ORNITHINE DECARBOXYLASE ACTIVITY IN TISSUES OF PROLACTIN-TREATED RATS

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Received January 17,1975

Summary: Treatment with prolactin caused increases of 3-100 fold in ornithine decarboxylase (EC 4.1.1.17) activity of kidney, adrenal gland and liver of female rats. The magnitude of the effect varied with age. In young male rats, activity of the enzyme was also stimulated markedly in spleen, as well as those tissues mentioned above, and there were smaller effects in thymus and heart.

INTRODUCTION: Several lines of evidence suggest that prolactin may be involved in regulating the activity of numerous tissues in addition to mammary gland. Physiological responses have been reported for cardiovascular tissue, aorta and kidney (1,2,3,4). Changes in serum prolactin levels in response to exercise and stress (5), to insulin-induced hypoglycemia (6,7), and to variation in serum osmolality (8) suggest a wider role for prolactin than its function in initiation and maintenance of lactation. Furthermore, it is generally accepted that peptide hormones exert their effects after becoming bound to receptors in the plasma membranes of their target cells. Studies with prolactin have shown that membrane fractions from several organs, including mammary glands, possess specific prolactin-binding sites, and might therefore be classed as target tissues (9,10,11,12). However the relationship of this binding to the biological function of the hormone is uncertain. Metabolic responses have not been correlated with the binding studies, and a direct stimulatory effect of prolactin on RNA synthesis by nuclei isolated from mammary gland has been reported (13). This effect was not observed with nuclei isolated from liver and kidney, yet these tissues

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have an affinity for prolactin equal to that of mammary tissue in some species (9,10,11,12).

One of the immediate aims of this investigation was to determine the tissues of rats which show a metabolic response to prolactin administration. The activity of the enzyme, ornithine decarboxylase, was chosen as an indicator of hormonal stimulation. Large increases in the activity of this enzyme have been observed in many mammalian tissues stimulated by hormonal or nutritional factors. Hormonally-induced increases have been reported in liver of growth hormone, insulin and glucocorticoid-treated animals, in ACTH and growth hormone-stimulated adrenal, in estrogen-stimulated uterus, and in ovary and testes of gonadotrophin-treated animals. Many of these studies have been reviewed and summarized recently (14,15). An effect of prolactin on the enzyme in brain and liver of young rats has also been reported (16) The present investigation demonstrates that ornithine decarboxylase activity of other tissues is also affected by prolactin treatment.

MATERIALS AND METHODS; Materials - L-ornithine, pyridoxal phosphate and dithiothreitol were purchased from the Sigma Chemical Co. Ovine prolactin was a gift from NIH. (DL-1-^{14}C) ornithine-HCl, 58 mCi/mm was obtained from Amersham Searle Corp.

Tissue preparation: Tissues from Hooded rats, of age and sex as indicated, were used. Animals were killed by cervical dislocation, and organs from each group were weighed and homogenized in 5 volumes of the following buffer - 50 mM Tris-HCl, 5 mM dithiothreitol, 0.1 mM EDTA, pH 7.3, using a Servall tissue disintegrator and motor driven glass-Teflon homogenizer. The adrenals were pooled and homogenized in 1 ml of buffer in a glass hand homogenizer. Homogenates were centrifuged at 10,000 x g for 15 minutes at 0°, and the supernatant fractions used for assay.

Ornithine decarboxylase assay: Aliquots of tissue preparation containing 0.2-6 mg protein were incubated in 25 ml flasks essentially as described by Jänne and Williams-Ashman (17). The flasks contained, in a final volume of 1 ml, 30 nmoles of L-ornithine, including 0.5 μCi of (DL-1-^{14}C) ornithine, and pyridoxal phosphate, 0.2 mM. Incubations were begun by addition of substrate. The flasks were closed with rubber stoppers, each holding a plastic well (Kontes Glass) containing 0.2 ml hyamine. After 30 minutes at 37°, 1 ml of 40% Trichloroacetic acid was injected to stop the reaction and release radioactive CO_2, which was collected during a further incubation of 60 mins with shaking. The centre wells and their contents were placed in vials with 10 ml of a PPO-POPOP-toluene scintillation cocktail, and radioactivity was measured in a Nuclear-Chicago Isocap 300 counter. Each tissue was assayed at two protein concentrations. Each experiment included controls in which acid was added before substrate. At this substrate concentration, radioactive CO_2 production was linear for 50-60 minutes with protein concentrations up to 7 mg.

RESULTS: Table 1 shows the effect of prolactin treatment on the activity of the ornithine decarboxylase in several tissues of young male rats. The very marked increase in the activity in kidney, adrenal, liver and spleen has been observed in many experiments with animals of this age. Quantitatively smaller, but definite, effects were found in thymus and heart. Our findings with liver agree with those of other workers, but this is the first report of the response of this enzyme to prolactin in the other tissues. The results in table 2 show the activity of the enzyme in the tissues of female rats of different ages, and the effects of prolactin on the activity. It is apparent that the

TABLE 1. Effect of prolactin on ornithine decarboxylase activity in tissues of male rats.

	Ornithine decarboxylase activity	
Tissue	Control animals	Prolactin-treated animals
Liver	32.4 ± 3.5	253.4 ± 12.8
Kidney	18.2 ± 4.4	859.6 ± 25.9
Adrenal	6.0 ± 2.1	378.1 ± 15.4
Spleen	7.8 ± 1.1	41.2 ± 4.1
Thymus	39.2 ± 3.1	100.6 ± 7.7
Heart	18.4 ± 3.6	49.6 ± 3.2
Lung	36.8 ± 4.8	38.4 ± 5.2
Perirenal fat	265.5 ± 40.9	208.2 ± 38.8
Brown fat	100.8 ± 20.2	95.6 ± 18.5

Animals were given ip injection of 3 mg of prolactin in saline, or an equivalent volume of saline, 5 hours before sacrifice. The animals in this series of experiments were males, age 36-37 days, and each group consisted of 3-4 rats in each experiment. Tissue preparations were assayed at 2 protein concentrations. Results are expressed as pmoles CO_2/mg protein/30 minutes, ± S.E.M.

levels of activity vary in control animals of different ages, with the highest values occurring in unweaned females. Except for the 31-day old group, the increases in enzyme activity in prolactin-treated animals were less than those found in males, and significant stimulation of the enzyme in spleen has not been observed in females, with the possible exception of the suckling animals. No change in decarboxylase activity has been observed in adipose tissue or thymus of female rats.

The data in table 3 show that a dose of prolactin as small

TABLE 2. Effect of prolactin on ornithine decarboxylase activity in tissues of female rats.

	Specific activity of ornithine decarboxylase					
	Liver		Kidney		Adrenal	
Age of animals	C	P	C	P	C	P
26-28 days- non-weaned	66.2 ± 6.6	144.7 ± 9.1	48.0 ± 6.6	1003.6 ± 40.8	78.3 ± 13.4	245.6 ± 18.8
31-33 days	24.9 ± 4.8	202.6 ± 15.2	29.2 ± 5.5	1090.6 ± 48.6	4.6 ± 2.1	408.5 ± 20.1
42-44 days	22.5 ± 4.1	69.8 ± 5.1	20.1 ± 3.2	149.5 ± 14.1	13.2 ± 2.1	57.6 ± 4.4
2 years	26.3 ± 3.9	75.4 ± 6.2	29.8 ± 4.1	105.8 ± 13.1	16.9 ± 2.2	156.6 ± 18.5

Animals were injected 5 hours before sacrifice. Control animals (C) received saline, while treated animals (P) received 3 mg prolactin/50 g body weight, in saline. Each group in each experiment consisted of 3 animals. Activity of the enzyme is expressed as pmoles CO_2/mg protein/30 mins ± S.E.M.

TABLE 3. Dose response relationship of ornithine decarboxylase activity to prolactin treatment

	Activity of ornithine decarboxylase Dosage of prolactin-mg/50 g body weight			
Tissue	0	0.5	1.5	3.0
Liver	26.5 ± 4.2	125.1 ± 14.8	125.8 ± 20.1	220.6 ± 18.8
Kidney	27.1 ± 6.1	477.9 ± 21.1	742.5 ± 28.8	1254.3 ± 51.1
Adrenal	6.1 ± 1.5	135.6 ± 12.2	326.7 ± 16.6	427.5 ± 20.2

Animals in this series of experiments were 32-33 days of age. Prolactin or saline was administered by intraperitoneal injection 5 hours before sacrifice. The activity of the enzyme is expressed as pmoles CO_2/mg protein/30 minutes ± S.E.M.

as 0.5 mg produced a significant effect on the activity of the enzyme in the 3 tissues studied, with kidney and adrenal showing the most dramatic responses. Increasing effects were obtained with larger doses. The results of abbreviated experiments investigating the time course of stimulation (table 4) show that the effect is evident by 2.5 hours after treatment. The pattern of response differed in the 3 tissues. Maximal stimulation seemed to occur before 5 hours in liver, and activity in the adrenals was still increasing at 7.5 hours. The activity in kidney seemed to have plateaued by 2.5 hours.

DISCUSSION: The data presented clearly show that ornithine decarboxylase activity increased in several tissues of rats treated with prolactin, and, in this regard, prolactin resembles other hormones in their stimulatory effects on tissues. These studies do not show that prolactin itself acts directly on the various tissues. However, administration of the hormone elicits a metabolic response in several tissues, and these results provide sup-

TABLE 4. Time course of stimulation of ornithine decarboxylase in female rat tissues

	Activity of ornithine decarboxylase			
	Time after prolactin administration-hours			
Tissue	0	2.5	5.0	7.5
Liver	23.4 ± 3.6	103.8 ± 18.1	71.6 ± 11.1	22.1 ± 3.1
Kidney	20.8 ± 2.5	214.5 ± 10.6	153.3 ± 14.1	186.3 ± 8.3
Adrenal	12.6 ± 3.1	30.1 ± 2.5	60.6 ± 4.8	82.6 ± 6.1

Animals were 42 day old female rats, and received prolactin or saline by ip injection. The animals were killed between 11 a.m. and 2 p.m. to eliminate changes due to diurnal variation. Animals received 3 mg prolactin/50 g body weight. Activity of the enzyme is expressed as pmoles CO_2/mg protein/30 minutes ± S.E.M.

port for the hypothesis, previously based only on the hormone-binding studies, that prolactin may have several target tissues. The retention of sensitivity by some tissues in adult females and young males suggests too that prolactin may be involved in physiological regulation of some tissues beyond the stage of early growth and development. The lack of effect of prolactin on the enzyme activity in adipose tissue is difficult to reconcile with the recently noted effect of the hormone on lipoprotein lipase of mammary and adipose tissue (18). However, it is possible that the hormone may function by different mechanisms in different tissues, which would also explain the disparity of the effects on isolated nuclei mentioned previously (13).

ACKNOWLEDGEMENTS: This work was supported by the British Columbia Medical Research Foundation.

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