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Stimulation of Ornithine Decarboxylase by Relaxin

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Summary: Relaxin, a protein hormone of pregnancy, stimulated ornithine decarboxylase activity (EC 4.1.1.17) in two of its target tissues. Both the mouse pubic symphysis and uterus respond to a single injection of relaxin; within 2-4 hours after hormonal treatment of the mice, ornithine decarboxylase activity was observed to increase 2-8 fold over control levels. This increase in enzymatic activity may represent one step in the mechanism by which relaxin exerts its effects.

INTRODUCTION

Relaxin, a protein hormone, has a profound effect on the connective tissues of the pubic symphysis and the uterus of various species including rodents (1-4). A single injection of relaxin given to non-pregnant steroid-primed animals brings about radical changes in the structure of the pubic symphysis (3,5). Increases in the dilatibility of the uterine cervix (3,6,7) due to relaxin exposure have also been observed. Recently it has been demonstrated that relaxin causes rapid increases in cAMP levels in the mouse pubic symphysis (8).

Studies on cells in culture, and an *in vivo* study on rat liver, suggest that there is a relationship between increases in cellular cAMP levels and ornithine decarboxylase (EC 4.1.1.17) induction (9-12). When cultured cells are treated with cAMP, cAMP derivatives or compounds that increase cellular cAMP levels, an induction of ornithine decarboxylase occurs (9,11). Based on these studies, and the knowledge that both steroidal and protein hormones induce ornithine decarboxylase activity in their respective target tissues (13), it seemed possible that an increase in ornithine decarboxylase activity

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is an intermediate step in the mechanism of relaxin action.

The occurrence of ornithine decarboxylase in connective tissue was demonstrated recently by Conroy et al. (14). They found that the levels of enzymic activities of pelvic cartilage from chick embryo and from costal cartilage from young rat and rabbit and human fetus were of the same order as those detected in other body tissues. Cultured fibroblasts derived from human skin and chick embryo contain measurable levels of ornithine decarboxylase (15,16). Treatment of the chick fibroblasts with insulin, serum or non-suppressible insulin-like activity (NSILA) stimulated ornithine decarboxylase activity.

The data in this paper show that the ornithine decarboxylase preparations obtained from targets of relaxin action, the pubic symphysis and the uterus, increases in activity in response to the hormone. This suggests that the enzyme may be involved in the structural changes induced by relaxin.

MATERIALS AND METHODS

Animals. Virgin female ICR mice obtained from Charles River Breeding Laboratories (Wilmington, MA), were housed in air-conditioned quarters that were illuminated between 0700 and 1900 hours. Three groups of mice were utilized: immature mice (16-20 g), intact mature mice (25 g) and ovariectomized mature mice. Where appropriate the animals were injected subcutaneously (sc) with 5 µg of estradiol benzoate (E_2B) in 0.1 ml sesame oil three days prior to treatment with either 0.1 ml of 1% benzopurpurin 4B (BP) (sc) or 3 µg of purified relaxin in BP (sc). When required, ovariectomies were performed through a small dorsal incision under Avertin anesthesia [2.5% solution of tribromoethanol/t-amyl alcohol (1 g/ml) in warm water] no sooner than three days after arrival from the supplier. The animals were then rested one week before experimentation.

Reagents. Relaxin was obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases as an ovarian acid-acetone extract. This relatively impure preparation was further purified by Dr. C. Schwabe of this department utilizing ion exchange chromatography on DEAE-cellulose followed by exclusion chromatography on Sephadex G-50 and G-75 Superfine in the presence of 6 M guanidine-HCl (17-19). All experiments described herein made use of relaxin obtained by this purification scheme (ca. 2500 units/mg). Benzopurpurin 4B was obtained from Eastman Organic Chemicals (Rochester, NY), DL-[1-^{14}C]ornithine (55 mCi/mmole) and Protosol from New England Nuclear Corporation (Boston, MA), dithiothreitol, L-ornithine and pyridoxal phosphate from Sigma Chemical Company (St. Louis, MO). All other chemicals (reagent quality) were used without further purification.

Assay of Ornithine Decarboxylase. Ornithine decarboxylase activity was assayed by a modification of the method of Russell and Snyder (20). At selected times after relaxin administration the mice were quickly killed and either the pubic symphysis (3-4 mm wide sections centered on the ligament) or the uteri (immediately above the vagina) removed. Following dissection, each

Table 1. ORNITHINE DECARBOXYLASE ACTIVITY IN THE PUBIC SYMPHYSES OF MICE

Test Group		pmoles CO_2 released / h / 5 pubic symphyses		
		Hours After Treatment		
		2	4	6
Immature Mice:	Control	23	27	27
	Relaxin	36	47	51
Estrogen Primed Immature Mice:	Control	14	19	-
	Relaxin	18	52	-
Intact Mature Mice:	Control	-	25	-
	Relaxin	-	72	-
Estrogen Primed Intact Mature Mice:	Control	-	41	-
	Relaxin	-	86	-
Ovariectomized Mature Mice:	Control	-	23	-
	Relaxin	-	160	-

The mice were treated as described in Materials and Methods. The assay for each treatment group was performed on the supernatant obtained from 5-pooled pubic symphyses and was done in triplicate. Estrogen primed mice were given 5 µg of estradiol benzoate in 0.1 ml sesame oil 3 days prior to treatment. The treatments were:

Control, 0.1 ml of 1% Benzopurpurin 4B.
Relaxin, 3 µg of relaxin in 0.1 ml of 1% Benzopurpurin 4B.

sample was placed in ice cold phosphate buffer (0.7 ml/pool of 5 symphyses and 1.5 ml/uterus), and immediately homogenized at 0-4°C for 10 seconds with a Polytron PCU2 (Brinkman Instruments) at a setting of 7. The buffer was 1 mM in DTT, 1 mM in EDTA, 50 µM in pyridoxal phosphate, 67 mM in Na_2HPO_4-KH_2PO_4, pH 7.2. The homogenates were centrifuged at 22,000 x g for 20 minutes at 4°C. The supernatants were then assayed for ornithine decarboxylase activity. Each assay tube (16 x 100 mm disposable test tube) contained 200 µl of 22,000 x g supernatant, 5 µl of 2.4 mM pyridoxal phosphate in 67 mM sodium-potassium phosphate buffer, pH 7.2, and 20 µl of 62.4 dpm/pmole DL-[^{14}C] ornithine solution (0.105 mM L-ornithine, final concentration). The tubes were fitted with rubber stoppers supporting polyethylene center wells (Kontes Glass Co., Vineland, NJ) which contained 200 µl of Protosol. The tubes were incubated at 37°C for 30 minutes under gentle agitation. The reaction was stopped by the addition of 1 ml of 1 M citric acid and incubated with gentle agitation for an additional 30 minutes at 37°C to release bound CO_2 for trapping in the Protosol. The center wells were removed and placed in 10 ml of toluene plus fluors followed by thorough shaking. The amount of radioactive CO_2 released was determined and the ornithine decarboxylase activity was calculated as pmoles CO_2 released per hour.

Table 2. ORNITHINE DECARBOXYLASE ACTIVITY IN THE UTERI OF MICE

Test Group		pmoles CO_2 released / h uterus, Hours After Treatment 2	4	6
Estrogen Primed Immature Mice:	Control	76 ± 7	52 ± 3	46 ± 5
	Relaxin	119 ± 17	166 ± 15	90 ± 12
Ovariectomized Mature Mice:	Control	-	27 ± 3	27 ± 4
	Relaxin	-	110 ± 19	247 ± 44

The mice were treated as described in Material and Methods. The assay was performed in duplicate on individual whole uteri and the value given represents the mean of 4 mice ± the standard error of the mean. Estrogen primed mice were given 5 µg of estradiol benzoate in 0.1 ml sesame oil 3 days prior to treatment. The treatments were:

Control, 0.1 ml of 1% Benzopurpurin 4B.
Relaxin, 3 µg of relaxin in 0.1 ml of 1% Benzopurpurin 4B.

RESULTS

The Effect of Relaxin Treatment on Ornithine Decarboxylase Levels in the Pubic Symphyses. Increases in symphyseal ornithine decarboxylase activity were observed following relaxin administration to both immature and mature mice (Table 1). The largest increases over control values were observed at 4 and 6 hours. Control levels of the enzyme activity were fairly constant with an average value of 22 pmoles CO_2 released per 5 pubic symphyses per hour. Responses to relaxin occurred whether the mice were previously treated with estrogen or not.

Elevation of the Uterine Levels of Ornithine Decarboxylase Following Relaxin Treatment. Relaxin treatment also caused an increase in ornithine decarboxylase levels of the uterus in both immature and mature mice (Table 2). Stimulation was observed at 2,4 and 6 hours following relaxin treatment. The observed increase in uterine ornithine decarboxylase activity occurred

in ovariectomized mice, suggesting a lack of an absolute requirement for estrogen for the response.

DISCUSSION

Relaxin stimulates ornithine decarboxylase activity in two of its target tissues. Increases of this enzymatic activity in the mouse pubic symphysis are consistently observed at 4 hours and remain elevated up to 6 hours after relaxin treatment. Stimulation of ornithine decarboxylase activity in the uterus is observed as early as 2 hours following relaxin treatment and is sustained for several hours thereafter. This temporal relationship is similar to that observed in other instances of hormonal stimulation of ornithine decarboxylase activity (13).

The results in this study provide additional information on the sequence of events which occur following a single injection of relaxin. Accordingly an early increase in the level of cAMP in the pubic symphysis (8) is followed by stimulation of ornithine decarboxylase activity in response to relaxin treatment. This is consistent with several studies (9-12) which suggest a relationship between increases in cellular cAMP levels and ornithine decarboxylase activity.

The biological response to a single injection of relaxin, widening of the pubic symphysis, does not occur unless the animals are primed with estrogen. The role for estrogen in this response has not yet been delineated. An early event which follows relaxin injection, elevation of symphyseal cAMP levels, does not require estrogen. The data in this paper (Table 1) suggest that estrogen priming is also not necessary for the increase in ornithine decarboxylase activity to occur but plays a role at a later stage in the sequence of events.

Increases in ornithine decarboxylase activity are noted in many hormonally stimulated tissues as well as those systems which involve rapid cell proliferation. A direct role in the control of RNA polymerase I was proposed for ornithine decarboxylase by Manen and Russell (21). They suggested that

RNA polymerase I activity is controlled through a modification of its structure by the binding of ornithine decarboxylase. Often associated with an observed ornithine decarboxylase increase is an increase in polyamine levels. Speculation as to the role of polyamines led to several hypotheses. One study suggested that spermidine is a controlling factor in DNA synthesis and cell division (22); another proposed that increased levels of polyamines stabilize polysomes (23). Collectively these and other studies provide evidence that either ornithine decarboxylase itself or the polyamine products of the metabolic pathway in which it functions may be involved in the processes of DNA, RNA and protein synthesis, and it therefore may act as an intermediary in eliciting a biological response to hormones in target tissues.

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