STIMULATION BY VASOPRESSIN AND ANGIOTENSIN OF PHOSPHOLIPID METHYLTRANSFERASE IN ISOLATED RAT HEPATOCYTES

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1. Introduction

Phosphatidylethanolamine and phosphatidylcholine are the two major phospholipid components of membranes from rat hepatocytes [1]. Phosphatidylcholine can be synthesized either by transcholination [2] or by transmethylation [3]. The transmethylation pathway involves the addition of 3 methyl groups to the polar head of phosphatidylethanolamine, the methyl donor being *S*-adenosyl-L-methionine. Two intermediates are formed during the synthesis of phosphatidylcholine by transmethylation: *N*-methylphosphatidylethanolamine and *N*,*N*-dimethyl-phosphatidylethanolamine [4]. In rat liver, phospholipid methyltransferase activity is associated with the microsomal fraction [5,6]. Although rat liver is one of the tissues were the specific activity of phospholipid methyltransferase is relatively high, the contribution of this pathway to the total synthesis of phosphatidylcholine is also minor (20–40%) in this tissue [7]. However, the finding that many signals acting on the cell surface, including hormones [8,9], immunoglobulins [10], attractants [11–13], and phagocytizable particles [14], modulate phospholipid methylation, suggest an important function for this process during signal transduction. We have shown that treatment of isolated hepatocytes with glucagon produces a time and dose-dependent activation of phospholipid methyltransferase, through a mechanism which seems to be dependent on cyclic AMP [9,15]. Rat hepatocytes possess at least 3 hormone receptors, α-adrenergic, vasopressin and angiotensin, in whose actions Ca^{2+} and not cyclic AMP, is involved [16–18]. This paper shows an activation of phospholipid methyltransferase by vasopressin and angiotensin in isolated rat hepatocytes which is dependent on Ca^{2+}. Furthermore, the ionophore A23187 mimics the effect of these hormones.

2. Methods

Hepatocytes were isolated from normally fed Wistar rats (250–300 g) as in [9]. Isolated hepatocytes were incubated in the presence of 2.5 mM $CaCl_2$ or, for the experiments under Ca^{2+}-depletion conditions, washed once and incubated in the presence of 1 mM EGTA. A 1.5 ml portion of this cell suspension, ~15 mg protein [19], was shaken (120 strokes/min) in stoppered 20 ml vials at 37°C in the presence of 10 mM glucose. The gas phase was 95% O_2: 5% CO_2. Cells were stimulated after 30 min pre-incubation. Vasopressin and angiotensin were dissolved in saline containing 0.1% dialyzed bovine serum albumin. Ionophore A23187 was dissolved in dimethylsulfoxide (DMSO). At the indicated time the hepatocytes were poured into pre-cooled centrifuge tubes and immediately centrifuged at 1000 × *g* for 20 s. The pellet was immediately frozen in a dry-ice–acetone bath. Phospholipid methyltransferase activity was assayed as in [9] except that sucrose and $MgCl_2$ were omitted from the incubation media. Angiotensin II and vasopressin were obtained from Ciba and Sigma, respectively. A23187 was from Lilly and *S*-[*methyl*-^{3}H]-adenosyl-L-methionine (15 Ci/mmol) from Amersham. Other reagents were of analytical grade.

3. Results and discussion

Treatment of isolated rat hepatocytes with vasopressin (10^{-7} M) or angiotensin (7×10^{-8} M) stim-

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Table 1
Effect on phospholipid methyltransferase activity of vasopressin, angiotensin and A23187 addition to isolated rat hepatocytes

Additions	Conc.	% Activity	
		2.5 mM $CaCl_2$	1 mM EGTA
None		100	104 ± 5
Vasopressin	10^{-7} M	198 ± 18	110 ± 6
Angiotensin	7×10^{-8} M	178 ± 13	100 ± 3
A23187	1.6×10^{-6} M	150 ± 7	–
DMSO	3.3% (v/v)	80 ± 7	–

Aliquots of hepatocytes for enzyme assay were taken 5 min after stimulation with the different additives. 100% activity corresponds to 84 ± 3 pmol *methyl*-^{3}H group . min^{-1} . mg $protein^{-1}$. Results are means ± SEM from 2 expt in triplicate

ulated ~2-fold the incorporation of *methyl*-^{3}H groups into phospholipids (table 1). The magnitude of activation was similar to that reported for glucagon [9]. When Ca^{2+} were removed from the incubation media, by the addition of 1 mM EGTA, none of the hormones had a significant effect on phospholipid methylation. The addition of the Ca^{2+} ionophore A23187 (1.6×10^{-6} M) also enhanced phospholipid methylation. The ionophore solvent, DMSO, decreased phospholipid methylation ~20%. Therefore, the activation by A23187 can be stimulated ~2-fold. The activation of phospholipid methylation by vasopressin and angiotensin was time dependent (fig.1). In both cases maximal activation was observed 5 min after the addition of the hormone. This pattern was similar to that reported for glucagon [9]. Basal phospholipid methyltransferase activity was ~3–4-fold higher than that in [9]. This could be due to the use of a new colony of rats in these experiments. The dose-dependent activation of phospholipid methylation by vasopressin and angiotensin is shown in fig.2. Levels $>10^{-7}$ M were not used. The concentration of both hormones

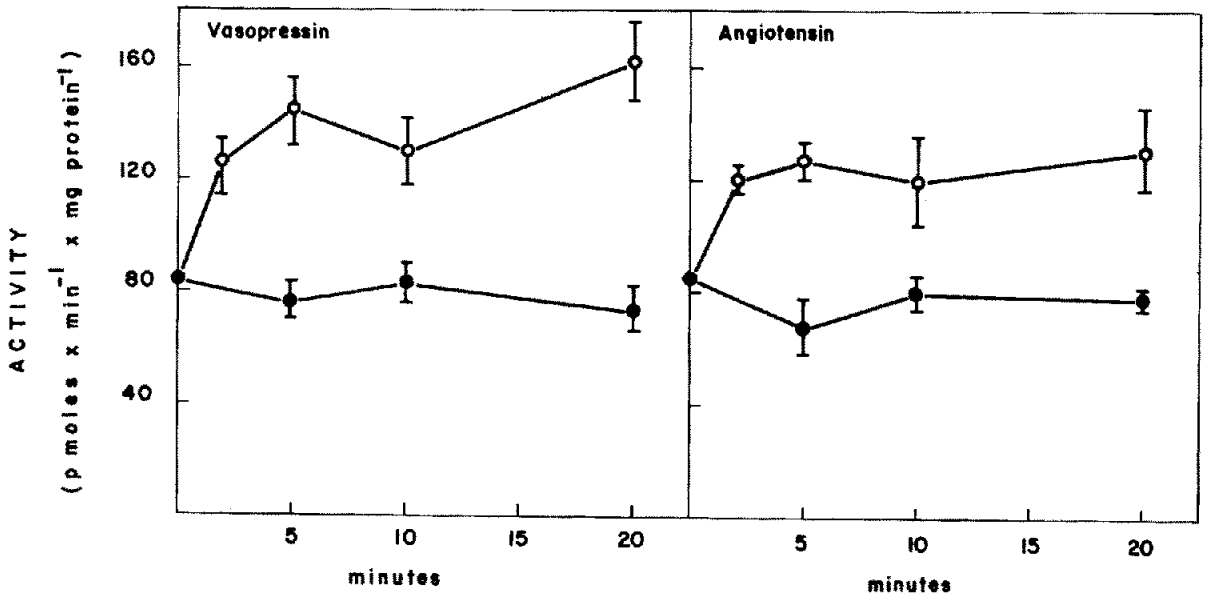

Fig.1. Time course of the effect of vasopressin and angiotensin on phospholipid methyltransferase activity in isolated rat hepatocytes: (•) control hepatocytes; (○) hormone (10^{-7} M vasopressin and 7×10^{-8} M angiotensin) treated cells. At zero time the hormone was added. Values are means ± SEM from 2 expt in triplicate.

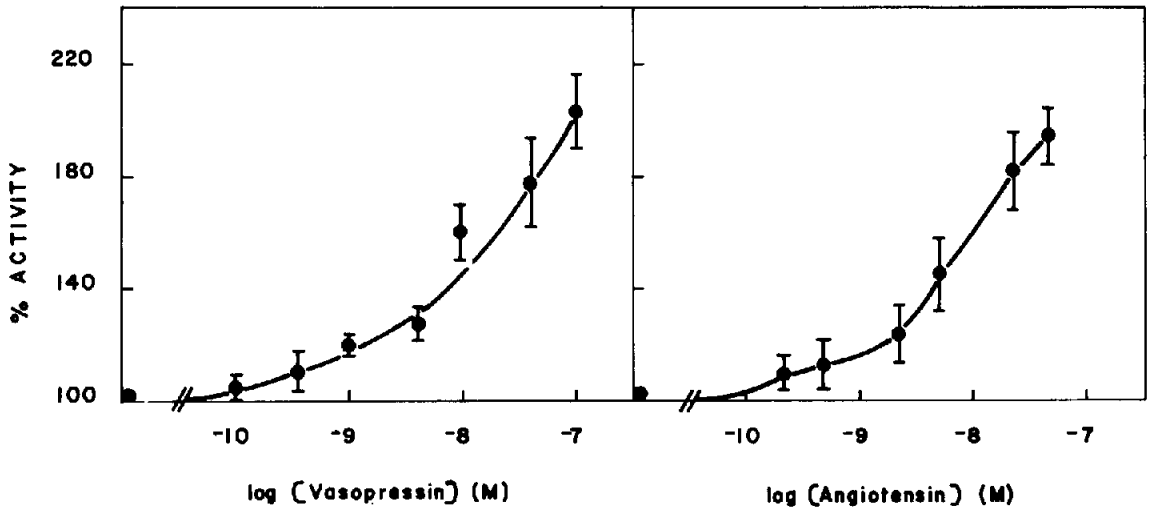

Fig.2. Effect of various concentrations of vasopressin and angiotensin on phospholipid methyltransferase activity of isolated rat hepatocytes. Aliquots of hepatocytes for enzyme assay were taken 5 min after addition of the hormone. 100% activity corresponds to 84 pmol *methyl*-^{3}H group . min^{-1} . mg $protein^{-1}$. Values are means ± SEM from 2 expt in triplicate.

necessary to see a significant effect on phospholipid methylation is higher than what has been shown to be effective in the control of the glycolytic–gluconeogenic flux in liver [18,20]. However, similar doses have been reported to activate phosphatidylinositol turnover [21,22].

In conclusion, these studies show a dose- and time-dependent activation of phospholipid methylation by vasopressin and angiotensin in isolated rat hepatocytes. Under conditions of Ca^{2+} deprivation (EGTA incubation of hepatocytes) phospholipid methyltransferase was not stimulated by vasopressin or angiotensin. These results suggest that the activation of phospholipid methylation is secondary to an increase in the intracellular Ca^{2+} concentration. In support of this hypothesis are the results with the Ca^{2+} ionophore A23187 which mimics the effect of these hormones. A23187 induces efflux of Ca^{2+} from an exchangable pool in isolated rat hepatocytes [23]. It seems therefore, that in rat hepatocytes cyclic AMP [9] and Ca^{2+} modulate phospholipid methylation. We have shown the activation of phospholipid methylation by Mg^{2+}-ATP + cyclic AMP in isolated rat liver microsomes [15], and some suggest that a cyclic AMP-stimulated protein kinase could be involved in the mechanism of activation of phospholipid methylation. It is therefore important to determine if a Ca^{2+}-stimulated protein kinase would mediate the activation of phospholipid methylation by vasopressin and angiotensin. It is interesting to notice that calmodulin modulates phospholipid methylation in *Dictyostelium discoideum* [23] and that Ca^{2+} fluxes have been reported in many of the processes where phospholipid methylation seems to play a role.

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