Vasopressin rapidly stimulates phosphatidic acid-phosphatidylinositol turnover in rat anterior pituitary cells

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Received 12 January 1985

In cultured rat anterior pituitary cells, the agonist [Asu[1,6], Arg[8]]vasopressin (AVP-A) increased by 1.5-fold $^{32}P_i$ incorporation into phosphatidic acid (PA), as early as 15 s after its addition. Increased phosphatidylinositol (PI) labeling became significant 4 min after AVP-A addition. Dose-response measurements with AVP-A showed ED_{50} values of 76 and 62 nM for PA and PI labeling, respectively. Peptide corticotropin-releasing factor (CRF) (0.1 μM) did not affect the stimulatory effect of AVP-A on PA and PI labeling. These data suggest that stimulation of PI metabolism in corticotrophs may be one of the early events involved in the stimulation of ACTH release induced by vasopressin.

Vasopressin *Corticotropin releasing factor* *Adrenocorticotropin* *Corticotroph* *Pituitary* *Phospholipid*

1. INTRODUCTION

The control of ACTH secretion is under the influence of both stimulatory and inhibitory factors. Positive regulators acting directly at the anterior pituitary level include vasopressin [1] and the peptidic corticotropin-releasing factor (CRF) [2]. It is well recognized that vasopressin not only stimulates ACTH release when present alone but also potentiates the stimulatory effect of CRF on ACTH secretion [3]. The potentiating action of vasopressin is possibly mediated by the stimulatory effect of the neuropeptide on CRF-induced cAMP accumulation.

In many endocrine cells, it has been demonstrated that ligand-receptor coupling triggers the rapid increase in phosphatidylinositol (PI) turnover [4–6]. In hepatocytes, convincing evidence indicates that vasopressin induces PI breakdown and its resynthesis through the phosphatidic acid (PA)-PI cycle [7–10]. At the anterior pituitary level, two hypothalamic hormones, namely luteinizing hormone-releasing hormone (LHRH) and thyrotropin-releasing hormone (TRH) were also recently found to stimulate PA-PI turnover in intact [11–13] or tumoral [14,15] cells in cultures. Thus we thought it of interest to examine the possibility that vasopressin exerts its early effect through changes of phospholipid metabolism in anterior pituitary cells.

2. MATERIALS AND METHODS

Anterior pituitaries obtained from adult female Sprague-Dawley rats at random stages of the estrous cycle were enzymatically dispersed and cultured (6×10^5 cells/ml per dish) as previously described [13]. Five days after plating, cells were washed 3 times with DMEM without serum before

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

a preincubation in triplicate for 20 or 30 min in 1.5 ml of MEM without phosphate containing 300 μCi $^{32}P_i$. Synthetic [Asu^{1,6},Arg^8]vasopressin (AVP-A, Beckman), synthetic CRF or the vehicle alone (50 μl) was then added for the indicated time periods or at increasing concentrations for dose-response studies. After incubation, the reaction was stopped by removing the media and cells were rapidly washed twice with 1 ml Hepes buffer before the addition of 1 ml of ice-cold 10% (w/v) trichloroacetic acid. Cells were then scraped from the dishes and the precipitated material was centrifuged at 27000 × g for 10 min.

Polar lipids were extracted as described previously [12,13]. Phospholipids were separated by 2-dimensional chromatography: in the first dimension, chloroform/methanol/28% ammonia (65:35:5, by vol.) and in the second dimension, chloroform/acetone/methanol/glacial acetic acid/water (6:8:2:2:1, by vol.). The different phospholipid fractions were scraped into scintillation vials and the radioactivity measured in a liquid scintillation counter. Statistical significance was determined by analysis of variance. Experiments were usually repeated 3 times.

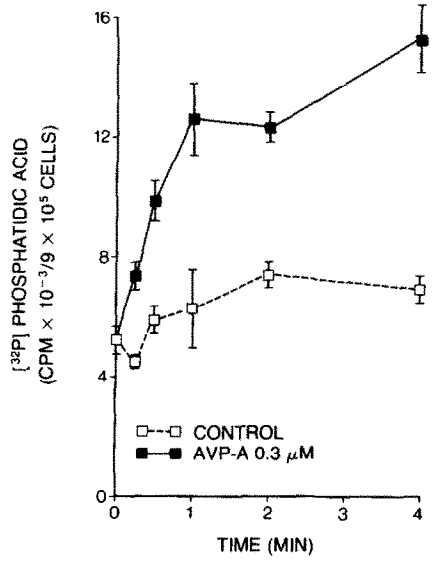

Fig.1. Short time course of the effect of AVP-A on phosphatidic acid (PA) labeling in anterior pituitary cells in culture.

3. RESULTS

As illustrated in fig.1, short time course experiments revealed that 0.3 μM AVP-A rapidly stimulates PA labeling. As early as 15 s after AVP-A addition to cells preincubated for 30 min with $^{32}P_i$, incorporation into PA was significantly increased (7340 ± 330 vs 4510 ± 140 cpm; $p < 0.05$). PI labeling was not significantly increased until 4 min of AVP-A treatment (8440 ± 900 vs 4710 ± 350 cpm; $p < 0.05$).

Longer time course experiments were then performed to analyze the duration and specificity of the stimulatory effect of vasopressin. As illustrated in fig.2, by 3 min after 0.3 μM AVP-A addition, $^{32}P_i$ incorporation was increased only into PA (fig.2B, inset). By 9 min, however, the stimulatory effect was also observed in PI (1790 ± 90 vs 1220 ± 130 cpm; $p < 0.05$) (fig.2A, inset) as well as in total phospholipids (34090 ± 190 vs 27850 ± 560 cpm; $p < 0.01$) (fig.2D, inset). In several time course experiments, this stimulatory effect of vasopressin was limited to PI, PA and total chloroform-soluble material; no significant effect of the neuropeptide could be detected on the labeling of phosphatidylserine, lysophosphatidylcholine, phosphatidylcholine, lysophosphatidylethanolamine and phosphatidylethanolamine (fig.2C).

Dose-response experiments were next performed to assess the sensitivity of the action of AVP-A on PA-PI turnover (fig.3). After a 60-min incubation period, 10 μM AVP-A led to an approx. 2-fold maximal enhancement of radioactivity incorporation into PA, PI and total chloroform-soluble material. ED_{50} values of 76 ± 20, 62 ± 18 and 63 ± 11 nM were measured for PA, PI and total labeling, respectively. In agreement with previous results, PC labeling remained constant up to the maximal dose of AVP-A tested (10 μM).

In other experiments, we have examined the possibility that CRF could interact with the labeling of PA-PI induced by AVP-A. Concomitant treatment of anterior pituitary cells with 0.3 μM AVP-A and 0.1 μM CRF for 20 or 60 min did not interfere with the stimulatory effect of AVP-A alone on PA-PI labeling (not shown). Also, CRF by itself did not induce any change in PA-PI labeling.

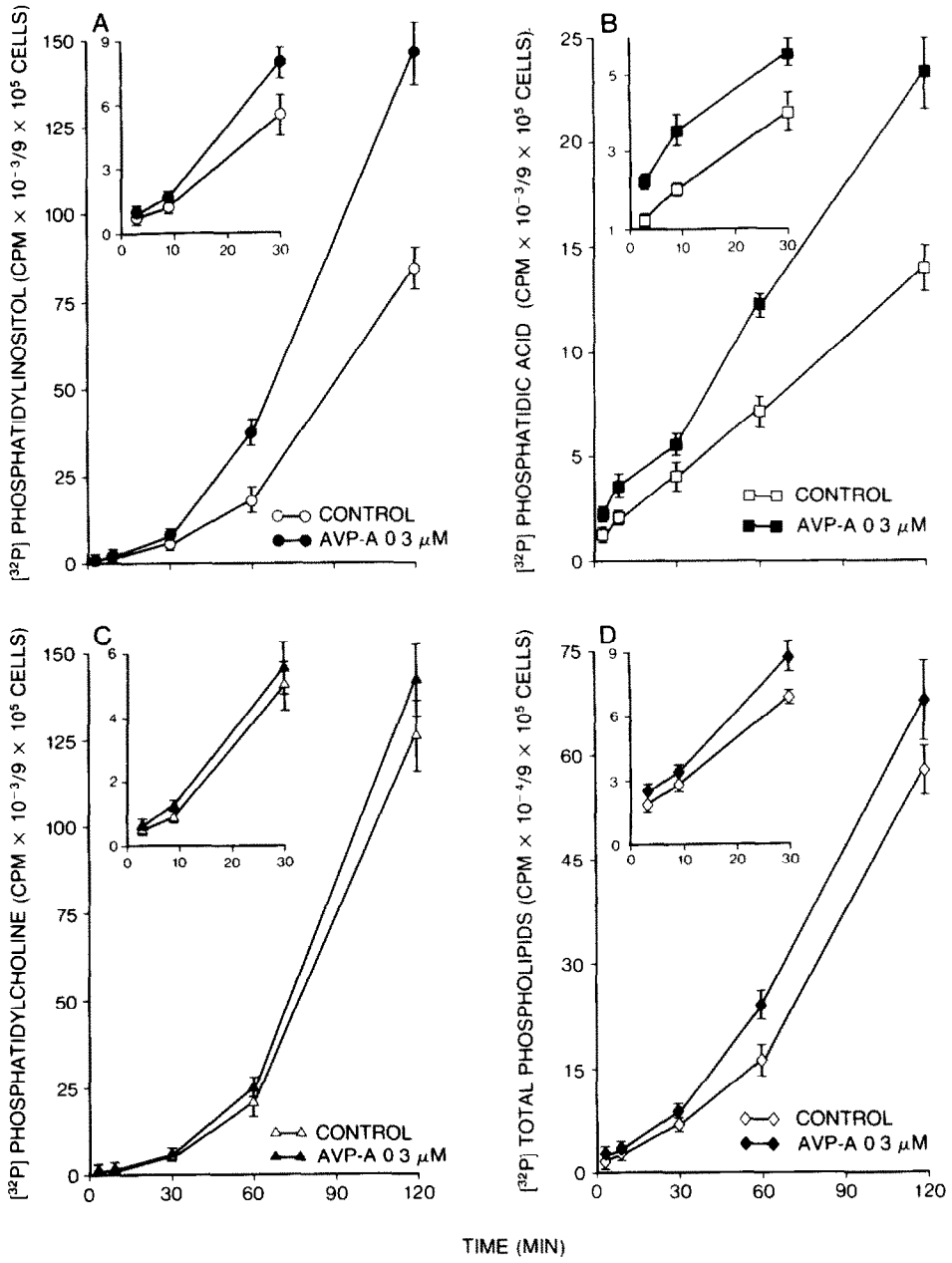

Fig.2. Long time course of the effect of AVP-A on phospholipid labeling in anterior pituitary cells in culture. Inset, enlargement of values obtained during the 3–30 min of incubation.

4. DISCUSSION

The present data clearly suggest that enhanced PA and PI turnover might be an initial post-receptor event in the action of vasopressin at the anterior pituitary level. In fact, the rapid onset of the stimulatory effect of AVP-A on phospholipid labeling detected into both PA and PI and in the first few minutes after its addition to pituitary cells (fig.1,2) represents one of the earliest biochemical

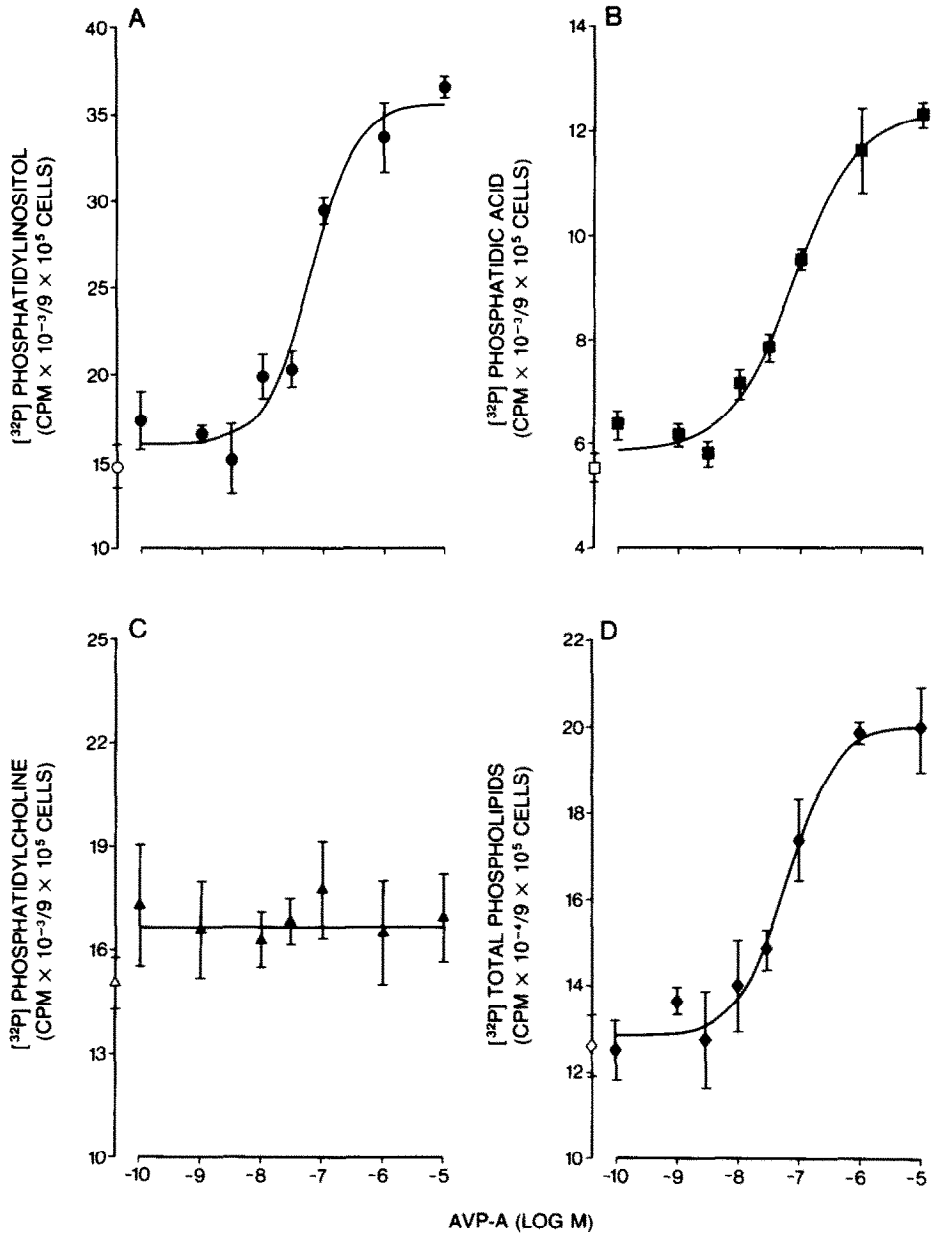

Fig.3. Effect of increasing concentrations of AVP-A on phospholipid labeling in anterior pituitary cells in culture.

responses to the hormone at the adenohypophyseal level reported to date. The kinetics of this effect is in close agrement with the speed of initial changes in the turnover of acidic phospholipids observed in many other systems [4,5].

The molarity for the stimulatory action of AVP-A at ED_{50} values of 76 and 62 nM for PA and PI labeling, respectively (fig.3), is 4- to 5-fold greater than the ED_{50} value of 14.7 nM for AVP-A-induced ACTH release in anterior pituitary cells in culture (Giguere and Labrie, unpublished data). In most systems where hormone effects are associated with enhanced PI metabolism, half-maximal physiological responses occur at lower concentra-

tions than the half-maximal effect on PI metabolism and, as suggested, this effect might more closely parallel receptor occupancy [4,5].

Using an enriched population of rat pituitary corticotrophs [16] or mixed pituitary cells in culture [3], we have demonstrated that 0.1 μM synthetic CRF causes a marked stimulation of intracellular cAMP accumulation as early as 60 s after its addition, an effect which lasts for at least 60 min [16]. These cAMP changes were also found to coincide with or precede the secretion of ACTH in response to CRF. Thus, the present observation of an increased PI turnover induced by vasopressin but not by CRF is consistent with a mechanism whereby vasopressin stimulates acidic phospholipid metabolism, probably through PI breakdown and its resynthesis via PA, while CRF acts in corticotrophs primarily through elevation of cAMP.

Stimulation of the PI cycle is a universal feature of hormones which exert their effects through elevation of intracellular Ca^{2+}. As discussed in many excellent reviews of this topic [17], the stimulated PI cycle is probably a metabolic consequence of an initial breakdown or phosphoinositides linked in some fashion to the entry of extracellular Ca^{2+} or mobilization of intracellular Ca^{2+}. The current hypothesis is that agonists (such as vasopressin) increase triphosphoinositide (TPI) hydrolysis, thus liberating inositol-triphosphate, which in turn mobilizes intracellular Ca^{2+} [17]. Consequently, TPI hydrolysis also seems to be the primary event which leads to increases in PA synthesis and PI labeling. The precise molecular mechanism of vasopressin action at corticotroph level remains to be elucidated.

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