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Vasopressin signaling in kidney cells

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One of the events that is most vital to organ and organism function is that of a hormone acting upon a cell to evoke a physiological response. The hormone evoking the cellular response is the "first messenger". The activation of a cellular response, however, involves "secondary messengers" that are generated in the cell as a result of hormone–receptor cell interaction and that are crucial to the expression of hormone action. The two most well–studied secondary messenger systems in hormone physiology are cyclic adenosine monophosphate (cAMP) and calcium. In this present chapter we will describe the biochemical mechanisms in which vasopressin interacts with its V1 and V2 receptors generating, in specific cells, cAMP and/or calcium as second messengers. We will focus on two cell culture models that have been utilized to identify vasopressin signaling: 1) the LLC-PK_1 cell which contains the V2 receptor and 2) the glomerular mesangial cell which contains the V1 receptor.

Vasopressin receptor (V2)-adenylate cyclase interactions

The antidiuretic action of vasopressin is mediated by the sequential steps of vasopressin binding to its cell surface receptor, followed by the receptor–mediated stimulation of adenylate cyclase. During the last decade it has become clear that adenylate cyclase is part of a complex regulatory system, consisting of at least three subunits for hormonal activation [1–3]. These subunits, receptor (R), guanine nucleotide regulatory protein (G), and catalytic unit (C) have been characterized by their individual biological properties using a variety of techniques [2, 4]. The G subunit in turn consists of two major subunits, termed α and β, with molecular weights of (45 to 52) $\times 10^{-3}$ and $35 \times 10^{-3,}$ respectively [4, 5]. A fundamental question is how the individual subunits are structured in the membrane before and after exposure to activating ligands [6]. In this regard several possible models for the sequence of subunit interactions involved in the activation of adenylate cyclase have been proposed [7, 8]. Depending on the critical step involved in the formation of the active enzyme species, these mechanisms can be classified as associative or dissociative reactions. For associative mechanisms, the hormone induces association of subunits to form an active enzyme, and for dissociative mechanisms the hormone disaggregates subunits into active enzymes. In order to define the mechanism of adenylate cyclase activation by vasopressin, we turned to approaches that could be applied to intact cells, thus avoiding possible disruption of subunit assembly which accompanies solubilization and purification techniques. The model system utilized was the cultured pig kidney epithelial cell line, LLC-PK_1, in which the vasopressin V_2 receptor is capable of stimulating cAMP production in intact cells and in isolated membranes.

Binding of tritiated vasopressin in intact LLC-PK_1 cells

The techniques used for establishing LLC-PK_1 monolayer cell cultures and the measurement of vasopressin binding and cAMP accumulation have been previously described [9–13]. The basic characteristics of the vasopressin V_2 receptor reported in these studies were similar to those found in rat and bovine renal membranes [10, 12]. However, it was possible to observe the regulation of receptor properties by hormone and by other effectors in intact cells. Scatchard analysis of the binding of tritiated lysine vasopressin to its receptor in LLC-PK_1 cells revealed a concave upward relation of bound/free versus bound hormone [10]. One potential explanation for such a concave upward plot is the occurrence of negatively cooperative site–site interactions among receptors [14]. Studies of hormonal dissociation have shown that true negative cooperativity is not likely in this system. Rather, a number of experimental maneuvers indicated that the concave upward equilibrium Scatchard plot observed for the vasopressin V2 receptor in intact LLC-PK_1 monolayers was most consistent with the presence of interdependent states of the receptor of varying hormone binding affinity [10, 12]. Furthermore, the transition from a complex concave upward to a linear Scatchard plot was observed with various manipulations of the cell, including cell suspension and exposure to hypertonic incubation media [10, 12]. These data led us to the conclusion that a complete understanding of the vasopressin receptor–adenylate cyclase interactions could not be achieved from hormone binding studies alone. Indeed, as will be demonstrated below, without a full definition of the nature of the interaction between the hormone receptor complex and other subunits of the adenylate cyclase system, defining the model for hormone action based on a description of nonlinear and/or linear Scatchard plots is impossible. For this reason, we turned to the technique of target analysis by radiation inactivation and applied it to intact, cultured cell monolayers to characterize the sequence of subunit interactions involved in activation of adenylate cyclase by vasopressin.

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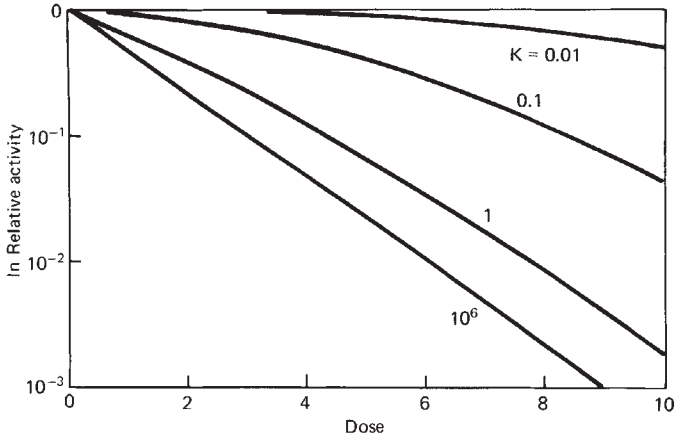

Fig. 1. *Radiation inactivation of the dissociative reaction AB ↔ A + B with B the active species.* The molecular weights of A and B are equal. Complete equilibration is allowed after radiation exposure. K is a dissociation constant; increasing K results in an increase in the quantity of active B. Reprinted with permission from [19].

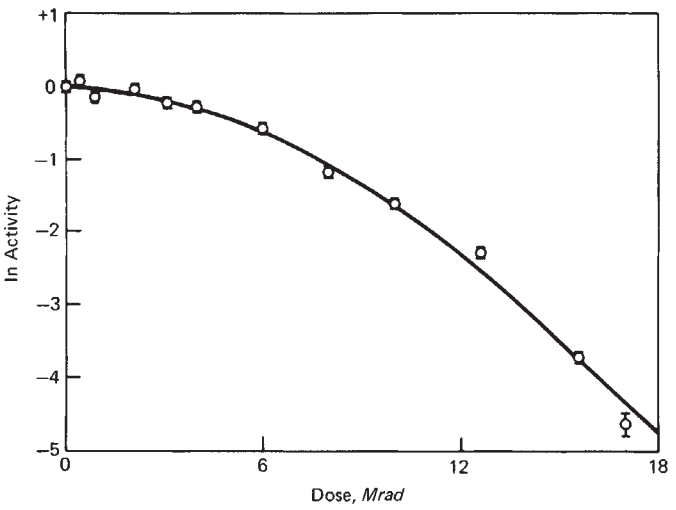

Fig. 2. *Activity–dose relation for the radiation inactivation of basal adenylate cyclase in LLC-PK$_1$ cells.* Reprinted with permission from [20].

The principles of radiation inactivation

When ionizing radiation is applied to an enzyme of interest, loss of activity occurs as a function of the number of molecules escaping destruction [15]. The probability of a molecule escaping destruction in turn is a function of its molecular weight and is given by a Poisson distribution, such that the logarithm (ln) of fractional activity surviving radiation is linearly related to radiation dose. When this technique has been applied to a large series of enzymes of known molecular weight (m), m was related to the D_o dose by the relation:

$$m = \frac{640}{D_o} \quad (1)$$

where D_o is the radiation dose in Mrad which reduced enzyme activity by 37% (1/e) of its initial value and where m is expressed in kDa. The majority of enzyme systems studied have given single exponential inactivation curves with excellent agreement between molecular weight determined by radiation inactivation and by classical biochemical techniques [15].

In contrast, target analysis of adenylate cyclase has yielded complex nonlinear ln(activity) versus dose relations which have presented problems in interpretation [16, 17]. For this reason we developed a systematic approach to interpret nonlinear ln(activity) versus dose relations in terms of the interaction of multimeric subunits of enzymes and then applied these methods to vasopressin–sensitive adenylate cyclase [18–20]. It was apparent that dramatic transitions of inactivation curve shape were predicted for enzyme activation models associated with disaggregation of relatively inactive multimers into active monomeric subunits. The nature of this transition depended upon specification of radiation inactivation assumptions, namely: 1) presence or absence of energy transfer; 2) multi-hit requirements for inactivation; and 3) equilibration of enzyme species following radiation. The detailed curve shapes depended also upon subunit molecular weights, multimer size, and relative activity of multimer and monomer. For a fully equilibrated system with no energy transfer, Figure 1 shows the ln(activity) versus dose relation which is predicted to occur with activation of the dissociative system,

$$AB \xleftrightarrow{K} A + B^* \quad (2)$$

in which all the activity resides in B* and in which the molecular weights of A and B are nearly equal. A concave downward ln(activity) versus dose relation is predicted for low K (dissociation constant) values typical of a basal state of the enzyme, with transition to a linear ln(activity) versus dose relation for high K (full enzyme activation). At high enough radiation dose and at high K, only the fully dissociated active B* subunit is probed and the inactivation curve becomes linear with a slope corresponding to the molecular weight of B. In contrast, for an associative reaction in which inactive subunits A and B combine to form the active multimer, AB, curve shape transition would be opposite that seen in Figure 1. In general, for a complex multistep activation process, the pattern of curve shape transition (associative vs. dissociative) will be determined by the step leading to the formation of the species whose concentration limits final activity [18–20].

Radiation inactivation of vasopressin–sensitive adenylate cyclase in intact LLC-PK$_1$ cells

We applied the technique of radiation inactivation to distinguish an associative versus dissociative mechanism for activation of adenylate cyclase by vasopressin [19, 20]. A pattern of curve shape transition consistent with a dissociative activation mechanism was clearly obtained (Figs. 2 and 3). In the basal state a concave downward inactivation curve was observed with a limiting slope corresponding to a molecular weight of approximately 180 kDa (Fig. 2). In contrast the addition of vasopressin (Fig. 3A), guanine nucleotides (Fig. 3B), or forskolin (Fig. 3C) resulted in curve shape transition to a linear ln(activity) versus dose relation with a slope corresponding to a molecular weight similar to that observed at basal activity (that is, 180 kDa). Based on the theory outlined in the previous section, this value was shown to correspond to the molecular

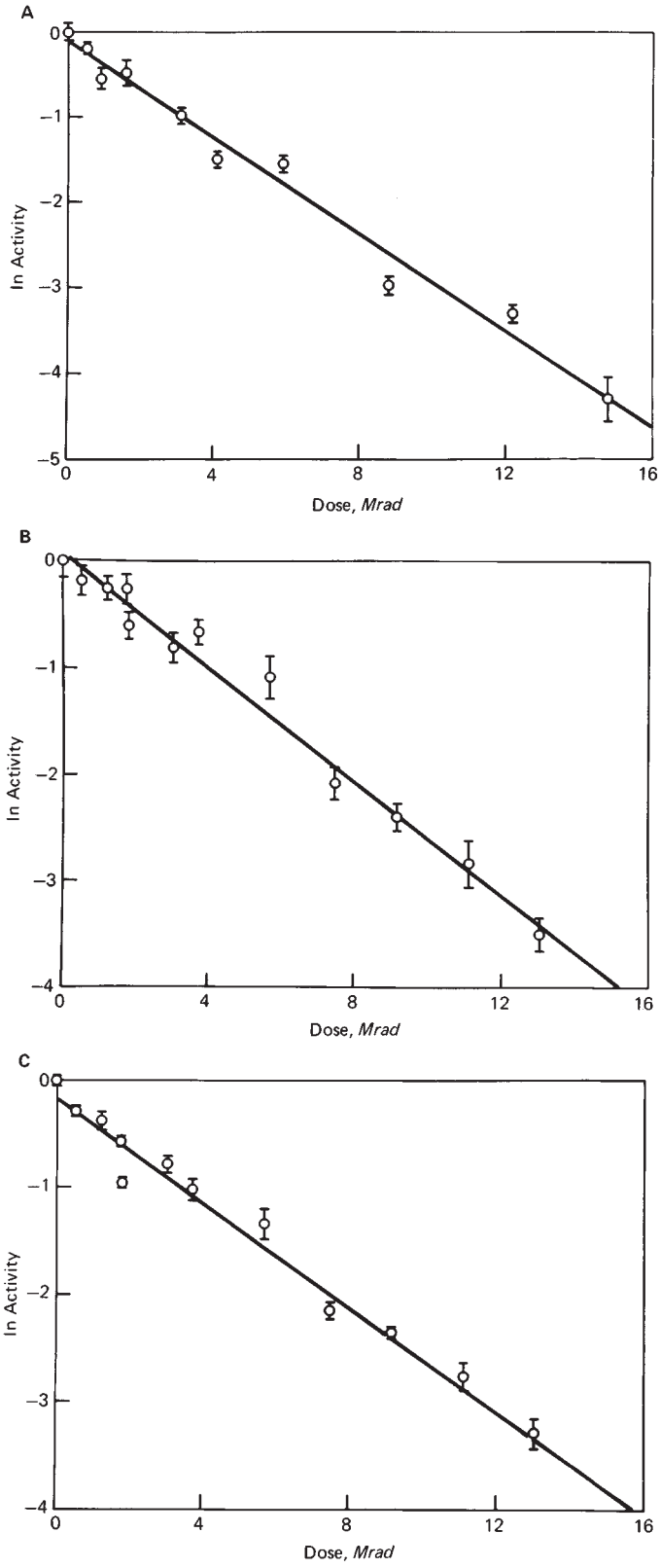

Fig. 3. *Activity–dose relation for the radiation inactivation of adenylate cyclase stimulated by* **A:** *2 μM vasopressin;* **B:** *10 μM guanylyl imidodiphosphate; and* **C:** *100 μM forskolin.* Reprinted with permission from [20].

Table 1. Criteria for adenylate cyclase activation mechanism

1. Non-linear coupling of receptor occupancy to enzyme activation
2. Non-linear Scatchard for equilibrium hormone binding under appropriate conditions of receptor–enzyme stoichiometry
3. GTP lowers hormone binding affinity
4. Molecular weight of active species: approximately 180 kDa
5. Shallow initial slope and prominent downward concavity for basal radiation inactivation curve

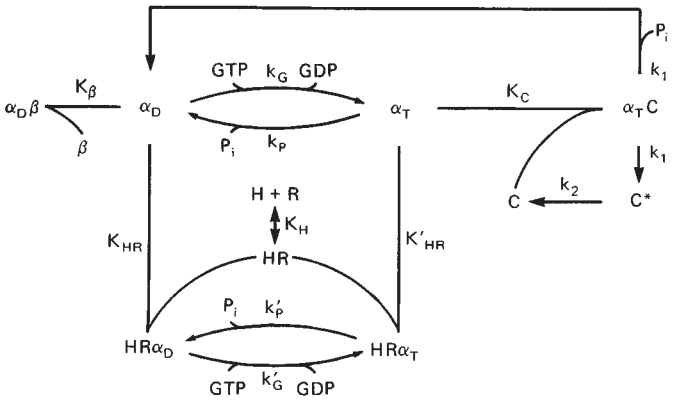

Fig. 4. *αβ Cyclic dissociation model for adenylate cyclase activation.* Abbreviations are: H, hormone; R, receptor; α, β, subunits of guanine nucleotide–regulatory protein; C, catalytic unit; GTP and GDP, guanosine triphosphate and diphosphate, respectively; P_i, inorganic phosphate; D and T subscripts denote GTP-and GDP-associated states of α, respectively. K_{HR}, K'_{HR}, K_C, and K_β are equilibrium dissociation constants and k_G, k_P, k'_G, k'_P, k_1, k_2 are kinetic constants. Reprinted with permission from [20].

weight of the species whose interaction limits final activity. The molecular weight of the catalytic unit (C), which clearly must be part of the final active species, has been estimated at 120 to 150 kDa [21–23]. Since the target size is greater than the estimates for C alone, constituents of the final active species include C plus another subunit component.

The above target analysis data were combined with known biochemical characteristics of the vasopressin adenylate cyclase system (Table 1) to formulate a unique pattern for the interaction of the adenylate cyclase subunits (Fig. 4). It should be noted that the biochemical criteria, 1 to 3, listed in Table 1 are general characteristics of a number of hormone–receptor adenylate cyclase systems, and not limited to the vasopressin activation of this enzyme. When the criteria in Table 1 were utilized to evaluate potential mechanisms for adenylate cyclase activation [19, 20], only the model illustrated in Figure 4 was able to satisfy all criteria. According to this model, hormone (H) interacts with its free receptor to form an HR complex which in turn can interact with either α_{GDP} or α_{GTP}. The α_{GDP} subunit is the high affinity subunit for HR binding, and GTP lowers the affinity of hormonal binding during the formation of HR α_{GTP}. The multiple interdependent binding sites for hormone account for the presence of a nonlinear Scatchard plot which, interestingly, could be converted to a linear Scatchard plot depending on the relative stoichiometry of R and α and the individual binding affinities. As noted above, such a transition in Scatchard plots for vasopressin binding has been observed [10, 12].

Table 2. Predictions from the model of vasopressin activation of adenylate cyclase

1. Hormone–receptor complex interacts with dissociated α subunit
2. GDP-GTP interconversion takes place on the dissociated α subunit
3. Effect of hormone is to accelerate GTP addition on α
4. $\alpha\beta$ dissociation occurs as part of the activation process in the intact membrane

The coupling ratio of receptor occupancy to enzyme activation will be a function of the lifetime of activated C* compared to the rate of unbinding of hormone. If C* is long lived, non-linear coupling will occur.

Finally the mechanism shown in Figure 4 satisfies the details of the radiation inactivation data. The species whose concentration limits overall activity is $\alpha_{GTP}C$. This corresponds to the target size of 180 kDa (α = 45 kDa; C = 120 to 150 kDa) obtained for the active species for radiation inactivation. A prominent degree of initial downward concavity at low radiation dose is predicted for basal enzyme activity, as was observed (Fig. 2). This arises because GDP-GTP interconversion limits the amount of α_{GTP} available for association with C in the steady state, so that the initial curve shape will reflect predominantly the molecular weight of α and therefore will appear shallow. At high radiation dose, which is conceptually equivalent to a large dilution of the components, $\alpha\beta$ will be predominantly in the dissociated state, resulting in transition toward a linear plot with composite target size of αC. We have generated the expected ln(activity) versus dose relations for this model, based upon an explicit delineation of the equations which define the mechanism, and confirmed this curve shape for basal activity and the linear transition with hormone and other activators [19, 20]. It is equally important to note that a number of other plausible models previously suggested in the literature as mechanisms of activation of adenylate cyclase can be excluded on the basis of their inability to satisfy all the criteria in Table 1. The models eliminated included those in which $\alpha\beta$ dissociation was assigned as the hormone sensitive step, as well as models in which there was precoupling of either R or C with the $\alpha\beta$ subunits [19, 20].

The model in Figure 4 yields a number of testable predictions for adenylate cyclase activation by vasopressin listed in Table 2. These features can now be evaluated by biochemical means in the intact membrane.

Use of the adenylate cyclase model to understand the antidiuretic action of vasopressin

Hormones, drugs, and the hypertonic environment of the renal medulla are known to modulate the vasopressin stimulation of adenylate cyclase. Recent studies have allowed us to define the mechanism of action of a variety of agents on the basis of their alteration of vasopressin–adenylate cyclase subunit interactions as illustrated in Figure 4.

Incubation with high concentrations of vasopressin for a few minutes is known to cause a "desensitization" of the vasopressin adenylate cyclase–response [11]. This actue desensitization is not associated with loss of receptor number, but rather is the result of an apparent uncoupling between the hormone receptor complex and the adenylate cyclase system. This phenomena is a common one for many hormones and it is one of the important ways that cells can rapidly regulate their responses to a hormone [24]. We have recently been able to demonstrate that desensitization to vasopressin in LLC-PK_1 cells is consistent with a decrease in the rate of the reaction defined by the rate constant, k'_G, in Figure 4 (unpublished observations). The implications of this conclusion are that some biochemical modification of either the vasopressin receptor or the α subunit results in a blunted ability of $HR\alpha_{GDP}$ to form the $HR\alpha_{GTP}$ complex. Recent observations by Sibley and Lefkowitz [24] have demonstrated that the β-adrenergic receptor can be phosphorylated by a variety of protein kinases, including protein kinase A and protein kinase C, resulting in the desensitization of the β-adrenergic response. A similar phenomena may occur for the vasopressin receptor. Indeed, early evidence from our laboratory [12] suggested that ATP had a facilitory effect on the desensitization phenomena, possibly related to a phosphorylation event. The phosphorylation of the vasopressin receptor could be associated with an altered hormone–binding affinity, as has been observed for the desensitized state [10, 12 and unpublished observations], as well as inhibit the ability of the HR complex to interact with the α subunit. Full definition of this process will require purification of the appropriate subunits.

Since vasopressin can exert its action upon its receptor and adenylate cyclase in a hypertonic environment, it is not surprising that hypertonic sodium chloride has been found to have profound effects on the ability of vasopressin to stimulate cAMP. When hypertonic sodium chloride is added to the incubation media with kidney medullary membranes, a several–fold increase in vasopressin–stimulated adenylate cyclase activity is observed [25]. Hypertonic sodium chloride can similarly enhance the vasopressin response in the cultured LLC-PK_1 cells, and, most interestingly, can prevent the desensitization induced by vasopressin described above [10–12]. The regulation of vasopressin–sensitive adenylate cyclase by hypertonic sodium chloride in vitro is consistent with a similar effect of hypertonicity observed in the Brattleboro rat with hypothalamic diabetes insipidus (DI) in vivo. In these rats the sensitivity of the medullo-papillary adenylate cyclase to vasopressin is reduced [26, 27]. Chronic administration of physiological concentrations of vasopressin in vivo induced a 30% increase in the responsiveness of the enzyme to vasopressin in vitro [27]. These data are consistent with the hypothesis that vasopressin receptor–adenylate cyclase interactions can be controlled by the chemical composition of the renomedullary interstitial fluid, as in the LLC-PK_1 cells. The inability of the DI rats to maximally concentrate urine, presumably as a consequence of minimal stimulation of adenylate cyclase, may be due to the subnormal solute concentration prevailing in the renal medulla. With prolonged treatment of the DI rats with vasopressin, which gradually increases medullary osmolality, there is a restoration of the concentrating ability of the kidney to normal [28].

In order to define the mechanism by which hypertonic sodium chloride stimulates adenylate cyclase, we conducted target analysis studies with radiation inactivation as described above [29]. Interestingly, activation of adenylate cyclase by

sodium chloride is not associated with a curve shape transition from downward concavity to linear as was observed with the other activators of the enzyme (Figs. 2 and 3). In addition, the combination of vasopressin plus hypertonic sodium chloride was not capable of fully linearizing the radiation inactivation curve shape. We have interpreted these data to mean that there is little if any dissociation of $\alpha\beta$ in the presence of sodium chloride despite activation of the enzyme. Hypertonic sodium chloride may well provide a means by which the intact $\alpha\beta$ complex is capable of undergoing GDP-GTP transition in the presence of hormone and facilitating coupling to the catalytic unit and activation of the enzyme. It may be that this altered state of subunit association can prevent desensitization and/or facilitate the association of a "desensitized" vasopressin receptor with the intact $\alpha\beta$ complex. These data provide a potential molecular mechanism for the physiological modulation of the vasopressin–adenylate cyclase interaction by hypertonic sodium chloride. This model of activation by sodium chloride may prove to be a key determinant of the maximal rate and duration of cAMP signaling induced by vasopressin in the renal medulla.

Interaction of calcium with the cAMP signal

It has become clear in the least several years that the majority of hormone–stimulated transport events involve interactions between the two major messenger systems, cAMP and Ca^{2+}. These interactions can occur at the level of synthesis of the messages themselves, or at a variety of steps leading to the physiologic response of the hormone. In this section we will describe the mechanisms by which Ca^{2+} – calmodulin can alter the vasopressin–stimulated cAMP signal, and cite physiological examples in which Ca^{2+} may be playing such a modulatory role.

Calmodulin (CaM) can have a significant effect on the synthesis and degradation of cAMP [30]. However, while CaM and adenylate cyclase are ubiquitous in mammalian cells, CaM regulation of basal adenylate–cyclase activity has been limited to a few tissues, including those from the brain, pancreas, and adrenal glands [31–33]. We have described the activation of vasopressin–sensitive adenylate cyclase by CaM [13]. Previous studies with oxytocin in adenylate cyclase preparations from the frog bladder [34] and with vasopressin in similar preparations from the kidney medulla [35] had demonstrated a biphasic adenylate cyclase response with increasing Ca^{2+} concentrations. Ca^{2+} in the micromolar range was required for activation; further increases in Ca^{2+} resulted in inhibition. At the time of those studies, the existence of CaM was unknown.

Using the LLC-PK_1 cell line we were able to demonstrate that the characteristics of CaM stimulation of vasopressin–sensitive adenylate cyclase were similar to those described for other enzymes [13], specifically: 1) activation required micromolar quantities of free Ca^{2+}; 2) maximum enzyme rates were altered, but the K_m for hormone activation was not; 3) activity was inhibitable by trifluoperazine; 4) activation was dependent on the Mg^{2+} concentration; and 5) CaM exerted a biphasic effect on vasopressin–sensitive adenylate cyclase, with activation occurring at low Ca^{2+} and inhibition at high Ca^{2+} concentrations.

The mechanism by which CaM regulates adenylate cyclase has not been firmly established, although it seems most likely that it stimulates the catalytic unit directly [30]. The fact that CaM stimulates adenylate cyclase from only a limited number of tissues suggests that these enzymes are distinct from adenylate cyclases that are not regulated by CaM.

The physiologic significance of the CaM regulation of vasopressin–sensitive adenylate cyclase also remains to be demonstrated. Such a regulatory system however can offer a potentially unifying hypothesis for vasopressin–stimulated water flow in the mammalian kidney and amphibian bladder. Several years ago, Petersen and Edelman [36] demonstrated that high Ca^{2+} concentrations in the serosal bathing medium of the toad urinary bladder selectively inhibited the hydroosmotic effect of vasopressin without altering the natriuretic response of the hormone. This inhibition of Ca^{2+} was overcome by exogeneous cAMP. These observations led the authors to propose that vasopressin activates two adenylate cyclases, one of which is Ca^{2+}-sensitive and coupled to the regulation of osmotic water flow. This hypothesis is consistent with the observation that large increases in extracellular Ca^{2+} blunt vasopressin stimulation of cAMP in toad bladder cells [37]. Studies have demonstrated that prostaglandins [37, 38], verapamil [39], general anesthetics [40, 41], and α-adrenergic agents [37, 42] selectively inhibit vasopressin–stimulated water flow in the toad bladder by decreasing the ability of the hormone to enhance cAMP production. In the mammalian kidney, it has been suggested that prostaglandins and verapamil inhibit vasopressin–stimulated water flow by a common mechanism [43]. It is thus possible that all of these agents share an ability to inhibit Ca^{2+}-sensitive adenylate cyclase. For CaM-sensitive enzymes studied to date [30, 44], a small increase in the Ca^{2+} concentration results in a Ca^{2+}-CaM complex that is capable of activating the enzyme. Our data in the LLC-PK_1 cells would suggest that an inhibitor might act on the vasopressin–sensitive enzyme by: 1) preventing the necessary increment in the Ca^{2+} concentration; 2) stimulating a large increase in the Ca^{2+}-CaM complex (the "biphasic" response); 3) preventing the interaction of the Ca^{2+}-CaM complex with the enzyme; and 4) altering the Mg^{2+} concentration. It is of interest that several substances that act as Ca^{2+} antagonists, such as anesthetics and verapamil, have been shown to inhibit Ca^{2+}-CaM in much the same manner as trifluoperazine [45, 46]. It is thus possible that the observed effects of prostaglandins, verapamil, and anesthetics on the physiologic action of vasopressin are mediated through the effects of these agents on the Ca^{2+}-CaM system.

Once cAMP is generated inside cells by adenylate cyclase, it is degraded by a variety of phosphodiesterases, at least one of which is activated by Ca^{2+}-CaM [30]. In contrast to Ca^{2+}-CaM-stimulated adenylate cyclase discussed above, Ca^{2+}-CaM-sensitive phosphodiesterase is a ubiquitous enzyme found in all mammalian cells. The observation that CaM stimulates the activities of both adenylate cyclase and phosphodiesterase may appear paradoxic. However, the sensitivity of adenylate cyclase and phosphodiesterase to Ca^{2+} is different [47]. Adenylate cyclase is activated by a lower concentration of Ca^{2+} and, at a concentration of Ca^{2+} that activates phosphodiesterase, adenylate cyclase is already inhibited. Thus, an increase in cellular free Ca^{2+} may cause a sequential activation of adenylate cyclase and phosphodiesterase, resulting in a transient accumulation of cAMP.

Vasopressin receptor V_1 calcium–phospholipid interactions

Vasopressin receptors have been divided into two groups, V_1 and V_2, based primarily on functional characteristics. While the V_2 receptor, as discussed above, is coupled to adenylate cyclase the V_1 receptor is not. Interactions between vasopressin and cells with V_1 receptors involve calcium as a second messenger system. V_1 receptors are present in three sites in the kidney: the glomerular mesangial cell, vascular smooth muscle cell, and the renomedullary interstitial cell. We will discuss the characteristics of the vasopressin–V_1 interactions in the mesangial and renomedullary interstitial cell.

Glomerular mesangial cell

Vasopressin induces contraction [48] and prostaglandin synthesis [49, 50] in cultured glomerular mesangial cells. It has been suggested that the glomerular mesangial cell is the primary regulator of glomerular capillary surface area, and therefore, K_f [51]. The contractile response to vasopressin may be responsible for the alteration in K_f observed with vasopressin in the intact glomerulus. The prostaglandin synthesis in turn has been postulated to modulate the contractile response on the mesangial cell itself, and both vasopressin–induced contraction and prostaglandin (PGE_2) synthesis are blocked by a V_1 receptor antagonist [49, 52], thus implicating the V_1 receptor in mediation of these events. Bonventre et al [53], using both quin2 and fura-2 as intracellular fluorescent Ca^{2+} indicators, have demonstrated that vasopressin increases cytosolic free calcium concentration ($[Ca^{2+}]_f$) in the cultured mesangial cell. This response is blocked by the V_1 receptor antagonist, $(CH_2)_5$Tyr(ME)V_D AVP. Basal levels of $[Ca^{2+}]_f$ were approximately 80 to 105nM. Vasopressin produced approximately an eightfold increase in $[Ca^{2+}]_f$ within seconds of addition of the hormone. This was followed by a rapid fall in $[Ca^{2+}]_f$ with levels returning to approximately 50% above baseline within one minute of addition of vasopressin (Fig. 5). The increase in $[Ca^{2+}]_f$ was due in large part to release of Ca^{2+} from intracellular storage sites since the $[Ca^{2+}]_f$ response was observed even when extracellular Ca^{2+} concentration was reduced to levels lower than 100 nM. There may, however, be an additional contribution to the $[Ca^{2+}]_f$ rise due to entry of extracellular Ca^{2+}. Reduction of extracellular calcium concentration to levels below 100 nM results in reduction in the initial rise of $[Ca^{2+}]_f$ and more rapid return of mean $[Ca^{2+}]_f$ to baseline. The time response of $[Ca^{2+}]_f$ to vasopressin depicted in Figure 5 does not necessarily reflect the time response in any individual cell. The response presented represents a mean response of a large number of cells that may not be activated in synchrony. It has been shown, for example, in single hepatocytes that vasopressin stimulates repetitive transient rises in $[Ca^{2+}]_f$ [54].

In other tissues, such as liver [55], vascular smooth muscle cells [56] and platelets [57], vasopressin results in a rapid phosphodiesterase–mediated hydrolysis of phosphatidylinositol-4-5-bisphosphate with the production of myo-inositol-1,4,5-trisphosphate (IP_3). Troyer et al [52] have demonstrated that vasopressin also increases metabolism of polyphosphoinositides in cultured mesangial cells. Since IP_3 releases Ca^{2+} from nonmitochondrial stores in mesangial cells (Bonventre and Cheung, unpublished observations) it is likely that IP_3 serves as an intracellular second messenger for hormone–induced Ca^{2+}

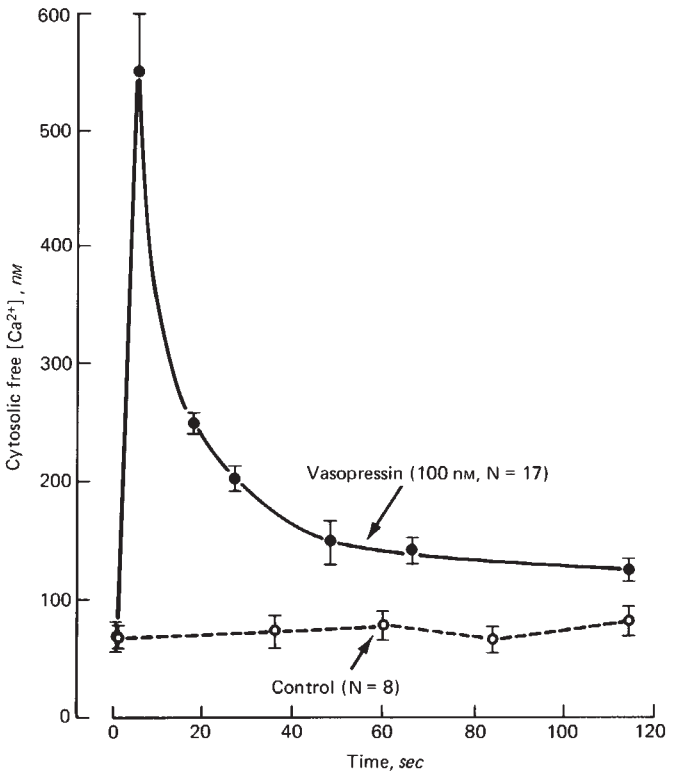

Fig. 5. *Effect of 100nM arginine vasopressin (AVP) on cytosolic free $[Ca^{2+}]$ ($[Ca^{2+}]_f$) in cultured mesangial cells.* $[Ca^{2+}]_f$ was measured by incorporation of a Ca^{2+} sensitive fluorescent dye, fura-2, into the cell. The AVP-treated cells are compared with time matched control cells. $[Ca^{2+}]_f$ increased by approximately eight-fold within seconds of addition of AVP. Within 60 seconds after addition of hormone $[Ca^{2+}]_f$ returned to a level approximately 50% higher than control. At each time point $[Ca^{2+}]_f$ in AVP-treated cells (●, $N = 17$) was statiscally higher than the control value (○, $N = 8$) ($P < .025$). Reprinted with permission from [53].

release. A working scheme for the elevation of $[Ca^{2+}]_f$ after hormone-V_1 receptor interactions, along with the varied cellular responses to this increase in $[Ca^{2+}]_f$, identified in various cell types, is presented in Figure 6. From work done in other cells, it has become apparent that the scheme depicted in Figure 6, for the Ca^{2+}-polyphosphoinositide second messenger system, is an oversimplification [58]. Inositol [1,3,4,5]P_4(IP_4) is formed as rapidly as inositol (1,4,5)P_3 (IP_3) in some stimulated cells and IP_3 may be metabolized via IP_4. IP_5 and IP_6 have also been isolated from mammalian cells [58]. It has also been established that 1,2 cyclic derivatives of inositol phosphates can be generated from phosphatidylinositol-4,5-bisphosphate and phosphatidylinositol-4-monophosphate by phospholipase C [59]. Each of these compounds may have its own, as yet undefined, action in the cell.

The increase in $[Ca^{2+}]_f$ observed with vasopressin is likely related to the vasopressin–induced contraction observed in the mesangial cell. By analogy to other contractile cells such as smooth muscle, an increase in $[Ca^{2+}]_f$ with an associated increase in calmodulin binding likely leads to enhance myosin light–chain phosphorylation.

Prostaglandin synthesis is also a result of vasopressin–V_1 receptor interactions. Diacylglycerol generated by phospholi-

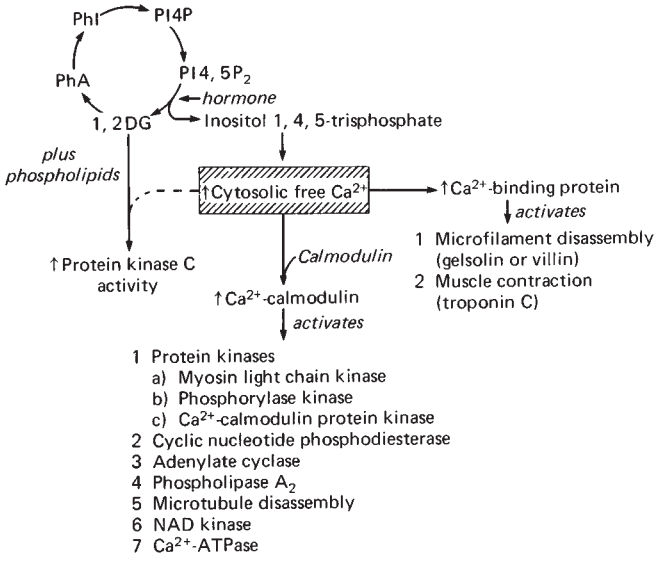

Fig. 6. *Simplified scheme for the elevation of cytosolic* free $[Ca^{2+}]$ after vasopressin-V_1 receptor interaction, along with the varied cellular responses activated by the system in many cell types. The Ca^{2+} signal may express its activity through a number of binding proteins including calmodulin, gelsolin or villin, troponin C or protein kinase C. As indicated by the dashed line, an elevation in cytosolic free $[Ca^{2+}]$ is not necessary for protein kinase C activation in the presence of diacylglycerol (1,2 DG) and phospholipids but may potentiate this kinase activity. The example of Ca^{2+}-dependent processes is not all inclusive. Abbreviations are: PhI, phosphatidylinositol; PI4P, phosphatidylinositol-4-phosphate; PI4,5P_2, phosphatidylinositol-4,5-bisphosphate; PhA, phosphatidic acid. Modified from: Ausiello and Bonventre, *Semin. Nephrology* 4:134–143, 1984

pase C can be sequentially hydrolyzed by diglyceride and monoglyceride lipases resulting in arachidonic acid release and subsequent prostanoid generation (60). The increased $[Ca^{2+}]_f$ associated with vasopressin activation can also explain the enhanced prostaglandin synthesis via activation of Ca^{2+}-dependent phospholipase A_2 liberating arachidonic acid from various phospholipids. Even when extracellular Ca^{2+} concentration is reduced to below 100nM there is a large increase in PGE_2 synthesis after vasopressin treatment (50), supporting the concept that activation of cellular phospholipases is not dependent on entry of Ca^{2+} from the extracellular milieu.

In contrast to the marked increase in $[Ca^{2+}]_f$ observed with vasopressin there was no increase in adenylate cyclase activity in the mesangial cell with this hormone. Furthermore $[Ca^{2+}]_f$ was not altered by activation of adenylate cyclase with isoproterenol or direct addition of 1 mM dibutyryl cAMP (53), nor was the vasopressin-induced increase in $[Ca^{2+}]_f$ affected by isoproterenol or dibutyryl-cAMP. The action of isoproterenol to prevent vasopressin-induced contraction in mesangial cells (61) is, therefore, likely due to a dissociation of the change in $[Ca^{2+}]_f$ from force generation. This might be explained by an inhibitory effect of cAMP on the Ca^{2+}-calmodulin induced phosphorylation of myosin light chains (53).

Medullary slices and the renomedullary interstitial cell

Vasopressin stimulates PGE_2 production in renomedullary interstitial cells [62] and possibly, to a diminished extent, in the medullary collecting duct [63], but does not appear to significantly alter prostaglandin synthesis in other medullary cells [64]. The increase in prostaglandin synthesis in the interstitial cell is due to a stimulation of cellular phospholipase activity and is not evoked by the V_2 receptor agonist, dDAVP [65, 66]. It is likely that vasopressin induces an increase in $[Ca^{2+}]_f$ in the renomedullary interstitial cell analogous to the vasopressin effect on mesangial cells. $[Ca^{2+}]_f$ has never been measured in this cell; however, the stimulation of prostaglandin synthesis in the cell is Ca^{2+} dependent in that reduction of extracellular Ca results in reduction in the rate of secretion of PGE_2 [67]. The calcium channel–blocking agent, verapamil, and the relatively non-specific Ca^{2+}-calmodulin inhibitor, trifluoperazine, also inhibited PGE_2 production implicating Ca^{2+} as an important factor in the vasopressin–induced response in the cell [67]. The role of the phosphoinositide system in the Ca^{2+} or prostaglandin response has not been evaluated.

Studies using medullary tissue slices also implicate Ca^{2+} and calmodulin in the regulation of prostaglandin production in the renal medulla. In kidney medullary slices it has been found that Ca^{2+} ionophore (A23187)-induced stimulation of prostaglandin production is abolished by reduction of extracellular Ca^{2+} concentration [68–72]. Furthermore, putative inhibitors of calmodulin, trifluoperazine and W-7 (N-6-aminohexyl-5-chloro-1 naphthalene sulfonamide hydrochloride) suppress vasopressin in A23187-induced prostaglandin production [68–72].

Implications of vasopressin–V_1 receptor interactions for urinary concentration

The interactions between vasopressin and cells possessing the V_1 receptor are complex and offer many potential implications for urinary concentration. The prostaglandins that form as a result of V_1 receptor activation can alter urinary concentrating ability by: 1) changing medullary blood flow; 2) altering solute transport into the medullary interstitium; or 3) altering the hydrosmotic effect of vasopressin. Vasodilatory prostanoids likely increase medullary blood flow which may enhance medullary solute washout, thereby reducing concentrating ability. Sodium transport in the medullary collecting duct is inhibited by PGE_2 [73]. Furthermore, PGE_2 antagonizes the action of vasopressin on the thick ascending limb as reflected by decreased cAMP production as well as chloride transport [74, 75]. Medullary NaCl concentration increases when prostaglandin synthesis is inhibited [76]. Although prostaglandins have little effect on basal water permeability in the collecting duct, they markedly impair the increased water permeability seen with vasopressin in the cortical collecting duct and the toad bladder. Therefore in many cases prostaglandins serve as antagonists of vasopressin action. By contrast it has been demonstrated that vasoconstrictor cyclo-oxygenase products such as thromboxane and cyclic endoperoxide analogs [77] can potentiate the effect of vasopressin in nonmammalian tissues; however, the relevance of these findings to the mammal remain unclear. In summary, vasopressin–V_1 receptor interactions are important to the function of the renal medulla. The net effect of prostaglandin synthesis is to decrease concentrating ability as demonstrated by the observation that blockage of prostaglandin synthesis with indomethacin results in an increase in urinary concentration and papillary solute content [75, 78].

Conclusion

The ability of vasopressin to induce antidiuresis is the result of its combined effects on V_1 and V_2 receptors in multiple renal cell types. The complex interactions of the V_2 receptor with the adenylate cyclase subunits have been described at the molecular level and help to provide an understanding of the regulation of this enzyme by hormone and other agents. The molecular events involved in V_1 receptor signaling are less well defined, but the response is characterized by significant changes in phospholipid metabolism and intracellular free $[Ca^{2+}]$. Our expanding knowledge of vasopressin signalling will eventually provide for the full molecular definition of vasopressin action.

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