Table 2. Effect of high dose (500 μg/kg) L-thyroxine on hepatic drug metabolism

	Cytochrome P-450	Lidocaine N-Deethylase	Lidocaine 3-Hydroxylase	N-Oxidase	Imipramine N-Demethylase	2-Hydroxylase
♂ Control (5)†	100 ± ‡	100 ± 21	100 ± 33	100 ± 22	100 ± 34	100 ± 24
♂ T4-Treated (5)	45 ± 18**§	63 ± 7*§	68 ± 22	84 ± 22	95 ± 22	97 ± 20
♀ Control (5)	100 ± 28	100 ± 24	100 ± 30	100 ± 21	100 ± 7	100 ± 5
♀ T4-Treated (5)	72 ± 23	75 ± 7	66 ± 16	170 ± 123	113 ± 28	105 ± 25

† No. of animals in each group.
‡ Values expressed as % of control. Control values are: ♂ 0.25 (N-deethylase), 0.06 (3OH), 0.11 (N-oxidase), 0.09 (N-demethylase), 0.08 (2OH) all nmole/min per mg protein. ♀:0.06 (N-deethylase), 0.06 (3OH), 0.04 (N-oxidase), 0.10 (N-demethylase), 0.08 (2OH) all nmole/min per mg protein.
§ Value significantly different from control values; * = $P < 0.05$, ** = $P < 0.01$.

[6] who only found sex-dependent effects at supraphysiological levels of L-thyroxine which could be due to the substrates used in that study not being appropriate to the problem under investigation due to the substrate-dependence of the effects.

Effect of thyrotoxic doses of L-*thyroxine.* The results are shown in Table 2. There are very few effects of hyperthyroidism and the effects are, with one exception, those that would be expected from the previous experiment: decrease in metabolism of lidocaine in both sexes and increase in imipramine N-oxidase activity in the female. The unexpected finding is the sharp decrease in cytochrome P-450 content seen in the male. This is the only effect which could possibly be explained in terms of a thyrotoxic action of excess L-thyroxine but in this case the sex-dependent effect of physiological doses of L-thyroxine is lost in the hypothyroid state.

In conclusion, these results substantiate the findings of Kato *et al.* [1] that the effects of the thyroid gland on drug metabolism are both sex- and substrate-dependent even at physiological levels of L-thyroxine and that, contrary to the results of Rumbaugh *et al.* [6], excess L-thyroxine leads to a disappearance of the sex-dependent effects with the substrates used in this study. The hypothesis that the sex-dependent effects of L-thyroxine are due to the induction of a hyperthyroid state can thus be discounted.

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α_2-Adrenergic inhibition of lipolysis and respiration in rat brown adipocytes

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Brown adipose tissue is a highly specialized organ with two known functions: (1) to produce regulative heat in order to keep body temperature constant in a cold environment (non-shivering thermogenesis, NST); (2) the combustion of excess calories in cases of prolonged intake of an unbalanced diet, i.e. rich in carbohydrate relative to protein (diet-induced thermogenesis, DIT) [1–4]. The major product under these two conditions is heat derived from a high rate of uncoupled mitochondrial respiration. The mechanisms by which the brown adipocytes are triggered for oxidation of fatty acids during NST and during DIT seem to be identical. Norepinephrine (NE) which is released from the dense sympathetic nerve supply binds to the adrenergic receptors of the adipocytes [5]. Lipolysis is then initiated through the 'cascade effect' of cyclic AMP production, activation of protein kinase and hormone-sensitive lipase. The fatty acids (or their derivatives) uncouple the mitochondria and are oxidized at a high rate within these organelles [3, 6, 7–9].

The brown adipocytes possess different kinds of adre-

nergic receptors; β-receptors have been regarded as the sole receptor type responsible for the increase in oxygen consumption due to NE stimulation and hence the only adrenergic receptors of importance in bringing about brown fat thermogenesis [6, 10]. However, in hamster brown adipocytes, α_1-adrenergic receptors have recently been shown to be present and of significant importance. About 20% of the maximal NE-evoked oxygen consumption is a consequence of α_1-stimulation [11, 12]. Another effect of α_1-catecholamine action on brown adipocytes from rats is an increased turnover of phosphatidylinositol [13].

A third kind of adrenergic receptors, the α_2, are present in human and hamster white fat cells and inhibit adenylate cyclase, thereby reducing the accumulation of cyclic AMP and the degree of lipolysis, but have not been detected in rat white adipocytes [9, 14–17]. However, Itaya [18] reported an inhibitory effect of α-catecholamines on lipolysis in slices of rat brown fat.

The brown adipocytes also have the capacity to undergo adaptational changes during NST and DIT. These trophic responses have been shown to be at least partly dependent on β-adrenergic stimulation [4, 19]. In the present investigation, inhibitory effects on cyclic AMP accumulation, lipolysis and respiration were seen with α_2-adrenergic stimulation of rat brown adipocytes. Additionally, the diterpene forskolin stimulated cyclic AMP accumulation and lipolysis as previously reported valid for human and rat white adipocytes [16, 20]. It should be noted that the mode of action of forskolin differs from true adrenergic agonists but that the events following accumulation of cyclic AMP seem to be similar.

Materials and methods

Female Sprague–Dawley rats weighing 200–250 g were used. The rats were kept at 20°, with food and water *ad libitum*. The light/dark cycle was 12/12. Brown adipocytes were isolated as originally described by Fain *et al.* [21]. Krebs–Ringer bicarbonate buffer with 4% bovine albumin and 10 mM glucose, pH 7.4, was used in all experiments throughout the study. A small sample of the cell preparation was diluted and the cells were counted in a Burker chamber. The isolated brown adipocytes were stored under an atmosphere of O_2–CO_2 (95:5) in a slowly shaking water bath at +37° for approximately 1 hr before use.

For the determination of cyclic AMP production, the isolated adipocytes (50–100,000) were incubated in 1 ml of buffer in a shaking water bath. A pilot study, where the cyclic AMP was measured at different time intervals, showed that maximal levels were reached after 15 min of incubation (not shown). Therefore, the reactions were stopped after 15 min of incubation by the addition of 100 μl of 2 N HCl, the samples were boiled for 1 min and then neutralized with 2 N NaOH. The level of cyclic AMP accumulated was determined using a modified protein kinase binding procedure [22] involving charcoal adsorption of the free cyclic AMP [23]. The release of free fatty acids and glycerol was determined using an identical experimental set-up as for cyclic AMP production (see above). The free fatty acids were extracted and the amount measured basically according to Dole and Meinertz [24]. Glycerol was measured fluorimetrically with an enzymatic assay using glycerokinase and α-glycerophosphate dehydrogenase [25]. Respiration of the isolated brown adipocytes was monitored using a Clark-type oxygen electrode. The volume was 3 ml and the cell concentration 100,000 cells/ml. The buffer was saturated with air and the oxygen content was taken to be 434 nmole O/ml at +37°. Statistically significant differences were evaluated using Student's *t*-test. Forskolin (7β-acetoxy-8,13-epoxy-1α,6β,9α-trihydroxy-labd-14-en-11-one) was obtained from Calbiochem. A stock solution of 10 mM with ethanol as solvent was further diluted with buffer to the desired concentration.

Results and discussion

The production of cyclic AMP during stimulation with forskolin of the brown adipocytes was increased in a concentration-dependent manner, reaching values more than 60 times higher (2250 pmole/10^6 cells, at 10 μM) than the basal value of 33 pmole/10^6 cells (Fig. 1). The response obtained with 10 μM isoproterenol was 2500 pmole/10^6 cells. However, if 10 μM forskolin was added together with 10 μM isoproterenol, cyclic AMP accumulation was 8500 pmole/10^6 cells. Qualitatively similar findings have been reported on rat and human white fat cells [16, 20].

Half-maximal stimulation of lipolysis in brown adipocytes was achieved with a forskolin concentration of less than 1 μM, which only caused a minor increase in cyclic AMP (Fig. 1). The respiratory response to forskolin was similar to the lipolytic response as shown in Fig. 1. We have also observed that a strict correlation exists between the degree of lipolysis and respiration (unpublished observations).

The stimulation of lipolysis due to 1 μM forskolin was inhibited by the α_2-catecholamine agonist clondine. Statistically significant inhibition required only 1 nM clonidine. (Fig. 2). The clonidine effect was reversed by the addition of the α_2-antagonist yohimbine (Fig. 2).

In experiments where glycerol release was measured, similar results were obtained: forskolin (1 μM)-induced glycerol release was significantly inhibited by 100 nM clonidine (66 ± 11%, $P \leq 0.01$, S.E., $n = 4$). Cyclic AMP accumulation in the presence of 1 μM forskolin was 174 ± 25 pmole/10^6 cells; the accumulation was inhibited with 100 nM clonidine (41 ± 9%). Burns *et al.* [16], who performed similar studies on human white fat cells, reported an α_2-mediated inhibition of forskolin stimulation on cyclic AMP accumulation and glycerol release.

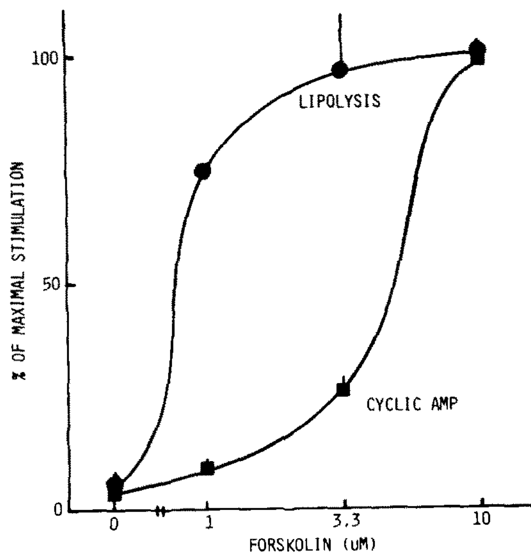

Fig. 1. Forskolin stimulation of cyclic AMP accumulation and free fatty acid release in isolated rat brown adipocytes over a 15 min incubation. Each point represents the percent of maximal stimulation; maximal stimulation was obtained with 10 μM forskolin. Cyclic AMP accumulation (squares) was 2250 pmole/10^6 cells in the presence of 10 μM forskolin and free fatty acid release (circles) was 1675 nmole/10^6 cells. The values are from 2–4 experiments with the vertical lines indicating the standard error.

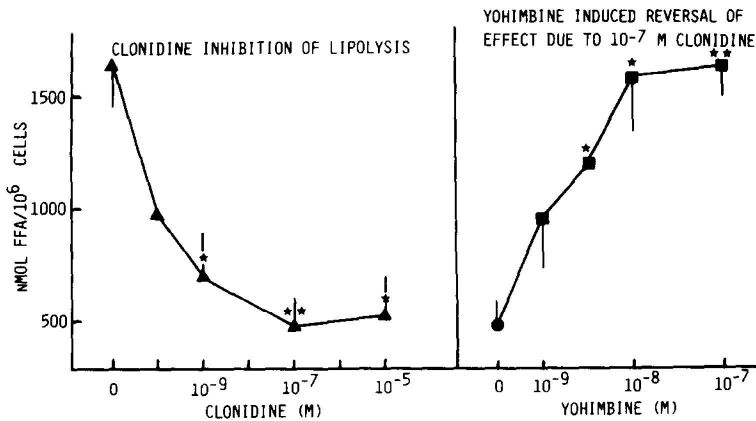

Fig. 2. Inhibition of forskolin (1 μM) stimulated lipolysis in rat brown adipocytes by clonidine (triangles, left panel) and yohimbine-induced reversal of lipolysis (squares, right panel) seen in the presence of 1 μM forskolin and 10^{-7} M clonidine. Lipolysis was measured as the release of free fatty acids over a 15 min incubation. The graph (left panel) starts at the level of lipolysis in the absence of clonidine. The x-axis is positioned at the level of basal lipolysis. Each point represents the mean from three separate experiments made in duplicate. The stars represent differences significant with a $P \leq 0.05$ (one star) or $P \leq 0.01$ (two stars) for forskolin vs clonidine or yohimbine vs forskolin + clonidine (100 nM).

In order to establish further the significance of the α_2-mediated metabolic effects on rat brown adipocytes, respiratory studies were undertaken. Clonidine at a concentration of 100 nM inhibited the increase in respiration due to isoproterenol or forskolin (Fig. 3). The similar magnitude of clonidine evoked inhibition of respiration with either isoproterenol or forskolin as stimulatory agent speaks in favour of the absence of any partial β-antagonistic effect of clonidine at this concentration (Fig. 3).

The present results are in contrast to a recent claim that there are no α_2-adrenergic effects on lipolysis or cyclic AMP accumulation in brown adipocytes from hamsters [26]. Curiously there are α_2-catecholamine effects on lipolysis and cyclic AMP accumulation in white adipocytes

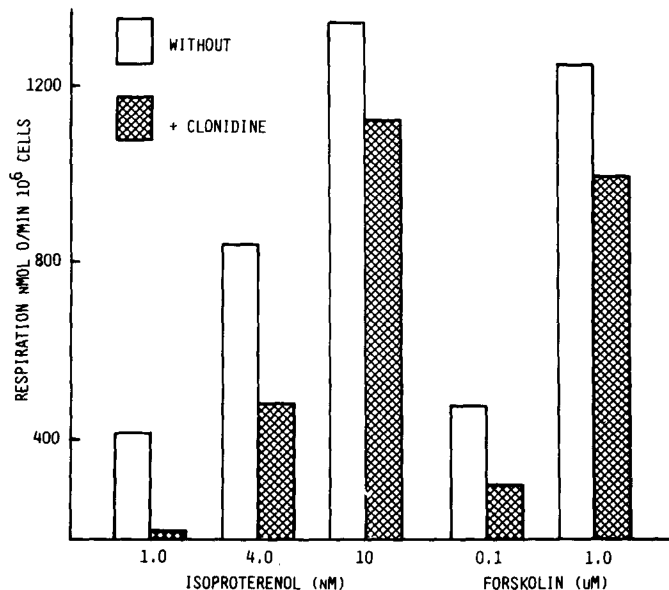

Fig. 3. Clonidine-induced inhibition of isoproterenol and forskolin-stimulated respiration in rat brown adipocytes. The open bars represent respiratory rates achieved with the indicated concentrations of isoproterenol and forskolin while the cross-hatched bars represent the respiratory rates in the presence of 100 nM clonidine. The graph starts at the level of basal respiration in the absence of added agents (170 nmole O min × 10^6 cells). The mean inhibition of respiration with clonidine (100 nM) of different concentrations of isoproterenol or forskolin was 238 ± 42 nmole O/min × 10^6 cells ($P \leq 0.01$, $n = 13$).

from hamsters but not those from rats [8, 17]. The present results demonstrate another difference between brown and white adipocytes from rats.

Summary. The main findings of this study were: forskolin stimulated, in a dose-dependent fashion, the accumulation of cyclic AMP, lipolysis and respiration as did isoproterenol. Clonidine (an α_2-agonist) inhibited forskolin-induced accumulation of cyclic AMP and lipolysis. The clonidine-induced inhibition of lipolysis was overcome by yohimbine (an α_2-adrenergic antagonist) in a dose-dependent manner. These results indicate that in adipocytes isolated from dorsal interscapular brown fat of rats there is an α_2-adrenergic reduction of cAMP accumulation and inhibition of lipolysis and respiration.

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The metabolism of *O*-acetyl-5-methoxy-tryptophol in the rat

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The metabolism of melatonin (aMT) (Fig. 1) is unique among pineal methoxy-indoles. It is hydroxylated at the six position [1] and subsequently excreted as the sulphate or the glucuronide conjugate. The 6-conjugates of melatonin account for over 80% of the administered dose; small quantities of unconjugated 6-hydroxy-melatonin and a kynurenine are also found. The metabolism of melatonin is rapid and relatively complex. By contrast, 5-methoxy-tryptophol (ML) and 5-methoxy-tryptamine (MT) are rapidly excreted as a single metabolite, 5-methoxy-indole-acetic acid (MIAA) with only trace quantities of unchanged ML or MT [2, 3].

In 1966 Delvigs and Taborsky [4] attempted to discover the precise structural alterations that would account for this difference of metabolism. They synthesized a novel compound, *O*-acetyl-5-methoxy-tryptophol (aML) (Fig. 1), arguing that its close structural similarity to melatonin should ensure that it would favour hydroxylation rather