Regulation of protein kinase by vasopressin in renal medulla in situ

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DOUSA, THOMAS P., AND LARRY D. BARNES. Regulation of protein kinase by vasopressin in renal medulla in situ. Am. J. Physiol. 232(1): F50-F57, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 1(1): F50-F57, 1977. — Results of this study demonstrate that vasopressin activates protein kinase in intact renal medullary cells as detected by measurement of the (–cyclic AMP/+cyclic AMP) protein kinase activity ratios in freshly prepared tissue extracts (40,000 × g supernates) from bovine renal medullary slices. The activation of protein kinase was specific for vasopressin since parathyroid hormone, histamine, angiotensin II, or the inactive analog of vasopressin did not activate protein kinase. There was a direct correlation between the extent of protein kinase activation and the elevation in tissue levels of cyclic AMP elicited by increasing doses of vasopressin or with an increase in incubation time. The elevation of tissue cyclic AMP level and maximum activation of protein kinase reached maximum level at a vasopressin concentration of about 2 × 10⁻⁸ M. Incubation of slices with vasopressin caused a dose-dependent decrease in the cyclic AMP-dependent protein kinase activity in the 40,000 × g supernate of homogenate from the renal medullary slices. This effect of vasopressin was specific for protein kinase since activity of lactate dehydrogenase or a specific [³H]colchicine-binding activity was not affected, and the decrease in the protein kinase was not due to the accumulation of a heat-stable protein kinase inhibitor. There was an increase in protein kinase activity extracted from 40,000 × g pellets of homogenate prepared from slices exposed to vasopressin. Results thus provide evidence that cyclic AMP-mediated protein kinase activation in the intact cells is an integral part of cellular response of the mammalian renal medulla to vasopressin.

ADH; kidney, in situ activation; cyclic AMP

IT IS GENERALLY ACKNOWLEDGED that vasopressin (VP) stimulates cyclic AMP formation and its accumulation in target cells, but the mechanism by which cyclic AMP elicits the increase in luminal plasma membrane water permeability (11, 13)—the ultimate functional response to the hormone—has not been elucidated (11, 13, 14). It has been proposed that cyclic AMP may regulate the water permeability of collecting ducts by influencing the phosphorylation of specific membrane proteins (10). This hypothesis is indirectly supported by the findings of some other hormone-sensitive tissues (12, 15, 23, 26, 28). It has been proposed that cyclic AMP-dependent protein kinase in the mammalian renal medulla (3, 10, 20, 22), by the fact that proteins of the plasma membrane fraction from renal medulla can serve as a substrate for this enzyme (10), and also by the fact that phosphorylated plasma membrane proteins can be dephosphorylated by a protein phosphatase which is also present in this tissue (3, 10). Some investigators have proposed that cyclic AMP-dependent protein kinase is localized directly in the luminal plasma membrane of the renal medulla (22). Studies on the subcellular distribution of cyclic AMP-dependent protein kinase and protein phosphatase in the renal medulla indicated that most of the cyclic AMP-dependent protein kinase and protein phosphatase activities are found in the fraction of soluble proteins (cytosol) (3). All the above-mentioned studies were performed on cell-free preparations from homogenized renal medullary tissue and indicate only indirectly that the proposed cyclic AMP-dependent phosphorylating mechanism can possibly occur in intact, VP-sensitive cells.

In the present study we examined whether VP, through mediation of cyclic AMP, activates protein kinase in situ, i.e., in intact, unbroken renal medullary cells. The extent of cyclic AMP-dependent protein kinase activation by VP in intact tissue was assessed by the ratio of protein kinase activities measured without or with addition of a maximal stimulatory dose of cyclic AMP in freshly prepared extracts from control tissues and from tissues after exposure to the hormone, an approach designed (7, 23) and previously used in studies of some other hormone-sensitive tissues (12, 15, 23, 26, 28).

MATERIALS AND METHODS

Tissue slices of 0.5 mm thickness from bovine renal medulla were prepared using a Stadie-Riggs tissue slicer and were thoroughly washed at least 5 times in a large volume of ice-cold medium of the following composition, in millimoles per liter: NaCl, 140; KCl, 5; MgSO₄, 1.2; CaCl₂, 0.8; glucose, 10; acetic acid, 10; sodium phosphate, 2; and Tris, 20 (pH 7.4). Slices were randomly distributed into 25-ml flasks (400 mg tissue per 4 ml of medium) and kept at 0°C until incubation.

Incubation. The slices were first preincubated for 20 min at 30°C in the medium described above. After preincubation, slices were transferred to 4 ml of the same medium which contained also 0.2 mM 1-methyl-3-isobutylxanthine (MIX). Slices were then incubated at 30°C without or with addition of VP or other agents to be tested, added at zero time. Unless otherwise stated, the incubation time was 20 min. At the end of the incubation period, slices were removed from the incubation
medium, quickly minced with scissors, and homogenized immediately in 2.8 ml of ice-cold medium of the following composition: 0.25 M sucrose, 5 mM potassium phosphate, 2 mM EDTA (ethylenediaminetetraacetic acid), and 0.5 mM MIX (pH 7.0), using a Teflon-pestle glass homogenizer. Also, all the following steps were carried out at 0–4°C. The homogenate was filtered through nylon cloth (mesh size, 390; Nytex Co.), and 0.5 ml of homogenate was immediately mixed with a buffer of the following composition: 3 mM guanosine 5’-triphosphate (GTP), 30 mM MgCl₂, and 30 mM potassium phosphate (pH 6.8) in a ratio of 2 volumes of supernate to 1 volume of buffer. This aliquot of extract was also quickly frozen in Dry Ice and used later for determination of the [3H]colchicine-binding activity. Frozen supernates were stored at −80°C until assayed for [3H]colchicine-binding activity or lactate dehydrogenase activity or used for preparation of heat-stable protein kinase inhibitor extracts.

Protein kinase assay. The protein kinase activity in the 40,000 × g supernate was assayed in an incubation mixture of 200 µl total volume containing the following ingredients in final concentration: 10 mM MgCl₂, 2 mM theophylline, 0.3 mM EGTA (ethylenediaminetetraacetic acid), and 0.5 mM MIX (pH 7.0) using a Teflon-pestle glass homogenizer. Also, all the following steps were carried out at 0–4°C. The homogenate was filtered through nylon cloth (mesh size, 390; Nytex Co.), and 0.5 ml of homogenate was immediately mixed with a buffer of the following composition: 3 mM guanosine 5’-triphosphate (GTP), 30 mM MgCl₂, and 30 mM potassium phosphate (pH 6.8) in a ratio of 2 volumes of supernate to 1 volume of buffer. This aliquot of extract was also quickly frozen in Dry Ice and used later for determination of the [3H]colchicine-binding activity. Frozen supernates were stored at −80°C until assayed for [3H]colchicine-binding activity or lactate dehydrogenase activity or used for preparation of heat-stable protein kinase inhibitor extracts.

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RESULTS

In a system originally developed for adipose tissue (7, 25), homogenization of tissue in a medium containing 0.5 M NaCl was of advantage since it apparently prevented reassociation of catalytic and regulatory subunits of protein kinase after homogenization and dilution. However, use of high NaCl concentration can inhibit the protein kinase or apparently cause dissociation of the enzyme in some other tissues (5, 15, 23). For the present study, use of homogenization medium containing 0.5 M NaCl was compared with a medium containing 0.25 M sucrose as described in Methods. Results summarized in Table 2 show that by using a medium of either composition, a significant increase was detected in the (-cyclic AMP/+cyclic AMP) protein kinase activity ratio after incubation of tissue slices with AVP. Extracts prepared in our standard sucrose medium and those prepared in medium containing 0.5 NaCl differed in that the control (-cyclic AMP/+cyclic AMP) protein kinase activity ratio was much higher in 0.5 NaCl medium and that the increase in the ratio after AVP was due solely to the increase in the activity assayed without addition of cyclic AMP. Total protein kinase activity (assayed in the presence of added maximum concentration of cyclic AMP) from slices incubated in the presence of AVP did not change. Total protein kinase activity for control slices is lower in 0.5 M NaCl extract, possibly due to the inhibitory effect of high salt concentration on protein kinase as observed also in other studies (7, 10). On the other hand, in a medium containing 0.25 M sucrose the increase in (-cyclic AMP/+cyclic AMP) protein kinase activity ratio was due to the simultaneous increase in activity without cyclic AMP addition and a decrease in total protein kinase activity (Table 2). The relative increase in the (-cyclic AMP/+cyclic AMP) protein kinase activity ratio with 2.5 × 10^-7 M AVP, a concentration of hormone which stimulates maximally bovine renal medullary adenylate cyclase (3), was not markedly different with use of 0.5 NaCl (about +34.4%) from the increase with standard sucrose medium (about +36.7%). Therefore, in all other experiments, a medium containing 0.25 M sucrose (described in methods) was used.

When tissue slices were incubated for different time periods, in the absence of AVP, the protein kinase (-cAMP/+cAMP) activity ratio as well as cyclic AMP levels remained constant up to 30 min (Fig. 1). A slight, insignificant increase in both parameters in the first few minutes of incubation is likely due to transfer of slices to medium containing 0.2 mM MIX. On the other hand, in the presence of added AVP there was marked increase in both cyclic AMP and protein kinase (-cAMP/+cAMP) activity ratios within the first 5 min, and both parameters leveled off between 5 and 30 min. Changes with time in the kinase activation and cyclic AMP levels went in parallel, and a significant correlation between the two parameters was found (Fig. 1).
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TABLE 2. Effect of vasopressin on protein kinase activity measured in extracts prepared in medium containing either sucrose or sodium chloride

<table>
<thead>
<tr>
<th></th>
<th>Extracts Prepared in 0.25 M Sucrose Medium</th>
<th></th>
<th>Extracts Prepared in 0.5 M NaCl Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>2.5 x 10^-4 M</td>
<td>P value</td>
</tr>
<tr>
<td>Without cyclic AMP addition</td>
<td>66.5±5.8</td>
<td>71.7±4.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>With 5 x 10^-6 M cyclic AMP added</td>
<td>240.8±10.9</td>
<td>187.9±5.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>(-Cyclic AMP+/cyclic AMP) protein kinase activity ratio</td>
<td>0.278±0.027</td>
<td>0.381±0.018</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

Values are means ± SE from 5 experiments. Extracts (40,000 × g supernate) were prepared from slices homogenized in a medium containing 0.25 M sucrose as described in METHODS. For other experimental conditions, see METHODS. Slices were incubated in the absence (control) and in the presence of [8-arginine]vasopressin. * For significance of difference from controls (paired t test); NS denotes P > 0.05. † Value significantly higher than for extract prepared in sucrose (P < 0.001; t test). ‡ Value significantly lower than for extract prepared in sucrose (P < 0.02; t test).

Increasing doses of AVP (10^-10 to 10^-6 M) elicited a gradual elevation of tissue cyclic AMP concentration and a parallel increase in the (-cyclic AMP+/cyclic AMP) protein kinase activity ratio (Fig. 2). There was also a progressive increase in protein kinase activity measured without addition of exogenous cyclic AMP and a decrease in the total and net cyclic AMP-dependent protein kinase activities (Fig. 2). All these changes in protein kinase parameters were significantly correlated with AVP-elicited increases in tissue cyclic AMP (Figs. 2 and 3). The maximum increases both in cyclic AMP levels and maximum changes in protein kinase parameters reached a plateau at an AVP concentration of about 2 x 10^-9 M in the present system (Fig. 2).

Protein kinase activation in renal medullary slices was specific for AVP. (Des-Gly^{4},NH^{2})-oxytocin, an inactive analog of VP^{1} and some other hormones which are known either to stimulate adenylate cyclase in other target tissues (PTH, histamine) or elicit biological effects probably without mediation of cyclic AMP (angiotensin II)^2 had no significant effect on protein kinase activation (Table 3).

To examine whether the decrease in total protein kinase activity is a specific feature of this cytosolic enzyme (3), the effects of AVP on cyclic AMP levels, on protein kinase activity, and on the activity of two other components of renal medullary cytosol were measured simultaneously. Results summarized in Table 4 show that while AVP markedly increased cyclic AMP levels and decreased total protein kinase activity, it had no effect on the activity of lactate dehydrogenase or on the [3H]colchicine-binding activity in the same extracts. The specificity of AVP-induced changes is further stressed by the observation that (des-Gly^{4},NH^{2})-oxytocin had no effect on the measured parameters (Table 4).

We explored whether AVP-induced decreases in total protein kinase activity and net cyclic AMP-dependent protein kinase activities were possibly due to accumulation of a heat-stable protein kinase inhibitor (1, 27) in tissue slices incubated with AVP. Tissue slices were incubated without or with AVP and protein kinase activity and cyclic AMP levels were determined. Aliquots of tissue extracts from these incubations were processed as described in METHODS to obtain preparations which were subsequently tested for the presence of the heat-stable protein kinase inhibitor. Samples were diluted in such a way that preparations from paired control and AVP-treated tissues had equal protein concentrations. No inhibition of protein kinase activity was detected after addition of extracts either from control or AVP-treated tissue preparations. On the other hand, protein kinase inhibitor prepared in a similar way from rabbit skeletal muscle, a tissue known to contain a high quantity of this inhibitor (27), completely inhibited cyclic AMP-dependent protein kinase activity in the present system (Table 1).

\footnote{In incubation of renal medullary slices with 5 x 10^-6 M angiotensin II did not change the tissue level of cyclic AMP (unpublished observations).
FIG. 2. Effect of increasing concentrations of AVP on tissue levels of cyclic AMP and protein kinase activation. Each point represents mean ± SE from 5 experiments. Abcissa: AVP concentration; PKA, protein kinase activity. Lower panel: (---●--●--), cyclic AMP/mg tissue protein; (●○●○), (–cyclic AMP/+ cyclic AMP) protein kinase activity ratio. Upper panel: ( – – – – – – ), protein kinase activity without addition of cyclic AMP; (● – ○ – ○ – ), total protein kinase activity; ( – – – – – – ), net cyclic AMP-dependent protein kinase activity. A close positive correlation was found between tissue cyclic AMP levels and (–cyclic AMP/ + cyclic AMP) protein kinase activity ratio (see Fig. 3), as well as between cyclic AMP levels and protein kinase activity measured without added cyclic AMP (r = 0.5145; P < 0.01). There was a significant negative correlation between cyclic AMP levels and total protein kinase activity (r = 0.3968; P < 0.05), and between cyclic AMP levels and net cyclic AMP-dependent protein kinase activity (r = 0.5392, P < 0.01).

Further, we tested whether the decrease in total protein kinase activity is due to attachment (or "translocation" (16)) of protein kinase from the supernate to the particulate fraction sedimenting at 40,000 x g. Slices were incubated with or without AVP, homogenized, and the homogenate was centrifuged at 40,000 x g for 10 min as described in METHODS. In the supernate, protein kinase activity as well as cyclic AMP binding capacity was determined. The 40,000 x g pellet was resuspended in the homogenization medium and centrifuged again at 40,000 x g for 10 min to wash out remaining supernate. This washed pellet was then suspended in a medium containing 300 mM NaCl and 5 mM sodium phosphate (pH 7.0). The suspended pellet was extracted by stirring in an ice-cold (0-2°C) bath for 60 min. The suspension was then centrifuged at 40,000 x g for 10 min, and the supernate was appropriately diluted and assayed for protein kinase activity without cyclic AMP and with 5 x 10⁻⁶ M cyclic AMP. Moreover, protein kinase both in the supernate and in the extract from pellets was also assayed with cyclic AMP and heat-stable protein kinase inhibitors prepared from rabbit skeletal muscle (see METHODS, Table 1) at the dose of inhibitor which completely suppresses the activity of cyclic AMP-dependent protein kinase (26). The results summarized in Table 5 show that the increases in the (–cyclic AMP/+ cyclic AMP) protein kinase activity ratio and in the activity of protein kinase measured without cyclic AMP, as well as the decrease in total protein kinase activity, were accompanied by marked decrease in cyclic AMP binding capacity in the same supernate (Table 5, part A). Protein kinase activities in extracts from pellets obtained from slices exposed to AVP were significantly higher than in extracts from controls (Table 5, part B). Protein kinase activities in extracts from pellets were only slightly stimulated by added cyclic AMP, and there was no change in the (–cyclic AMP/+ cyclic AMP) protein

### TABLE 3. Hormonal specificity of increase in protein kinase activity ratio by vasopressin

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration</th>
<th>n</th>
<th>Δ% in (–Cyclic AMP)/ (+Cyclic AMP) Protein Kinase Activity Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>[8-arginine]vasopressin</td>
<td>2.5 x 10⁻⁸ M</td>
<td>6</td>
<td>+39.1 ± 9.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>(Des-Gly⁹-NH₂)-oxytocin</td>
<td>10⁻⁷ M</td>
<td>8</td>
<td>+1.1 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Histamine</td>
<td>10⁻⁷ M</td>
<td>6</td>
<td>+8.8 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>5 U/ml</td>
<td>6</td>
<td>+3.6 ± 4.1</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>5 x 10⁻⁸ M</td>
<td>6</td>
<td>+2.4 ± 4.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE; n denotes number of paired observations. Changes in the protein kinase activity ratio (–cyclic AMP)/ (+ cyclic AMP) after addition of tested agents are expressed as percent of control incubations. (–cyclic AMP/+ cyclic AMP) ratio of controls was taken as 100%. * For significance of changes; paired t-test; NS denotes P > 0.05.
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DISCUSSION

In mammalian renal medulla, cyclic AMP-dependent protein kinase is predominantly a cytosolic enzyme (3) and studies on rabbit renal medullary protein kinase indicate the presence of at least two cyclic AMP-dependent subfractions of the enzyme (21). In order to examine whether VP activates protein kinase in situ we used measurement of the (cyclic AMP/cyclic AMP) protein kinase activity ratio (7, 28), a method designed originally for adipose tissue. In spite of differences in stoichiometry (5, 17), the basic mechanism of protein kinase activation by cyclic AMP appears to be similar for different organs and animal species (7, 17, 23), and the method using measurement of (cyclic AMP/cyclic AMP) protein kinase activity ratio (22) appears to be generally applicable (7, 12, 15, 20, 24, 28).

In the present study, we observed that incubation of renal medullary slices with AVP leads to an increase in (cyclic AMP/cyclic AMP) protein kinase activity ratio, and that this increase is hormone specific (Table 3) and is dependent on the dose of AVP. Furthermore, a close quantitative correlation between the protein kinase activity ratio after incubation with AVP. The activity of protein kinase assayed in the presence of added cyclic AMP plus protein kinase inhibitor, which represents protein kinase not regulated by cyclic AMP (26), was not changed after incubation with AVP either in the supernate or in the extract from pellets (Table 5).

| TABLE 4. Specificity of changes in cyclic AMP levels and protein kinase activity parameters after vasopressin |
|-------------------------------------------------|------------------|-----------------|-----------------|
| Cyclic AMP level, pmol/mg protein               | Controls         | 8-Aarginine/Vasopressin, 2.5 x 10^-3 M | P Value*         | Des-Gly-NH–Oxytocin, 10^-3 M | P Value*         |
| Protein kinase activity, pmol P_i/5 min per mg protein | 12.7 ± 2.4       | 48.5 ± 3.9      | < 0.001         | 13.2 ± 1.7          | NS              |
| Without addition of cyclic AMP                  | 84.3 ± 2.1       | 94.2 ± 4.8      | < 0.05          | 84.4 ± 4.9          | NS              |
| Total protein kinase activity (with 5 x 10^-4 M cyclic AMP) | 302.3 ± 21.4     | 241.6 ± 9.9     | < 0.02          | 305.0 ± 27.4        | NS              |
| (-Cyclic AMP/+cyclic AMP) ratio                 | 0.28 ± 0.02      | 0.39 ± 0.01     | < 0.005         | 0.29 ± 0.02         | NS              |
| Lactate dehydrogenase activity, µmol DPNH/min per mg protein | 1.06 ± 0.04      | 1.22 ± 0.12     | NS              | 1.17 ± 0.07         | NS              |
| Colchicine-binding activity, pmol/90 min per mg protein | 23.7 ± 2.5       | 28.5 ± 3.3      | NS              | 21.6 ± 3.1          | NS              |

Values are means ± SE of 6 experiments. * For significance of difference from controls (paired t test); NS denotes P > 0.05.

| TABLE 5. Protein kinase activity (PKA) in 40,000 x g supernate, in extract from 40,000 x g pellet, and [3H]cyclic AMP binding in 40,000 x g supernate |
|-------------------------------------------------|------------------|-----------------|-----------------|
| PKA without cyclic AMP added                   | 0.205 ± 0.02     | 0.288 ± 0.01    | < 0.001         |                             |                 |
| PKA with 5 x 10^-6 M cyclic AMP                | 118.5 ± 5.5      | 139.0 ± 7.5     | < 0.005         |                             |                 |
| PKA with 5 x 10^-6 M cyclic AMP and with protein kinase inhibitor | 69.0 ± 3.0       | 74.5 ± 4.0      | NS              |                             |                 |
| Cyclic [3H]AMP binding, pmol/mg protein        | 1.49 ± 0.13      | 0.65 ± 0.03     | < 0.001         |                             |                 |
| PKA with 5 x 10^-6 M cyclic AMP and with protein kinase inhibitor | 73.0 ± 4.5       | 70.0 ± 3.5      | NS              |                             |                 |

Values are means ± SE from 6 experiments. Protein kinase activity is expressed in picomoles [3P/Pi/5 min per mg protein. Rabbit skeletal muscle protein kinase inhibitor was prepared as described in METHODS. Amount used in this experiment was 235 µg per tube. * For significance of difference from controls (paired t test); NS denotes P > 0.05.
cyclic AMP and protein kinases from other cell types contained in renal medulla remain unaffected.

It is also possible that factors such as a high rate of cyclic AMP breakdown, efflux of cyclic AMP from cells, or some as yet unknown negative feedback regulatory mechanism at the cellular level may limit the maximal increase in cyclic AMP tissue levels by VP and prevent maximal activation of protein kinase. That these factors may indeed play a role is suggested by the finding that incubation of slices with 8-[[chlorophenylthio]-cyclic AMP, a potent cyclic AMP analog which is resistant to cyclic AMP breakdown, activates protein kinase in renal medullary slices to a much greater degree than maximal doses of AVP (2).

The relative changes in tissue cyclic AMP levels in response to AVP (in terms of percent increase above control) are larger than the relative changes in protein kinase activation. This suggests that only a certain portion of cyclic AMP generated in response to VP is available for and operative in the protein kinase activation. Nevertheless, the very close correlation between tissue levels of cyclic AMP and the extent of protein kinase activation, both in time and in relation to the dose of VP, as well as the specificity of VP effect, suggest that protein kinase activation is an integral step in the cellular action of VP in the renal medulla.

Protein kinase activation by VP in renal medullary slices is accompanied by a marked decrease in total cyclic AMP-dependent activity in the tested preparation. This decrease in the protein kinase activity appears to be specific for cytosolic protein kinase since other cytoplasmic components—lactate dehydrogenase (3) or free tubulin, assessed by [3H]colchicine binding activity (10)—are not affected by VP. The decrease in protein kinase activity is apparently not due to the accumulation of a heat-stable protein kinase inhibitor (27), and since such a decrease in the total protein kinase activity does not occur in high-salt medium, it may reflect the translocation of protein kinase on the particulate material contained in the 40,000 g pellet (16).

Such a translocation is documented by the observation that in preparations with AVP-induced decreases of total protein kinase in the supernate, a significantly higher activity of protein kinase can be extracted from 40,000 x g pellets (Table 5). The protein kinase which can be extracted from pellets was only slightly stimu-

In conclusion, the present results provide the first evidence that cyclic AMP-dependent protein kinase is specifically and in a dose-dependent way activated by VP in intact cells and that this activation is correlated with VP-induced changes in tissue cyclic AMP level. The mechanism by which activated soluble protein kinase elicits the ultimate functional response to VP remains to be elucidated. Although the proteins of plasma membranes from the renal medulla (10, 22) or cytosolic proteins (8, 9) can be phosphorylated in a cell-free system, the natural specific protein substrate for soluble renal medullary protein kinase has not yet been identified. It is possible that AVP-activated cytosolic protein kinase may phosphorylate proteins in situ which are directly or indirectly involved in the control of luminal plasma membrane water permeability (11). Conceivably, these protein substrates could be an integral component of the membrane or could be closely associated. Alternatively or additionally, protein kinase may activate phosphorylase (14) and other metabolic adjustments required for the energy supply for a continuous VP effect (11, 14). However, regardless of the exact role for protein kinase in VP action, it appears that activation of this enzyme by VP represents an integral and perhaps obligatory step in the cellular response of mammalian renal medulla to VP.

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L. D. Barnes is a Senior Mayo Research Fellow.

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