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STIMULATION OF RAT PITUITARY PHOSPHOLIPID METHYLTRANSFERASE BY VASOPRESSIN BUT NOT OXYTOCIN

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SUMMARY: Rat pituitary extracts contain two methyltransferases that catalyze stepwise methylation of phosphatidyl-ethanolamine to phosphatidylcholine using S-adenosylmethionine as the methyl donor. The activities of both of these enzymes were stimulated by 40 μM lysine or arginine vasopresin but not oxytocin, arginine vasotocin and Pro-Leu-Gly NH_2. The concentration of lysine-vasopressin required for the half-maximal stimulation of phospholipid methylation was 27 μM. A comparison of the chemical structure of different peptides with their ability to stimulate phospholipid methylation suggests that the stimulatory activity resides in the covalent ring structure (pressinoic acid) of the vasopressin molecule.

INTRODUCTION

Phosphatidylcholine (PC)[1] is the major phospholipid of many endocrine tissues, including pituitary gland (1). The synthesis of PC can be achieved by two alternative pathways, the incorporation of CDP-choline to α, β-diglycerate (2), or the stepwise methylation of PE to PC utilizing AdoMet as the methyl donor (3). While the relative contribution of the second pathway to the biosynthesis of PC is minor (4-5), recent studies from several laboratories have shown profound biological effects of this transmethylation pathway on processes such as calcium transport (6), chemotaxis (7), lymphocyte mitogenesis (8), and histamine secretion (9).

Recently, our laboratory has shown that rat pituitary extracts contain two methyltransferases that methylate PE to PC using AdoMet as the methyl donor (10). The first enzyme methylates PE to PME and has a high Km (40-42 μM) for AdoMet, whereas the second enzyme catalyzes two successive methylations of PME to PMME

[1]The abbreviations used are: ACTH, adrenocorticotropic hormone; AdoMet, S-adenosyl-L-methionine; AVP, arginine vasopressin; AVT, arginine vasotocin; BHT, butylated hydroxytoluene; TKM buffer, 40 mM tris (hydroxymethyl) amino methane (pH 7.4) + 7.5 mM KCl + 2 mM $MgCl_2$; LVP, lysine vasopressin; MIF, Pro-Leu-GlyNH_2; OT, Oxytocin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PME, phosphatidyl-N-monomethylethanolamine; and PMME, phophatidyl-N, N-dimethylethanolamine.

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and then to PC and has a low Km (6.7 μM) for AdoMet. The Km (for AdoMet) of both methyltransferases from pituitary differs from those of bovine adrenal medulla and brain enzymes. Adrenal (11) or brain (12) methyltransferase I has low Km (2-4 μM), whereas methyltransferase II has high Km (100-110 μM) compared with pituitary enzymes.

MATERIALS AND METHODS

Therefore, we decided to explore the potential effect of vasopressin, a neurohypophyseal peptide known to partially stimulate ACTH (13-14) and under certain conditions growth hormone (15) secretion from adenohypophysis, on phospholipid methylation by pituitary extracts. The data presented here show that pituitary phospholipid methyltransferase activity is stimulated by LVP and AVP but not three other structurally related peptides-OT, AVT and MIF.

Materials: S-Adenosyl-L-[methyl-^{3}H] methionine (14 Ci/mmol) was purchased from New England Nuclear (Boston, MA). PE, PME, PMME, and PC were obtained from GIBCO (Grand Island, NY), whereas AdoMet was a product of Sigma Chemical Co. (St. Louis, MO). Oxytocin, LVP, AVP, AVT and MIF were purchased from Chemical Dynamics (S. Plainfield, NJ).

Methods:

Preparation of Crude Extract: Sprague-Dawley rats (male, 150-200 g) were killed by decapitation; then, their pituitary glands were removed and rinsed in ice-cold 0.9% saline. For the preparation of crude extract, pituitaries were homogenized in cold TKM buffer (117 μl buffer/pituitary) by means of a small glass homogenizer.

Assay of Phospholipid Methylation: The methylation of phospholipids was measured by incorporation of [^{3}H] methyl group from S-adenosyl-L-[methyl-^{3}H]-methionine into phospholipids (11). The reaction mixture (50 μl) contained: buffer (2.5 μmol, pH 9.5), $MgCl_2$ (0.5 μmol), sodium EDTA (5nmol), S-adenosyl-L-[methyl-^{3}H]-methionine (2 μCi, 10 nmol) and tissue extract (10-50 μg protein). The reaction was initiated by the addition of tissue extract and run in a 12-ml stoppered glass tube at 37°C for 5 min. To stop the reaction, 3 ml of chloroform/methanol/hydrochloric acid (2/1/0.02, v/v/v) containing BHT (50 μg/ml) followed by 2 ml of 0.1M KCl in 50% methanol was added. The mixture was vigorously vortexed twice and then centrifuged at 200 x g for 10 min. The aqueous phase was aspirated, the chloroform phase was rewashed with 2 ml of 0.1M KCl in 50% methanol, and 1 ml of the chloroform phase was transferred to a miniscintillation vial. After the solvent was evaporated to dryness at 80-85°C, 4 ml of scintillation fluid (Formula-963, New England Nuclear) was added and the radioactivity was measured in a Packard Liquid Scintillation Counter.

Identification of Reaction Products: To identify the products of phospholipid-methylation, the chloroform phase was evaporated to dryness under nitrogen gas at 23°C and the residue was dissolved in a small volume of chloroform. The sample was applied on a silica gel G plate and the chromatogram developed in solvent #32 (chloroform/propionic acid/n-propyl alcohol/water - 3/2/6/1, v/v/v/v) at 23°C in ascending mode. The phospholipid standards were chromatographed simultaneously and

Table 1

Effect of Lysine Vasopressin and Related Peptides on Rat Pituitary Phospholipid Methyltranferase Activity

Peptide (40 μM)	Structure	Phospholipid methyltranferase fmole [^{3}H]-methyl incorporated/min/mg protein	P-Value compared to control
Control	——	216.0 ± 45.9 (5)	——
Lysine Vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys--Pro-Lys-GlyNH$_2$	408.3 ± 67.2 (5)	<0.02
Arginine Vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys--Pro-Arg-GlyNH$_2$	366.7 ± 57.8 (5)	<0.05
Arginine Vasotocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Lys-GlyNH$_2$	194.8 ± 56.8 (5)	>0.6
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys--Pro-Leu-GlyNH$_2$	221.0 ± 61.5 (5)	>0.8
MIF	Pro-Leu-GlyNH$_2$	185.8 ± 44.5 (5)	>0.5

their positions were visualized by spraying a saturated solution of iodine in chloroform. The areas corresponding to standard phospholipids (PC, PMME and PME) were scrapped separately and then the radioactivity was extracted with chloroform/methanol (2:1).

RESULTS

To determine the effect of neurohypophyseal peptides and their fragments on phopholipid methylation, rat pituitary extracts were incubated with [methyl-^{3}H]-AdoMet in the absence or presence (40 μM) of various peptides as described in "Materials and Methods" and then the amount of [^{3}H]-methyl radioactivity incorporated into phospholipids was determined. The data presented in Table 1 show that both AVP and LVP stimulated phospholipid methylation whereas OT, OVT and MIF had no effect. The LVP activation of phospholipid methylation was dose-dependent (Fig. I) and the half-maximal activation was observed at 27 μM LVP, which is in the range of 2-3 times the physiological concentrations of the hormone (16). To identify the products of phospholipid methylation, the standard reaction mixture (50 μl) was scaled up to 250 μl. The [^{3}H-methyl]-phopsholipids were extracted into chloroform phase, evaporated to dryness under nitrogen gas at 23°C, and then various products were

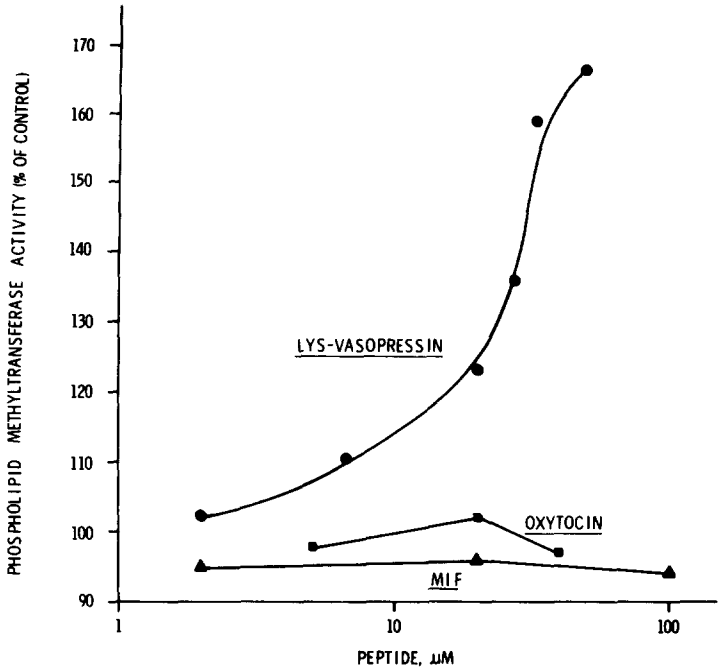

Fig. 1. Effect of varying concentrations of lysine-vasopressin, oxytocin and MIF on phospholipid methyltransferase activity. Each point represents the mean of quadruplicate determinations in one experiment. The experiment was performed three times with similar results.

identified as described under "Materials and Methods." The data presented in Fig. 2 show that LVP (30 μM) increased the incorporation of [^{3}H]-methyl AdoMet into various phospholipids. In a typical experiment LVP-mediated increase in the incorporation of labeled methyl groups in to PME, PMME and PC was 26, 22 and 23% respectively. These data suggest that phospholipid methyltranferase I as well as II are stimulated by LVP. Lysine-vasopressin (30 μM) stimulated phospholipid methylation by both adenohypophyseal and neurohypophyseal extracts, suggesting a lack of any apparent intrapituitary difference in LVP effect (adenohypophysis: control = 240.4 ± 12.4, LVP = 341.0 ± 15.1; neurohypophysis: control = 210.4 ± 11.8, LVP = 262.9 ± 14.7 fmoles/min/mg protein; n = 5; $p < 0.02$).

DISCUSSION

The data presented here show for the first time the dose-dependent stimulation of pituitary phospholipid methylation by LVP. An analysis of the chemical structure and

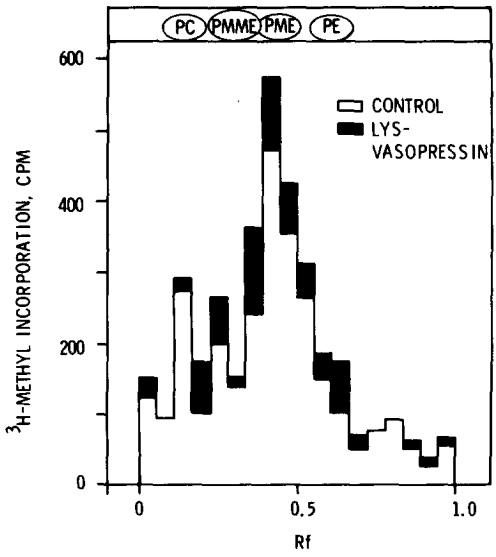

Fig. 2. Chromatographic pattern of [^{3}H]-methylated phsopholipids after incubation of rat pituitary extracts with [^{3}H-methyl]-S-adenosylmethionine in the presence (30 μM) or absence of lysine-vasopressin. The reaction products were isolated and chromatographed as described under "Materials and Methods."

the effects of LVP, AVP, AVT, OT and MIF on pituitary phospholipid methylation suggests that the covalent ring structure of vasopressin is more important than the linear part of the molecule (Table 1).

Vasopressin is a neurohypophyseal peptide which partially modulates the release of ACTH (13, 14) and under certain conditions growth hormone (15) from the adenohypophysis as well as exerts profound effects in the process of acquisition, consolidation, and the maintenance of learned behavior (17-19). The behavioral studies using these vasopressin analogues have suggested that the covalent ring structure of the neurohypophyseal hormones may predominantly affect consolidation processes, while the linear portions may be involved in retrieval mechanisms (17-19).

The possible physiological role of the stimulation of pituitary phospholipid methyltranferase by vasopressin in relation to ACTH or growth hormone secretion remains to be established. Since phospholipid methylation has been shown to affect membrane structure (20-21), it is possible that an increase in phospholipid methyltranferase activity could affect hormone secretion.

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