Metyrapone-induced Thermogenesis in Cold- and Heat-acclimated Rats

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Summary Metyrapone caused significant increase of $\dot{V}_{O_2}$ in cold-acclimated rats, but not in warm controls and heat-acclimated rats. Plasma FFA decreased, while plasma glycerol increased in all groups. Glycerol increase was less in heat-acclimated rats than in other groups. FFA/glycerol molar ratio decreased most in cold-acclimated rats.

Key words: metyrapone, cold acclimation, heat acclimation.

Metabolic acclimation to cold is characterized by an enhanced nonshivering thermogenesis (NST), which is developed and maintained mainly by noradrenaline (NA), and brown adipose tissue (BAT) is known to be a major site of NST (FOSTER and FRYDMAN, 1979). However, humoral factors other than NA may be involved in this mechanism (DOI and KUROSHIMA, 1984; KUROSHIMA *et al.*, 1984; SHIBATA and NAGASAKA, 1984). An involvement of corticosteroids seems to be important in NST, possibly through their permissive effects (DEAVERS and MUSACCHIA, 1979). However, recent studies suggest that corticosteroid would inhibit NST, and adrenalectomy could recover the reduced activity of BAT in the genetically obese animals (see ROTHWELL and STOCK, 1985, for references). On the other hand, it has been suggested that adrenocorticotropin (ACTH) through its direct action on BAT is involved, at least in part, in NST (LAURY and PORTET, 1980; ROTHWELL and STOCK, 1985). The ACTH increased heat production in rats and the effect appeared not to be mediated by the adrenal cortex, since it occurred in the adrenalectomized animals (JANSKÝ, 1983). WERNER and VENS-CAPPELL (1985) proposed that ACTH and/or deoxycorticosteroids may be related to thermoregulatory NST, at least in a hibernator, the hedgehog, since metyrapone, an inhibitor of adrenal 11 β -hydroxylase, could induce NST. The present study examined the effects of metyrapone

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on heat production and blood metabolites in the thermally acclimated rats in order to see whether the same mechanism functions in a nonhibernator, the rat.

Young adult male Wistar rats, 7 weeks old, were used. They were given commercial rat chow (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum. They were caged at $25 \pm 1^\circ\text{C}$ at ca. 50% relative humidity (warm controls, WC), $5 \pm 1^\circ\text{C}$ (cold-acclimated rats, CA), or $33 \pm 1^\circ\text{C}$ at ca. 30% relative humidity (heat-acclimated rats, HA) under 12 h artificial lighting from 7:00 to 19:00 for 4 to 5 weeks. The CA and HA were placed at a control temperature of 25°C about 18 h before experiments. Oxygen consumption ($\dot{V}_{\text{O}_2}$) was measured using a gas monitor (1H21A, NEC-San-ei, Tokyo) in an open system at an air flow of 500 ml/min. The animal was placed in an airtight metabolic box (ca. 1500 ml) and immersed in a water bath to maintain the inside temperature at 25°C . The $\dot{V}_{\text{O}_2}$ was expressed as ml/h $\cdot$ metabolic size ($\text{kg}^{0.75}$) in terms of dry gas at STP. After the stable resting $\dot{V}_{\text{O}_2}$ was obtained (usually in 40 to 60 min), the rat was removed from the box and metyrapone (Metopirone ditartrate, Ciba-Geigy Ltd., Basel, 20 mg/ (100 g $\cdot$ 0.1 ml) saline) or saline was administered i.p. and $\dot{V}_{\text{O}_2}$ was again measured for 40 min. Immediately after $\dot{V}_{\text{O}_2}$ measurement, blood was obtained by decapitation. Plasma free fatty acids (FFA) and glycerol were determined by the methods of ITAYA and UI (1965), and LAURELL and TIBBLING (1966), respectively. The significance of the difference was tested by Student's *t*-test.

Increases of body weight were significantly less in CA and HA than those in WC as previously reported (DOI and KUROSHIMA, 1984) (Table 1). Initial $\dot{V}_{\text{O}_2}$ before saline or metyrapone was significantly greater in CA ($1,040 \pm 27.2$, $p < 0.05$) and did

Table 1. Effect of a single injection of metyrapone distrate (20 mg/100 g, i.p.) on resting metabolism in thermally acclimated rats.

	Body weight (g)		$\dot{V}_{\text{O}_2}$ (ml/(h $\cdot$ kg ^{0.75}))			
			Saline		Metyrapone	
	Initial	At experiment	Initial	After 40 min	Initial	After 40 min
Warm controls (10)	196 ± 0.9	309 ± 4.5	954 ± 44.9	943 ± 38.7	969 ± 34.0	$1,058 \pm 42.2$
Cold-acclimated (10)	195 ± 1.2	$265 \pm 4.4^*$	$1,077 \pm 28.3$	984 ± 40.7	$1,004 \pm 45.1$	$1,590 \pm 91.2^a$
Heat-acclimated (10)	195 ± 1.2	$200 \pm 3.6^*$	924 ± 54.7	935 ± 46.7	917 ± 41.6	822 ± 49.8

Values are mean $\pm$ standard errors. Number in parentheses indicates the number of animals. Body weight was mean $\pm$ standard error of 20 animals in each group, since the animals in saline- and metyrapone-injected groups were combined. ^a*p* vs. initial < 0.001 . **p* vs. warm controls < 0.001 .

Fig. 1. Effects of a single injection of metyrapone on plasma free fatty acids (FFA) and glycerol. WC, warm controls; CA, cold-acclimated rats; HA, heat-acclimated rats. *., **., ***. p vs. WC <0.05 , <0.01 , and <0.001 , respectively. ****. p vs. saline <0.05 , <0.01 , and <0.001 , respectively.

not differ in HA (920 ± 33.5) as compared with the value of WC (961 ± 27.4). Metyrapone did not affect the resting control value in either WC or HA, while it increased the resting control value of 1,004 to 1,590 by about 60% 40 min after the injection when the effect reached almost the maximum. Metyrapone decreased plasma FFA levels in all experimental groups, while it increased plasma glycerol levels in all groups (Fig. 1). The FFA/glycerol molar ratio was 3.41 to 3.87, being similar among the saline group; it decreased in all metyrapone-injected groups. The extent of glycerol elevation was 164% in WC, 320% in CA, and 46% in HA; the FFA/glycerol molar ratio was significantly lower in CA (0.52 ± 0.05 , $p < 0.01$) than in WC (0.72 ± 0.05) and HA (0.92 ± 0.10).

It is well documented that relatively high concentrations of metyrapone inhibit adrenal 11 β -hydroxylase, resulting in a marked drop of serum cortisol and corticosterone, and a compensatory increase of ACTH as well as deoxycorticosteroids 30–45 min after administration (see WERNER and WÜNNENBERG, 1980 for references). In a hibernator, the hedgehog, metyrapone-induced thermogenesis was observed even in the euthermic state (WERNER and VENS-CAPPELL, 1985), while in a nonhibernator, the rat, it occurred only in the cold-acclimated state as shown in the present study. Injection of ACTH induced thermogenesis and improved BAT function in rats (ROTHWELL and STOCK, 1985), and furthermore it potentiated the metabolic effects of NA on BAT (LAURY and PORTET, 1980). Deoxycorticosterone

was reported to stimulate heat production in the hedgehog (WÜNNENBERG *et al.*, 1974). Although plasma ACTH or deoxycorticosteroids was not measured in the present study, the results may indicate that ACTH and/or deoxycorticosteroids is involved, at least in part, in cold-induced NST of the rat as well. Metabolic cold acclimation is characterized by an enhanced responsiveness to the calorogenic action of NA and other hormonal factors such as adrenaline and glucagon (DOI and KUROSHIMA, 1984). Therefore, modified metabolic responsiveness to metyrapone observed here seems to indicate that the same phenomenon mentioned above also operates for ACTH-deoxycorticosteroids system in relation to NST, although the direct evidence has not yet been fully presented for a final conclusion.

Metyrapone administration lowered plasma FFA level, while it elevated plasma glycerol, suggesting an increased utilization of FFA, since circulatory glycerol would be a better index of overall lipolysis than FFA. The result may indicate that metyrapone directed the metabolism preferentially toward lipid utilization. Plasma glycerol increased less in the heat-acclimated rats than in WC and CA, suggesting lesser mobilization of lipid under the presence of metyrapone. Moreover, FFA/glycerol ratio which may allow estimation of utilization of mobilized FFA (PORTET *et al.*, 1974) was significantly lower in CA as compared with those in WC and HA, suggesting that metyrapone-induced metabolic increase in CA is closely associated with an enhanced lipid metabolism. Major energy substrate for NST has been demonstrated to be lipids, that is, FFA (LAFRANCE *et al.*, 1980). The present study indicates that the same metabolic strategy also may serve for the metyrapone-induced thermogenesis. Depressed NST was observed in HA as assessed by the decreased thermogenesis stimulated by NA (PETROVIĆ and MARKOVIĆ-GIAJA, 1973) and glucagon (DOI and KUROSHIMA, 1984). Since metyrapone did not influence the resting metabolism in HA and metyrapone-induced NST was not observed in WC, its significance in HA remains unknown in the present study.

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