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INSULIN STIMULATION OF IODOTHYRONINE 5'-DEIODINASE IN RAT BROWN ADIPOCYTES

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SUMMARY: Insulin (100-3333 μU/ml) stimulates iodothyronine 5'-deiodinase 3 to 4 fold in dispersed rat brown adipocytes. Deiodinase activity increased steadily from 1 to 4 hours. Insulin increased enzyme activity via an increase in the Vmax while the Km remained unchanged. Omission of glucose from the medium did not affect the insulin response. Studies with α-amanitin suggested that the increase in deiodinase activity was not due to an increase in the rate of transcription. The insulin effect was not additive to that of α_1-catecholamines, suggesting the two stimulators might have one or more common elements.

Brown (but not white) adipose tissue contains Type II iodothyronine 5' monodeiodinase (5'D-II) which is stimulated *in vivo* by cold stress and by norepinephrine through an α_1 adrenergic mechanism (1). The activity of this enzyme, which provides about one half of the nuclear T3 to the brown fat cell (2), has recently been found to increase 5 to 10 fold 4 h after insulin injection (3). Insulin is required for both cold and diet-induced thermogenesis in brown adipose tissue and for the trophic response of this tissue to chronic cold exposure (4). In the present study, we have examined the effect of insulin on 5'D-II activity in an *in vitro* system in order to determine if insulin can increase deiodinase activity directly in these cells.

Abbreviation: 5'D-II, 6-n-propyl-2-thiouracil insensitive iodothyronine 5' deiodinase.

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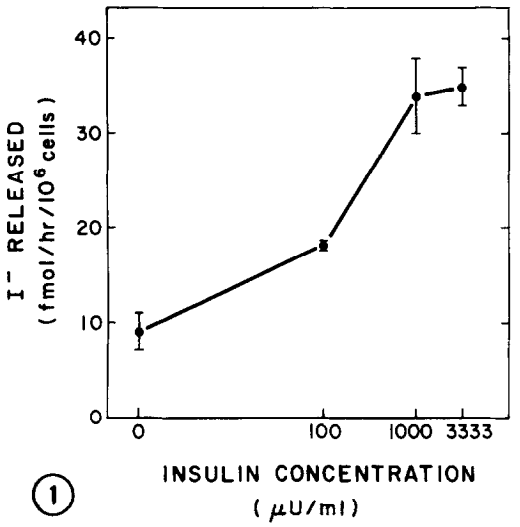
MATERIALS AND METHODS: Male Sprague-Dawley rats (Zivic Miller, Allison Park, PA) weighing between 150-200 g were used. Preparation of adipocytes was done according to Fain et al (5) with a 10 min filtration of digested material as described by Pettersson and Vallin (6). The digestion buffer consisted of minimum essential medium with 2 mM glutamine, 24 mM sodium bicarbonate (pH 7.4), 10 mM glucose, 10 mM fructose, 1 mM pyruvate, 4% albumin (Calbiochem, fatty acid poor) and 2 mg/ml collagenase (Type I, Cooper Diagnostics). Incubations were performed with the indicated agents for 3-4 h with approximately 400,000 cells/ml in the same buffer system but without collagenase. DTT (2 mM) was added 5 min prior to the conclusion of the incubation to improve recovery of active enzyme. Iodothyronine 5'-deiodinase activity was assayed as described previously (7) with the following modifications: The incubation buffer (200 μl) contained 5 mM Hepes (pH 7.5), 125 mM sucrose, 20 mM DTT, 1 nM 5' [^{125}I] T4 (200,000 cpm), 1 mM EDTA, 100 mM KH_2PO_4, 2 mM NaOH, 2 nM unlabeled T4 and 1 mM 6-n-propyl-2-thiouracil PTU, pH 7.0; after 1 h, 50 μl of a 1/1 mixture of human plasma and 10 mM PTU in dilute NaOH was added followed by 350 μl of 10% TCA. The tubes were centrifuged at 2,000 rpm for 10 min and 400 μl of the supernatant was passed over Dowex AG50W-X2 columns (8). The columns were washed with 2 ml of 10% acetic acid and the eluate was counted. Blank values (no protein added) were less than 0.5% of total tracer and less than 50% of basal 5'D-II activity. Results are expressed as fmol T4 deiodinated/h/10^6 cells. The assay was monitored periodically to ensure that $^{125}I^-$ released was equal to [^{125}I] T3 generated.

RESULTS: Insulin stimulated 5'D-II activity in a concentration dependent manner (Fig. 1). A two-fold stimulation was observed with 100 μU/ml, whereas 333 and 1000 μU/ml stimulated 5'D-II activity three and four fold, respectively. Maximal stimulation of 5'D-II activity was demonstrated at 1000 μU/ml insulin. The effect of insulin required several hours. After one to two hours exposure, the increase in 5'D-II activity by 3333 μU/ml insulin was only two fold, whereas three to four fold increases were present at four hours, the longest time studied. Concanavalin A also increased deiodinase activity with a 2 fold stimulation at 10 μg/ml and a 3 to 4 fold increase at 100 μg/ml (data not shown). Kinetic analysis (Figure 2) showed that the increase in 5'D-II activity occurred due to an increase in the Vmax (from 67 to 167 fmol/h/10^6 cells) with no change in the apparent Km for T4 (2.2 nM).

To evaluate the role of glucose transport in the insulin stimulation of 5'D-II, we studied this response in the presence



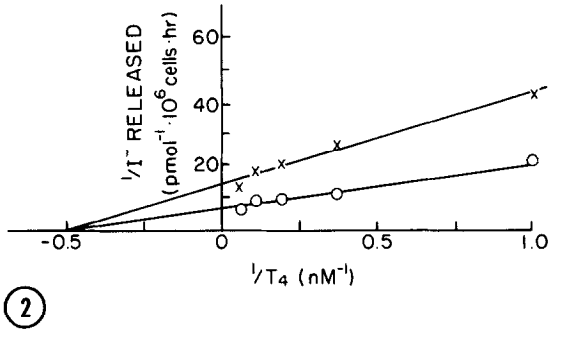


Figure 1. Effect of insulin of 5'D-II activity in rat brown adipocytes. Values shown are the Mean ± SE from 3 experiments performed in duplicate. Cells were exposed to insulin for 4 hours.

Figure 2. Double-reciprocal plot of the rate of 5'D-II activity as a function of T4 concentration in the presence (0) or absence (X) of 3333 μU/ml insulin for 4 hours. Values shown are the mean of duplicates from a representative experiment.

or absence of 10 mM glucose in Krebs Ringer bicarbonate buffer. As shown in Table 1, omission of glucose did not affect the stimulation by insulin. In addition, incubation in the presence of 10 mM 3-0-methylglucose had no effect on the insulin stimulation of 5'D-II (data not shown).

Since insulin stimulation of 5'D-II required several hours, we examined the effect of α-amanitin on this response (Table 2). In control cells, 1000 μU/ml insulin caused an 80% increase in

TABLE 1

Insulin stimulation of 5'D-II activity in rat brown adipocytes in the presence or absence of glucose in the incubation medium

Agent	5'D-II Activity (fmol/hr/10^6 cells)	
	-Glucose	+Glucose (10 mM)
Control	33 ± 9	39 ± 10
Insulin (3333 μU/ml)	107 ± 21	135 ± 32

Values shown are the Mean ± SE from 3 experiments performed in duplicate.

TABLE 2

Effect of α-amanitin on insulin stimulated 5'D-II activity in rat brown adipocytes

Agent	5'D-II Activity After 3 h Exposure (fmol/h/10^6 cells) Without	+α-Amanitin (10 μg/ml)	% of Control
Control	18 ± 3.3	9 ± 0.9	52 ± 5
Insulin (1000 μU/ml)	34 ± 9.0	15 ± 1.5	48 ± 9
INS/Control	1.8 ± 0.1	1.6 ± 0.1	

Values shown are the Mean ± SE from 3 experiments performed in duplicate.

5'D-II activity. Incubation with 10 μg/ml α-amanitin for 3 hours caused a 48% decrease in basal enzyme activity, but insulin still resulted in a 60% stimulation over this value, which is not different from the relative stimulation in the absence of α-amanitin. Thus the stimulatory effect of insulin was not altered by α-amanitin. Incubation with 10 μg/ml cycloheximide causes a rapid loss of enzyme activity (t 1/2 ~ 30 min, ref. 7), precluding studies of its effect its effect on insulin stimulation.

We have previously observed that adrenergic agonists cause a 2 to 4 fold stimulation of 5'D-II in these cells through an α_1 pathway (7). The studies in Table 3 show that the effects of

TABLE 3

Insulin and alpha-1 adrenergic stimulation of 5'D-II activity in rat brown adipocytes

Agent	% of Control
Control	100
Insulin (3333 μU/ml)	252 ± 18
Norepinephrine (1.0 μM)	196 ± 52
Insulin (3333 μU/ml) + Norepinephrine (1.0 μM)	224 ± 47

Values shown are the Mean ± SE from 3 experiments performed in duplicate. Alprenolol (5.0 μM) was added to block β-adrenergic effects of norepinephrine.

maximum concentrations of an α_1 adrenergic agent and insulin are not additive.

DISCUSSION: The present studies demonstrate that insulin directly stimulates 5'D-II activity in dispersed brown adipocytes. This stimulation requires supraphysiological concentrations of insulin, is time dependent and also occurs during exposure to the insulinomimetic lectin, concanavalin A. In this regard, this insulin effect is similar to that on ornithine decarboxylase in perfused rat liver (9) and lipid synthesis in MCF-7 cells (10) which also requires several hours for a maximal response. The stimulation of 5'D-II activity by insulin does not appear to require new mRNA synthesis since basal and insulin stimulated 5'D-II activities were inhibited to a similar extent by α-amanitin. Insulin stimulation of lipogenesis in MCF-7 cells is also not inhibited by actinomycin D (11). In addition, stimulation of glucose transport does not appear to be involved, since omission of glucose from the incubation medium or the addition of 3-O-methylglucose did not alter the insulin stimulation of 5'D-II. This has also been shown for other insulin responses such as activation of adipocyte glycogen synthase (12), lipogenesis in MCF-7 human breast cancer cells (10,11)) and the antilipolytic effect of insulin in adipocytes (13).

The mechanism of insulin stimulation of 5'D-II remains to be established. These data suggest a non-nuclear event independent of glucose transport. The present results also show that the stimulation of 5'D-II by insulin and α_1 catecholamines are not additive. This could occur because the stimulation of 5'D-II by these agents has a common element which is maximally stimulated by either agent. Alternatively, the activation of the enzyme itself may be limiting under these conditions preventing full stimulation. With respect to the former, recent studies

have indicated that both phorbol esters and insulin can stimulate ribosomal S6 phosphorylation in a murine myocyte cell line (14). These two agents apparently act by different mechanisms, though insulin could activate protein kinase C to some extent in myocytes through increases in diacylglycerol (15). In white adipocytes, insulin does not cause depletion of cytosolic protein kinase C (16). More knowledge of the mechanism for 5'D-II activation is required to determine whether insulin and α_1 adrenergic agents act through the same pathway in the brown adipocyte.

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