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Arginine-vasopressin stimulates the formation of phosphatidic acid in rat Leydig cells

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Arginine-vasopressin (AVP) stimulated the formation of labelled phosphatidic acid (PA) in [^{14}C]arachidonic acid-prelabelled rat Leydig cells. After addition of 10^{-6} M AVP [^{14}C]arachidonoylphosphatidic acid reached a maximum within 2 min. The increase was dose-dependent (10^{-11}–10^{-6} M). No change in labelling of other phospholipids and diacylglycerol could be detected. The V_1 antagonist dPTyr(Me)AVP inhibited in a dose-dependent manner the AVP-stimulated accumulation of PA. The V_2 agonist dPVDAVP was without effect. The present results suggest that AVP binds to V_1 receptors in rat Leydig cells resulting in stimulation of PA turnover. We suggest that the AVP-stimulated PA formation is an indication of phosphoinositide turnover.

Arginine-vasopressin; Phosphatidic acid; V_1 receptor; Arachidonic acid; Diacylglycerol; (Leydig cell)

1. INTRODUCTION

The neurohypophysial nonapeptide AVP is known to act via at least two distinct receptors [1]. The V_1 receptor is assumed to be coupled to the calcium-phosphoinositide system (hepatocytes [2]; smooth muscle cell line [3], platelets [4]; mesangial cells [5]) whereas there is evidence that the V_2 receptor is linked to adenylate cyclase [1].

It has been demonstrated that an AVP-like peptide is present in rat testes [6]. As specific AVP receptors of the V_1 type have been located in Leydig cells [7] and as an inhibitory effect of AVP on human chorionic gonadotropin-stimulated steroidogenesis [8] has been demonstrated, it is of interest to study the mechanism by which the AVP signal is transduced across the plasma membrane in Leydig cells.

We have studied the turnover of [^{14}C]arachidonoylphospholipids in rat Leydig cells during stimulation of the cells with AVP, with dPTyr(Me)AVP (a V_1 antagonist) and with dPVDAVP (a V_2 agonist).

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Abbreviations: AVP, arginine-vasopressin; dPTyr(Me)-AVP, [1-deaminopenicillamine,(*O*-methyl)Tyr^2,Arg^8]-vasopressin; dPVDAVP, [1-deaminopenicillamine,Val^4, Arg^8]vasopressin; PI, phosphatidylinositol; PIP + PIP$_2$, phosphatidylinositol 4-phosphate + phosphatidylinositol 4,5-bisphosphate; DG, diacylglycerol; PA, phosphatidic acid

2. MATERIALS AND METHODS

2.1. *Materials*

dPVDAVP, dPTyr(Me)AVP, bovine serum albumin (essentially fatty acid-free) and collagenase (type IA) were from Sigma (St. Louis, MO). [1-^{14}C]Arachidonic acid (59.6 mCi/mmol) was from Amersham International (England). Fetal calf serum was from Gibco (Paisley, Scotland). Lipid standards were from Serdary Research Laboratories (London, Ontario). Silica gel 60, high-performance thin-layer plates were

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from Merck (Darmstadt). AVP was from A/B Ferring (Malmø, Sweden).

2.2. *Methods*

Sprague-Dawley rats (35–40 days old) from Møllegård (Lille Skensved, Denmark) were used. Leydig cells were obtained after collagenase (0.1%) treatment of decapsulated testes as described [9,10]. The cells were allowed to attach to Costar multiwell dishes (diameter 35 mm) in 2 ml Krebs-Ringer bicarbonate buffer containing 0.2% (w/v) glucose (KRB) supplemented with 1% (v/v) fetal calf serum. Incubations were carried out at 32°C under air/CO_2 (95:5, v/v).

After 1 h the buffer was removed and the cells attached to the dishes were used for the experiments [120.0 ± 34.8 μg protein/well, mean ± SD (n = 14)]. Cells were incubated with [^{14}C]arachidonic acid (2×10^5 cpm/well) in KRB containing 0.05% (w/v) defatted bovine serum albumin. After incubation for 1 h the cells were washed in 1.5 ml KRB containing 0.5% (w/v) defatted bovine serum albumin, and once in 2 ml KRB.

Peptides were added and the cells were incubated as described in section 3. Immediately after removal of the medium the cells were lysed with 2 ml ice-cold 1 N HCl and harvested. The cell suspensions were sonicated and an aliquot was used for protein determination after neutralization with NaOH [11]. The remainder (1.5 ml) was extracted with 4 ml chloroform/methanol (2:1) containing PA (3 nmol/tube) and DG (3 nmol/tube). The lower phase was collected and the upper phase was reextracted with 2 ml chloroform. The combined extracts were evaporated under nitrogen. The residue was resuspended in chloroform/methanol (2:1). An aliquot was used for liquid scintillation counting. The rest was applied to a high-performance thin-layer plate. The plates were developed with chloroform/acetone/methanol/acetic acid/water (40:15:13:12:8, v/v) to a distance of 8 cm above the application line, and then with hexane/diethyl ether/acetic acid (70:30:2, v/v) to a distance of 15 cm. The lipids were visualized after spraying with 10% (w/v) copper sulfate in 8% (w/v) aqueous phosphoric acid and charring at 180°C for 10 min [12]. Spots were compared to standards, scraped into liquid scintillation vials and counted in a mixture of 0.1 ml water and 1.0 ml Aqualuma^R (Lumax Application Laboratory, The Netherlands).

3. RESULTS AND DISCUSSION

The distribution of [^{14}C]arachidonic acid among the neutral lipids and phospholipids in unstimulated rat Leydig cells is shown in table 1. The label was incorporated mainly in the phospholipids.

Exposure to AVP (10^{-6} M) induced a rapid rise in labelled PA (fig.1). Within 2 min the labelling in PA reached a maximum 1–2-fold over control. It was necessary to add PA as carrier before extraction in order to obtain maximum recovery of endogenous PA. Under the circumstances of these experiments we could not with certainty detect changes in labelling of other phospholipids (not shown). Even with addition of DG as carrier prior to lipid extraction no change in the level of labelled

Table 1

Distribution of [^{14}C]arachidonic acid in rat Leydig cell lipids

Lipid class	Radioactivity (% of total incorporated)
Free fatty acid	2.3 ± 0.6
Diacylglycerol	1.7 ± 0.4
Triacylglycerol	4.4 ± 0.6
Phosphatidic acid	0.9 ± 0.1
Phosphatidylethanolamine	10.8 ± 1.6
Phosphatidylcholine + phosphatidylserine	42.8 ± 1.1[a]
Phosphatidylinositol	27.0 ± 1.7[a]
Phosphatidylinositol 4-phosphate + phosphatidylinositol 4,5-bisphosphate	2.5 ± 0.5
Others	7.2 ± 1.0

[a] For these phospholipids the results are from only two experiments, as in the third experiment phosphatidylinositol cochromatographed with phosphatidylcholine + phosphatidylserine

Rat Leydig cells were incubated with [^{14}C]arachidonic acid for 1 h, washed and incubated for 2 min with medium only. The lipids were extracted and separated as described in section 2. Results are means ± SD of three experiments

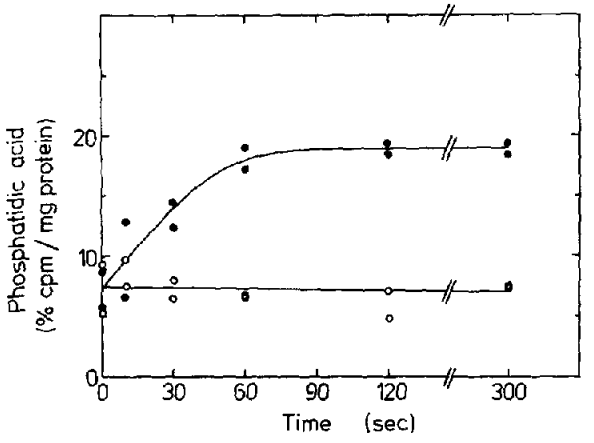

Fig.1. Effect of AVP with time on labelled PA accumulation. Rat Leydig cells were preincubated with [^{14}C]arachidonic acid for 60 min and then incubated in the absence (○) or presence (●) of AVP (10^{-6} M). One experiment, typical of four, is shown, each value in duplicate. Results (^{14}C in PA) are expressed as the percentage of cpm incorporated in the total lipid extract per mg protein.

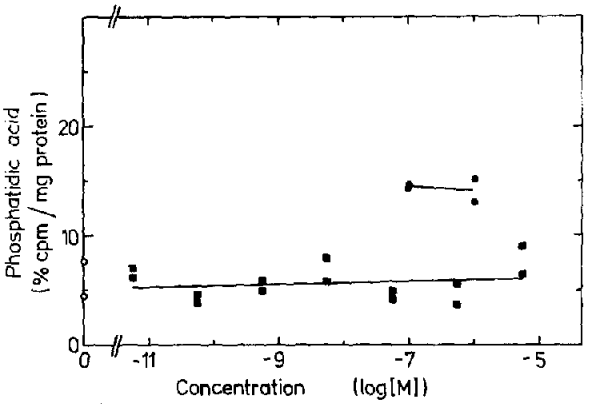

Fig.3. Effect of increasing AVP and V_2 agonist dPVDAVP concentrations on [^{14}C]arachidonoyl-PA formation. Rat Leydig cells, prelabelled with [^{14}C]arachidonic acid, were incubated for 2 min in the absence (○) or presence of either AVP (●) or dPVDAVP (■). One experiment, typical of three, is shown, each value in duplicate. For other details, see the legend to fig.1.

DG was observed between 10 s and 5 min (not shown). This is in contrast to studies in rat mesangial cells [5] where stimulation with AVP induces an increase in both [^{14}C]arachidonic acid-labelled DG and PA for up to 5 min after stimulation. The lack of changes in DG could be due to the fact that DG is rapidly phosphorylated to PA.

Formation of PA has been used to monitor phospholipase C activity [13].

The effect of AVP was dose-dependent (fig.2) in the range 10^{-11}–10^{-6} M. In contrast, the V_2 agonist dPVDAVP did not affect the amount of labelled PA (fig.3). dPVDAVP was used in a con-

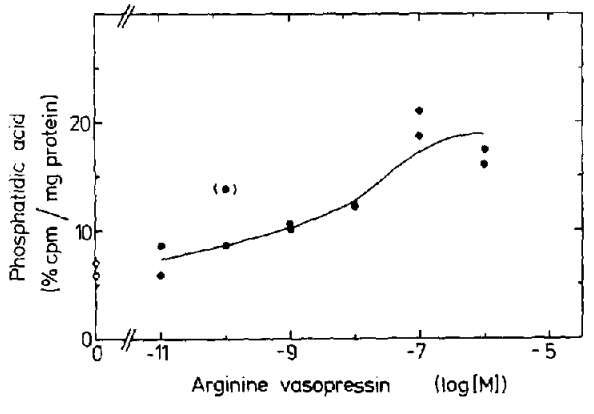

Fig.2. Effect of increasing AVP concentrations on [^{14}C]arachidonoyl-PA formation. Rat Leydig cells were preincubated with [^{14}C]arachidonic acid for 60 min and then incubated for 2 min in the absence (○) or presence (●) of AVP. For other details, see the legend to fig.1.

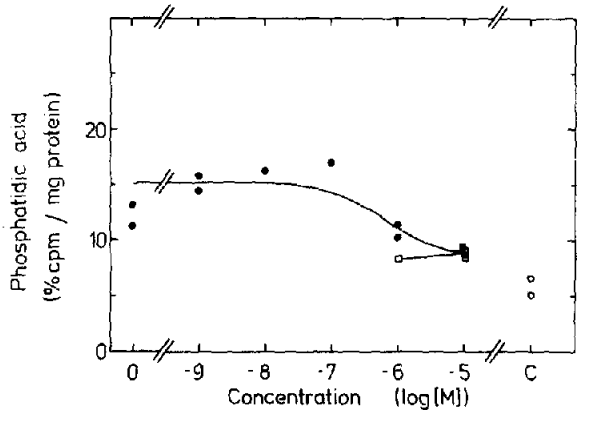

Fig.4. Inhibition by the V_1 antagonist dPTyr(Me)AVP on AVP induced [^{14}C]arachidonoyl-PA formation. Cells were incubated with different amounts of dPTyr(Me)AVP for 2 min in the absence (□) or presence of 10^{-6} M AVP (●). C, control (medium only) (○). For other details, see the legend to fig.3.

centration range where it can displace [^{3}H]AVP binding to Leydig cells [7].

The stimulatory effect of AVP (10^{-6} M) on formation of labelled PA was abolished by the V_1 antagonist dPTyr(Me)AVP in a dose-dependent manner (fig.4). When dPTyr(Me)AVP was added to Leydig cells alone it stimulated a small response, indicating that it could be a partial agonist.

Our results suggest that AVP binds to V_1 receptors in rat Leydig cells resulting in stimulation of PA turnover. Although in the time range studied we could not detect changes in the labelling of other lipids, including PI, PIP + PIP_2 and DG, we suggest on the basis of studies in other cell types [1–5] that the AVP-stimulated PA formation is an indication of phosphatidylinositol turnover.

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