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INHIBITION OF ORNITHINE DECARBOXYLASE AND GLUTAMIC ACID DECARBOXYLASE ACTIVITIES BY PHOSPHORYLETHANOLAMINE AND PHOSPHORYLCHOLINE

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SUMMARY: Ornithine decarboxylase, which catalyzes the first step in polyamine biosynthesis, is rapidly and transiently increased in various tissues during growth and after various hormonal or noxious stimuli, prior to an elevation in choline kinase activity. Polyamines themselves have been demonstrated to activate choline kinase. The present study sought to determine the effect of phosphorylcholine, the product of the reaction catalyzed by choline kinase, on ornithine decarboxylase activity. The data demonstrate that ornithine decarboxylase activity is inhibited by phosphorylcholine and more potently by the related compound phosphorylethanolamine. The inhibition by both compounds led to decreased affinity of partially purified ornithine decarboxylase for ornithine. The inhibition is not time dependent and reversible. Both compounds also inhibit glutamic acid decarboxylase activity. The results suggest that high intracellular levels of phosphorylethanolamine and phosphorylcholine can serve as natural inhibitors of decarboxylases.

The enzyme ornithine decarboxylase (EC 4.1.1.17; ODC) catalyzes the first step in the biosynthesis of polyamines which are involved in the control of cell growth and in the response to hormonal and toxic stimuli (1,2). ODC activity is well known to undergo very large, rapid and transient increases during growth and in response to different environmental stimuli (2). One of the unique features of ODC is its short half-life, *in vivo*, which ranges from 8-30 min (3), the shortest half-life yet reported in eukaryotes. To account for such rapid changes in activity several post-translational regulatory mechanisms have been proposed, among them are the antizyme protein, phosphorylation and transamidation (2).

Abbreviations: ODC, ornithine decarboxylase; GAD, glutamic acid decarboxylase; CK, choline kinase; PCh, phosphorylcholine; PEth, phosphorylethanolamine; PLP, pyridoxal-5'-phosphate.

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In several tissues choline kinase activity (EC 2.7.1.32; CK), which catalyzes the conversion of choline to phosphorylcholine (PCh) in the first step of phosphatidylcholine biosynthesis (4,5), is elevated after the initial surge in polyamine biosynthesis (6-9). Furthermore, polyamines have been observed to activate CK (6,7). The present study therefore sought to determine if PCh, in turn, exerts any effect on ODC activity. The data demonstrate that PCh and the related compound phosphorylethanolamine (PEth) exert an inhibitory effect on CDC activity in various tissues. Furthermore, these compounds exert similar inhibitory effect on glutamic acid decarboxylase (EC 4.1.1.5; GAD) activity.

METHODS: ODC activity was measured in the following tissues of Sprague-Dawley rats: 7d old cerebellum (10); adult (60d old) superior cervical ganglion 8h after postganglionic nerve crush (11), and in adult liver 20h after thioacetamide injection [150 mg/Kg in 0.9% (wt/vol) NaCl, intraperitoneally](12). In adult (60d old) ICR mice ODC activity was measured in the kidneys 13h after testosterone injection (100 mg/Kg in peanut-oil subcutaneously) (13). GAD activity was measured in adult rat cerebellum (9).

Tissues were removed, frozen on dry ice and stored for subsequent biochemical assays. For enzyme assays tissues were weighed and homogenized in 10 vol of ice cold 5mM Tris-HCl buffer (pH 7.2) containing 60μM pyridoxal-5'-phosphate (PLP) with an all-glass homogenizer. The homogenates were centrifuged at 10,000xg for 15 min and the supernatant decanted for assays. ODC and GAD activities were assayed as described before (10, 14). The concentration of proteins was determined by the method of Lowry et al. (15).

Ornithine decarboxylase was partially purified, according to the method described by Seely et al. (16). Kidneys from testosterone injected mice were homogenized in 3 vol of ice cold 25mM Tris-HCl buffer (pH 7.4) containing 2.5mM dithiothreitol and 0.1mM EDTA. The homogenate was centrifuged at 45,000xg for 75 min, at 4°C. Fractionation of the supernatant with ammonium sulfate was carried at 0-4°C as following: 16.5g of solid ammonium sulfate was added to 100ml of supernatant with stirring, following centrifugation at 10,000xg for 15 min additional ammonium sulfate (13.5g/100 ml) was added to the supernatant. After centrifugation the resulting precipitate was dissolved in minimal volume of the homogenizing buffer and dialyzed against 100 volumes of the same buffer containing 60 μM PLP. The dialyzed preparation was centrifuged and aliquots of the supernatant were frozen for subsequent ODC assays. The specific activity of this preparation was 6.6nmol CO_2/mg protein/min, about 2-fold purification of the original homogenate.

RESULTS AND DISCUSSION: PCh and PEth inhibited partially purified ODC in a dose dependent manner (Fig. 1A). The inhibition by PEth was more potent than that of PCh. The inhibition of ODC by PCh and by PEth was not time dependent (Fig. 1B). Dialysis for 4h of enzyme preparations which

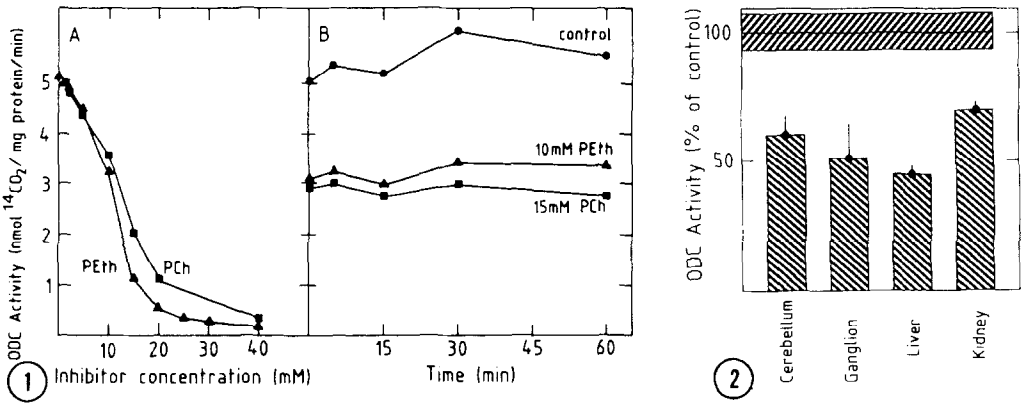

Fig. 1. A. Effect of varying concentrations of phosphorylcholine (PCh) and phosphorylethanolamine (PEth) on ornithine decarboxylase (ODC) activity. B. Effect of preincubation time with 15mM PCh or 10mM PEth on ODC activity. Preincubations were conducted for varying time intervals and the assay started by the addition of ornithine. Enzyme assays were conducted in the presence of 0.25mM ornithine for 30 min at 37°C.

Fig. 2. Effect of 15mM phosphorylcholine (PCh) on ornithine decarboxylase activity (ODC) in homogenates from 7d old rat cerebellum (cerebellum), rat superior cervical ganglion 8h after postganglionic nerve crush (ganglion), rat liver 20h after thioacetamide injection (liver) and mouse kidney 13h after testosterone injection (kidney). Results, mean (±SEM) of 5 animals, are expressed as percent of ODC activity measured in the absence of PCh (horizontal shaded area). Control values, nmol $^{14}CO_2$/mg protein/min were: cerebellum, 0.0270±0.0020; ganglion, 0.0070±0.0005; liver, 0.0310±0.0015, and kidney, 3.2000±0.2000. Reduction in ODC activity in all tissues was statistically significant ($p<0.001$).

were preincubated for 1h with 20mM of either PCh or PEth eliminated the inhibitory effect of these compounds completely (results are not shown).

Phosphorylcholine (15mM) inhibited ODC activity to a different degree in homogenates of various rat tissues and mouse kidney (Fig. 2). In 7d old rat cerebellum 40% inhibition was observed; 50% inhibition in rat superior cervical ganglion 8h after postganglionic nerve crush; 45% inhibition in rat liver 20h after thioacetamide injection, and 30% inhibition in mouse kidney 13h after testosterone injection.

In Fig. 3, the dependency of partially purified ODC activity upon ornithine concentration, was determined in the presence of 15mM PCh or 10mM PEth by the double reciprocal plot method. Both compounds increased Km values, PCh by 3.7-fold and PEth by about 5.0-fold (Table I). The effects of PCh, PEth, choline, hydroxylamine, O-methylamine and sodium phosphate buffer (pH 7.4) on the apparent Km and Vmax values of ODC

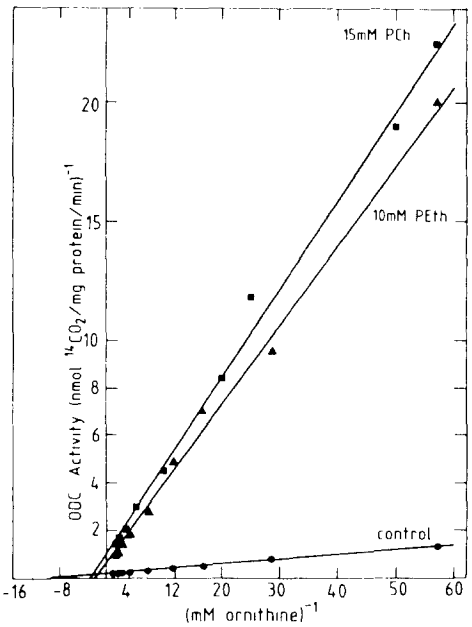

Fig. 3. Double reciprocal plots of ornithine decarboxylase (ODC) activity as a function of ornithine concentration in the presence of 15mM phosphorylcholine (PCh), or 10mM phosphorylethanolamine (PEth). Incubations were carried out for 10 min. Km and Vmax values are given in Table I.

activity as a function of ornithine concentrations, are summarized in Table I.

The present study demonstrates that PCh, the reaction product of CK, is a weak inhibitor and the related compound PEth is a potent inhibitor of ODC. Whether the biosynthesis of PCh and PEth are catalyzed by a single or separate kinase enzymes is controversial (7, 17-19). The activity of CK has been demonstrated to increase in several tissues during development and after toxin or hormonal stimuli, following the initial increase of ODC activity and polyamine biosynthesis (6-9). The demonstration that polyamines activate CK together with our findings that PCh and PEth inhibit ODC activity, suggest a reciprocal relationship between these two biochemical pathways, whereby the first reaction products (polyamines) to be increased, activate a second reaction (CK activation) which, through its reaction products (PCh and PEth), then inhibits the former reaction (ODC inhibition). The operation of such a feedback con-

TABLE I

Compound	Km (mM)	Vmax (nmol/mg protein/min)
Control	0.104	5.9
PCh	0.385	1.1
PEth	0.526	1.7
Choline	0.124	5.7
Ethanolamine	0.098	5.9
Na-PO_4	0.115	5.4
NH_2OH	0.515	1.5
NH_2OCH_3	0.125	5.5

Kinetic constants for partially purified ornithine decarboxylase (ODC) activity as a function of ornithine concentration in the presence of 15mM phosphorylcholine (PCh), 10mM phosphorylethanolamine (PEth), 20mM choline, 15mM ethanolamine, 0.25mM hydroxylamine (NH_2OH), 2mM O-methylamine (NH_2OCH_3) or 50mM sodium phosphate (Na-PO_4) buffer (pH 7.4).

Results are the mean values of two experiments ran in triplicates and calculated by linear regression analyses of double reciprocal plots.

trol mechanism is conceivable during development or during metabolic alterations in the mature state. Although the regulation of ODC activity by synthesis and degradation of enzyme molecules is a major regulatory mechanism (1), several post-translational inhibitory mechanisms (2) may have important roles in controlling the extremely short half-life of this enzyme (2,3). The inhibition by PCh and PEth described in the present study, may be involved in such post-translational control of ODC activity. The tissue levels of PCh and PEth are high. In the adult brain for example, the levels of PCh are 0.38 and of PEth are 0.94-1.8 umol/g wet wt (20). It is therefore reasonable to assume that the concentrations used presently for the in vitro studies may exist in vivo as well, especially in situation when CK activity is enhanced (21).

The mechanism by which PCh and PEth inhibit ODC activity is unclear. However, the observations that PEth is a more potent inhibitor than PCh and that hydroxylamine, a PLP scavenger (22), is an extremely potent inhibitor while methylamine, choline or ethanolamine are devoided of inhibitory effects, suggest that the inhibition may occur through competition with the binding of the substrate ornithine to the coenzyme PLP (22). The fact that these compounds inhibit GAD activity with similar

TABLE II

Compound	GAD activity	% inhibition
Control	1.60	
PCh	1.10	31.5
PEth	0.53	66.0
NH_2OH	0.33	79.4

Effect of 15mM phosphorylcholine (PCh) 10mM phosphorylethanolamine (PEth) and 0.5mM hydroxylamine (NH_2OH) on glutamic acid decarboxylase (GAD) activity (nmol/mg protein/min) in cerebellar homogenates.

Results are the mean values of three experiments ran in duplicates.

potency (Table II) corroborates this suggestion. Moreover these findings suggest that PCh and PEth can serve as natural inhibitors of decarboxylases in general.

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