PLC) and perhaps a second (PC-specific PLC) and simultaneously inhibit the effects of two relaxants. As we have not observed reciprocal effects of beta-adrenergic agonists or ANF on vasopressin-induced effects, the system appears to be designed to rapidly regulate the activities of endogenous vasodilators and to assure maximal contraction of smooth muscle cells in response to vasopressin.

Obviously many questions remain to be answered. Are receptors other than beta-adrenergic and ANF receptors regulated by vasopressin? What is the G protein(s) that is involved? Can the effects of PKC be defined more precisely? What is the role of arachidonic acid release? Are there receptors that regulate $V_1$ receptor activities in these cells? Additionally, we wonder whether the processes defined are generic and involve other combinations of various receptors in various cell lines.

We suggest that a pattern in which a dominant receptor, such as the $V_1$ receptor, regulates the activity of a set of subservient receptors and signal transduction processes is likely. Moreover, signal transduction systems may be arranged in one or more hierarchies. For example, receptors coupled to $Ca^{2+}$/inositol signals may consistently dominate receptors coupled to cyclic nucleotides. Despite the many obvious questions that remain, we believe our studies suggest that this is an important potentially fruitful area for additional research that may provide new insights into cellular regulatory processes.

Obviously, the relevance of such processes *in vivo* is not clearly defined as yet. Nevertheless, inter-receptor regulation may be one method to manage many complex phenomena such as blood pressure and cardiac output, and it will be of interest to explain these questions in appropriate *in vivo* experimental systems.

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