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Dexamethasone-induced reduction of phospholipase D activity in the rat

Possible role of lipocortin

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Subcutaneous injection of dexamethasone resulted in a reduction of demonstrable phospholipase D activity of rat brain and liver microsomes. Partially purified rat lung lipocortin inhibited the activity of both microsomal and partially purified rat brain phospholipase D. These results show that phospholipase D activity is suppressed by dexamethasone and one of the possible mechanisms of inhibition may be a phospholipase inhibitory protein, lipocortin.

Phospholipase D; Lipocortin; Dexamethasone

1. INTRODUCTION

Phospholipase D (PL-D) of mammalian tissues is a membrane-bound enzyme that possesses both hydrolytic and transphosphatidylation activities [1]. Mammalian PL-D, which is detectable in all tissues examined [2], does not require added divalent cations for activity. The synaptic plasma membrane has the highest specific activity compared to other membranes of rat brain [3]. Recently, several investigators have suggested that PL-D may play a role in the processes of cell activation. A variety of Ca^{2+}-mobilizing hormones were reported to result in the accumulation of phosphatidic acid via a PL-D mechanism with hepatocytes [4]. The release of choline rather than phosphorylcholine from phosphatidylcholine by cultured cells is stimulated by the tumor-promoting phorbol ester, TPA [5]. TPA also stimulates the accumulation of phosphatidylethanol in the presence of ethanol in animal cells [6]. Phosphatidylethanol can be formed by the transphosphatidylation activity of PL-D [7,8]. In addition, PL-D may function in providing choline for acetylcholine formation in brain [3].

Glucocorticoid hormones have a permissive effect on the action of other hormones. The anti-inflammatory action of glucocorticoid hormone may be explained by the induction of phospholipase A_2 inhibitory proteins termed lipocortins which in turn block prostaglandin precursor release [9]. The lipocortins have been the subject of several reviews [10–12]. Lipocortins can inhibit bacterial phospholipase C, cabbage PL-D and several soluble phospholipase A_2 activities in vitro [13]. We examined the effect of dexamethasone injection on the PL-D activity and the effect of partially purified lipocortin on the activity of solubilized partially purified PL-D.

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2. EXPERIMENTAL

Groups of male Sprague-Dawley rats weighing 80–100 g were either adrenalectomized (ADX) or sham-operated (NL). The completeness of adrenalectomy was confirmed by visual inspec-

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tion after killing. On the 14th post-operative day some of the ADX and NL animals were injected subcutaneously with 250 μg/100 g wt of dexamethasone acetate (DEX). Control animals were injected with 0.9% NaCl solutions and the adrenalectomized animals had 0.9% NaCl in their drinking water. The animals were injected for 4 successive days. 24 h after the last injection, the animals were fasted overnight and then the brain and liver microsomal fraction prepared as in [2].

Lipocortin was partially purified from rat lung tissue according to [14] and found to possess 2 bands on SDS-PAGE. Inhibition of snake venom phospholipase A_2 activity was measured as in [13]. Rat brain PL-D was solubilized and partly purified according to a previous procedure [15] and its activity assayed as described [16]. CDP-choline, 1,2-diacylglycerol cholinephosphotransferase activity was determined with CDP-[^{14}C]choline as in [17]. Phospholipid *N*-methyltransferase and base-exchange enzyme activities were as previously described [18]. NADPH cytochrome *c* reductase measurement was as described [19].

Statistical evaluation was performed using Student's *t*-test.

3. RESULTS

The effect of adrenalectomy and dexamethasone injections on the activities of the enzymes is shown in table 1. As previously reported, phosphatidic acid produced by PL-D is capable of being hydrolyzed by endogenous phosphatidic acid phosphatase to yield diglyceride. Diglyceride formation was also measured because phosphatidic acid phosphatase activity is only partially blocked by NaF. Hepatic PLD activity is reduced approx. 70% in both groups of DEX-treated animals. There is only a 40% reduction of the brain activity in the ADX-DEX treated animals and 16% in the NL-DEX animals. There was a reduction of liver phospholipid *N*-methyltransferase activity in both groups of DEX-treated animals when assayed with

Table 1

Phospholipase D, phospholipid *N*-methyltransferase and CDP-choline:choline phosphotransferase activity of brain and liver P_3 fraction from normal, adrenalectomized and dexamethasone-treated rats

	Normal	NL + DEX	ADX	ADX + DEX
(1) PLD				
(1.1) Liver				
PA	15.5 ± 0.9	5.3 ± 0.6[c]	20.1 ± 1.0[a]	4.9 ± 0.1[d]
Diglyceride	24.9 ± 1.3	6.6 ± 1.9[c]	17.5 ± 0.4[b]	8.1 ± 1.5[c]
PA + diglyceride	40.3 ± 0.4	11.8 ± 2.5[d]	37.6 ± 1.1	13.0 ± 1.5[d]
(1.2) Brain				
PA	52.3 ± 1.4	40.3 ± 1.0[c]	46.1 ± 3.1	34.0 ± 0.2[d]
Diglyceride	47.2 ± 1.9	43.8 ± 0.8	49.3 ± 2.2	27.3 ± 1.0[d]
PA + diglyceride	99.4 ± 3.4	84.0 ± 0.2[b]	95.4 ± 5.4	61.2 ± 0.7[d]
(2) PMT				
(2.1) Liver				
No addition	37.8 ± 1.6	18.0 ± 3.6[c]	49.1 ± 2.9[a]	9.0 ± 1.0[d]
+ PMME	93.1 ± 5.6	62.2 ± 7.5[a]	98.4 ± 3.7	37.0 ± 3.0[d]
(2.2) Brain				
No addition	27 ± 8	13 ± 9	22 ± 4	16 ± 4
+ PMME	27 ± 7	23 ± 12	51 ± 7	37 ± 6
(3) CDP-choline cholinephosphotransferase				
(3.1) Liver	2.2 ± 0.2	1.5 ± 0.04[a]	3.6 ± 0.2[c]	1.2 ± 0.08[c]
(3.2) Brain	1.4 ± 0.03	1.4 ± 0.01	1.9 ± 0.08[c]	1.8 ± 0.04[c]
(4) NADPH cytochrome *c* reductase				
(4.1) Liver	42.7 ± 0.4	60.8 ± 4.0[b]	36.0 ± 2.5	51.6 ± 1.0[c]

[a] $p < 0.05$; [b] p 0.02; [c] p 0.01; [d] p 0.001

PA, phosphatidic acid; PMT, phospholipid *N*-methyltransferase; PMME, phosphatidylmonomethylethanolamine. Data are shown as means ± SE of duplicate determinations from 3 separate experiments. Enzyme activities were expressed as nmol/mg protein per h for PL-D, pmol [^{3}H]CH_3 group incorporated/mg protein per min for liver PMT, pmol [^{3}H]CH_3 group incorporated/mg protein per h for brain PMT, nmol/mg protein per min for cholinephosphotransferase and nmol/mg protein per min for NADPH cytochrome *c* reductase

Table 2

Effect of lipocortin on membrane bound and partially purified rat brain phospholipase D

Enzyme source	PLD activity
Microsomal (24 μg protein), no addition	129 ± 4
+ lipocortin a (15 μg protein)	12 ± 1[b]
+ lipocortin b (7.7 μg protein)	27 ± 4[b]
Partially purified PLD (3.7 μg), no addition	714 ± 50
+ lipocortin a (15 μg protein)	254 ± 19[a]
+ lipocortin b (7.7 μg protein)	248 ± 36[a]

[a] $p < 0.002$; [b] $p < 0.001$

Data are means ± SE of duplicate determinations from 3 separate experiments. Enzyme activities expressed as nmol lecithin hydrolyzed/mg protein per h. Lipocortin was partially purified according to Shadle et al. [14]. The fractions of DEAE-cellulose (lipocortin a) and Sephadex G-100 (lipocortin b) column chromatographies were used as partially purified lipocortin

either endogenous or exogenous phospholipid acceptors but the phospholipid *N*-methyltransferase activity of brain tissue was less affected. The hepatic CDP-choline:cholinephosphotransferase activity of DEX-treated rats was reduced by 41–52% and the activity in ADX animals increased by 63%. The brain cholinephosphotransferase activity was only slightly affected. Adrenalectomy was reported to increase rat liver microsomal CDP choline:cholinephosphotransferase and phospholipid *N*-methyltransferase activities [20]. The base-exchange enzyme activities of brain and liver were essentially identical in all groups. The result on NADPH cytochrome *c* reductase activity in liver agreed with that of a previous study [21].

4. DISCUSSION

One of the possible explanations for the reduction of PL-D activity of the DEX-treated animals is the induction of a phospholipase inhibitory protein such as a lipocortin. Lung lipocortin was partially purified and fractions which were capable of inhibiting snake venom PLA_2 were tested with both microsomal bound as well as solubilized and partially purified PLD (table 2). A 78–90% reduction of the microsomal enzyme activity and a 65% reduction of the partially purified enzyme by lipocortin were observed.

The reduction of PL-D activity induced by dexamethasone injection might be due to the reduction of the quantity of this enzyme. The other explanation for the reduction of the activity is the induction of phospholipase inhibitory proteins such as lipocortins which inhibit PL-D.

Since PL-D may play an important role in cell-activation processes by many agents, the change in PL-D activity may contribute to the modulation of these processes. This alteration of PL-D activity conceivably could also affect the level of diacylglycerol, a potent activator of protein kinase C. We have previously shown that endogenous phosphatidylcholine can yield diglyceride upon activation of PL-D [22].

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