Prepubertal unresponsiveness of androgen-dependent microsomal enzyme activities* of rat liver towards exogenous testosterone

	Day of life	Male rats Untreated	Treated	Female rats Untreated	Treated
Δ^4-3β-Hydroxysteroid dehydrogenase	15	26 ± 6	42 ± 7	27 ± 8	45 ± 7
	20	38 ± 5	28 ± 13	35 ± 6	35 ± 8
	25	34 ± 8	31 ± 10	26 ± 8	26 ± 8
	30	45 ± 3	38 ± 6	45 ± 6	56 ± 3
	75	323 ± 46		48 ± 15	
20-Ketoreductase	15	4.6 ± 0.4	5.1 ± 0.9	3.9 ± 1.2	3.9 ± 1.5
	20	5.4 ± 0.9	4.0 ± 0.7	4.2 ± 0.7	3.7 ± 1.3
	25	7.8 ± 1.1	6.6 ± 1.1	8.6 ± 0.6	7.1 ± 1.3
	30	8.3 ± 0.6	6.4 ± 1.4	9.1 ± 1.3	6.7 ± 1.3
	75	28.0 ± 3.0		8.0 ± 1.2	
Seminal vesicles**	30	12 ± 4	125 ± 17		

*nmol × min^{-1} g^{-1} liver wet weight; means ± SD; in each group liver was pooled from 8 rats, 6 determinations per pool. **Wet weight, mg.

values was determined by Student's t-test. The degree of significance was set at $p < 0.001$.

Results and discussion. Up to day 30 of life, the activities of Δ^4-3β-hydroxysteroid dehydrogenase and 20-ketoreductase of testosterone-treated animals did not show any substantial deviation from the normal developmental course (table). In contrast, the activity of 3α-hydroxysteroid dehydrogenase responded in a biphasic manner in both sexes; within 5 days of administration, a 3fold increase had occurred and was followed by a further rise in activity between day 25 and 30 when the level normally found in mature male rats was reached (figure). No significant differences in the protein content of the microsomal fractions were noted for any of the groups, and thus the expression of the enzyme activities in terms of wet weight reflects changes in the 'specific' activities of these enzymes.

The results of this investigation indicate that the sexual indifference in the prepubertal phase of enzyme activity ontogenesis is partly the result of low androgen levels and partly the result of unresponsiveness to androgens. The refractoriness of prepubertal liver to sexual hormones has also been observed for the androgen-dependent synthesis of α_{2U}-globulin[10] and for the oestrogen-dependent synthesis of 'renin substrate'[11]. It is possible that androgen unresponsiveness may be due to the lack of androgen receptors. However, the existence of such receptors in the liver of the mature rat remains controversial[12, 13].

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Catecholamine-sensitive adenylate cyclase of human fat cell ghosts. Inhibition of catecholamine stimulation by phenylephrine

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Summary. The alpha-adrenergic agonist phenylephrine (up to 1 mM) did not affect basal and NaF-stimulated adenylate cyclase activities of human fat cell ghosts, but caused a dose-dependent inhibition of cAMP formation in the presence of catecholamines.

Catecholamines are thought to act via alpha- and beta-adrenergic receptor sites[1–4]. It has been suggested that both receptor types are coupled to the membrane-bound adenylate cyclase system in adipocytes[5–9]. According to this concept, binding of catecholamines to the beta-adrenergic receptor leads to an activation of the enzyme system, whereas interaction with alpha-adrenergic receptor sites is associated with inhibition of cAMP formation. We have previously shown that the human fat cell adenylate cyclase is coupled to beta-adrenergic receptors[10]. In an attempt to clarify the role of alpha-adrenergic stimulation, the effects of phenylephrine – an alpha-adrenergic agonist – on the human fat cell adenylate cyclase activity were tested.

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Methods. Biopsies of s.c. adipose tissue were obtained from 8 patients undergoing surgical treatment. The patients were operated after an overnight fast. Anesthesia was induced with a short acting barbiturate and maintained with halothane, nitrous oxide and oxygen. The biopsies were usually obtained after the skin incision at the start of the operation. Adipose tissues and fat cell ghosts were prepared essentially according to Rodbell[11] as described previously[10]. The adenylate cyclase activity of fat cell ghosts was determined according to Salomon et al.[12]. Data are given as nmoles of cAMP formed per mg protein per 15 min. Statistical analysis was by the Wilcoxon-test for paired samples. The protein content of the samples was determined according to Lowry et al.[13] using bovine serum albumin as standard.

Materials. (α-^{32}P) ATP (2–6 counts/mmole) and cyclic (^{3}H) AMP (27 counts/mmole) were purchased from Radiochemical Centre Amersham Bucks, England. Epinephrine, isoproterenol and phenylephrine were obtained from Boehringer & Söhne, Ingelheim am Rhein (BRD).

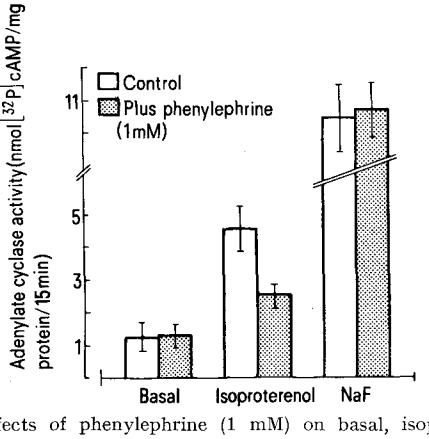

Fig. 1. Effects of phenylephrine (1 mM) on basal, isoproterenol (0.1 mM)- and NaF (20 mM)-stimulated adenylate cyclase activities. 1–20 μg of ghost protein were assayed in the absence and presence of phenylephrine. White columns denote controls, hatched bars show the effects of phenylephrine. Mean value $\pm$ SEM of 6 separate experiments are shown.

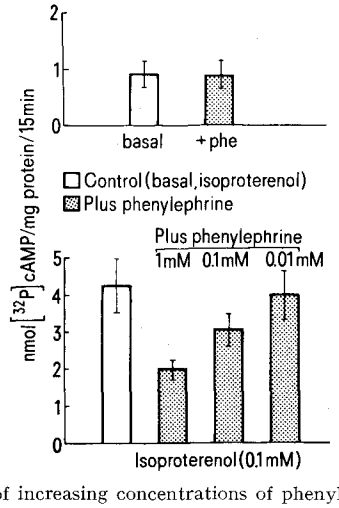

Fig. 2. Effect of increasing concentrations of phenylephrine on the catecholamine-stimulated adenylate cyclase activity of human fat cell ghosts. Isoproterenol concentration was 0.1 mM, phenyleprine concentrations were as indicated. The upper part of the diagram shows basal activities in the presence and absence of 1 mM phenylephrine.
For symbols see legend to figure 1. Mean value $\pm$ SEM of 8 separate experiments are shown.

Results. Figure 1 shows the effects of 1 mM phenylephrine on basal, isoproterenol- as well as NaF-stimulated adenylate cyclase activities of human fat cell ghosts from 6 patients. In the absence of phenylephrine, basal activities averaged 1.20 nmoles of cAMP formed per mg protein per 15 min. Isoproterenol (0.1 mM) increased enzymic activity by about 350–500%. The mean value was 4.50 nmoles cAMP/mg protein 15 min. NaF (20 mM) enhanced cAMP formation 7–9 fold. The mean figure was 10.5 nmoles cAMP/mg protein 15 min. Phenylephrine (1 mM) had no substantial effect on basal (102 $\pm$ 5% of control) as well as NaF-stimulated (105 $\pm$ 10%) adenylate cyclase activities. However, the isoproterenol (as well as adrenaline) activated rates of cAMP formation were decreased by about 45%. Inhibition of catecholamine-activated adenylate cyclase activities became apparent at 0.01 mM phenylephrine (figure 2). Increasing concentrations of this alpha-adrenergic agonist caused a dose-dependent inhibition of isoproterenol stimulated enzyme activities up to 55% at 1 mM phenylephrine in these experiments ($p \leq 0.05$).

Discussion. Evidence for a dual adrenergic receptor system in *human* adipose tissue is based on the following observations: Adrenaline, which usually increases lipolysis and the intracellular cAMP concentrations in isolated fat cells, decreased both lipolysis as well as cAMP formation below basal levels when added together with propranolol – a beta-adrenergic blocking agent[5,6]. Conversely, alpha blockade by phentolamin tends to potentiate the stimulatory effect of adrenaline action[5,6]. These findings have been interpreted as suggesting that the mixed agonist adrenaline has 2 opposing actions: The stimulatory effects are thought to be mediated via interaction with β-receptors. The opposite alpha-adrenergic effect of this catecholamine is supposed to be normally masked, but can be revealed by the 2 different classes of adrenergic blocking agents[5,9]. Attempts to demonstrate an augmentation of catecholamine action by phentolamin in human fat cell ghosts were not nearly as pronounced as one might expect from lipolytic studies[9]. However, our results obtained with phenylephrine are more convincing. Phenylephrine is known to possess some β-activity[14,15] when applied to whole cell preparation. The β-effect of this agonist is not apparent in our experiments as indicated by the finding that basal enzyme activity was not affected by this compound even at concentrations up to 1 mM. Moreover, the lack of influence on basal enzyme activity suggests that the effects of phenylephrine are not mediated via interaction with the catalytic component of the enzyme system. This conclusion is supported by the finding that the action of NaF which is thought to interact with the catalytic subunit of the system[16] is not influenced by phenylephrine, too. Thus, the observation that phenylephrine caused a dose-dependent inhibition of catecholamine-induced enhancement of cAMP accumulation is suggestive that alpha-sites coupled to the adenylate cyclase system are in fact present in human adipocytes membranes.

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