Thermogenic Response to Glucagon in Cold-acclimated Mice

Katsuhiko DOI and Akihiro KUROSHIMA

Department of Physiology, Asahikawa Medical College,
Asahikawa, Hokkaido, 078-11 Japan

Abstract Heat production after injection of glucagon and norepinephrine was measured in warm- and cold-acclimated mice. Glucagon and norepinephrine in a dose of 100 μ g/100 g caused a marked increase in oxygen consumption. Heat production after administration of glucagon and norepinephrine was significantly potentiated by cold acclimation. A significant positive correlation was observed between the increase in oxygen consumption after injection of glucagon or norepinephrine and the weight of brown adipose tissue in warm- and cold-acclimated mice. These findings indicate that glucagon is a calorogenic hormone in mice and its effect is potentiated by cold acclimation possibly due to an activation of thermogenic mechanism(s) in brown adipose tissue.

Key Words: glucagon, oxygen consumption, cold-acclimation, brown adipose tissue, nonshivering thermogenesis.

Current evidence indicates that glucagon functions as an energy-yielding hormone by supplying metabolic substrates such as glucose, free fatty acids, and ketone bodies under various physiological conditions (KUROSHIMA and DOI, 1976; KUROSHIMA *et al.*, 1981; LEFEBVRE, 1975). The calorogenic action of glucagon has been shown in several species of animals such as rats (DAVIDSON *et al.*, 1960), rabbits (COCKBURN *et al.*, 1968), guinea pigs (COCKBURN *et al.*, 1968), dogs (WEISNER and GRANDE, 1974), quails (KRIMPHOVE and OPITZ, 1975), fowls (FREEMAN, 1975), pigs (INGRAM and KACIUBA-USCILKO, 1980), and men (TRISOTTO *et al.*, 1970). However, in mice, glucagon has been reported as not eliciting any rise in energy metabolism although elevation of metabolism after administration of adrenaline has been found in this animal (KRIMPHOVE and OPITZ, 1975; MILLER and KRAKE, 1963). Glucagon stimulates heat production in brown adipose tissue of small animals such as rats *in vivo* (COCKBURN *et al.*, 1968) and *in vitro* (KUROSHIMA and YAHATA, 1979). Since mice also possess copious amounts of brown adipose tissue (SMITH and HORWITZ, 1969), it is expected that glucagon could stimulate heat production in this species. Recently, we observed that glucagon-induced elevation in the temperature of brown adipose tissue *in vivo* was significantly potentiated in cold-acclimated rats (DOI and KUROSHIMA, 1982).

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土居勝彦, 黒島晨汎

Therefore, the thermogenic action of glucagon in mice might be modified by cold acclimation. This report is concerned with the calorigenic action of glucagon in warm- and cold-acclimated mice.

MATERIALS AND METHODS

The experiments were performed on 68 male ddN strain mice, weighing 40 ± 1.3 g and 32 DHS strain mice, weighing 28 ± 1.4 g, at the age of 6 weeks after birth. Animals fed on the laboratory chow (Oriental M.F., Oriental Yeast Co., Ltd., Tokyo) were acclimated for 3 weeks to 25°C (warm-acclimated mice) (WA mice) or to 5°C (cold-acclimated mice) (CA mice) at 40% relative humidity under artificial lighting from 7:00 a.m. to 7:00 p.m. CA mice were kept at 25°C for about 18 hr before taking measurements to obtain the resting metabolic state. To investigate the calorigenic action of glucagon and norepinephrine (NE), oxygen consumption ($\dot{V}_{\text{O}_2}$) was measured before and after the administration of these test agents ($40 \mu\text{g}$, $100 \mu\text{g}/100$ g, i.p.) in unanesthetized and unrestrained fed mice. Crystalline glucagon (Sigma Chemical Co.) was dissolved in 0.01 N HCl. Glucagon and NE were diluted with saline to $100 \mu\text{g}/0.1$ ml immediately before injection. The saline control group was injected with vehicle solution only.

$\dot{V}_{\text{O}_2}$ was continuously measured using an automatic manometric apparatus (MORRISON, 1951). A fixed aliquot of oxygen of 15 to 20 ml was admitted through a solenoid valve controlled by an attached mercury manometer, and the time required to consume this aliquot was recorded continuously. The mouse was placed in an airtight metabolic box containing both silica gel and a carbon dioxide absorbent, Baralyme (Chemetron Corporation, U.S.A.). The metabolic box was immersed in a water bath whose temperature was controlled at $25 \pm 0.1^\circ\text{C}$, and the initial resting $\dot{V}_{\text{O}_2}$ was measured for about 60 min, the animal subsequently being removed from the metabolic box to be injected with the test agents. The animal was replaced in the metabolic box and thereafter $\dot{V}_{\text{O}_2}$ was again measured. $\dot{V}_{\text{O}_2}$ was expressed in S.T.P.D. The Student *t*-test was used to calculate statistical significance.

RESULTS

Body and brown adipose tissue (BAT) weights (Table 1)

The body weights of ddN strain mice before and after temperature acclimation were not significantly different between CA and WA mice, indicating that the growth rate in CA animals was similar to that in WA ones during temperature acclimation. The weights of BAT were significantly greater in CA mice than in WA ones.

Increase of $\dot{V}_{\text{O}_2}$ after glucagon and NE injections in WA mice

Table 2 summarizes the resting $\dot{V}_{\text{O}_2}$ and the changes of $\dot{V}_{\text{O}_2}$ from the resting

Table 1. Body and brown adipose tissue weights in ddN mice.

	Body weights (g)		BAT weights	
	Initial	At experiment	(mg)	(mg/100 g)
Warm controls (24)	30.5±1.52	38.5±1.03	142.7±12.85	415.3±40.5
Cold-acclimated mice (22)	30.9±1.33	36.7±1.26	333.3±19.17	924.5±30.56
<i>p</i> vs. warm controls	N.S.	N.S.	<0.001	<0.001

Mean±standard error. Numbers in parentheses indicate the numbers of animals.

BAT: interscapular brown adipose tissue. N.S.: not significant.

level after glucagon and NE injections in WA ddN mice. There was no significant difference in the resting $\dot{V}_{O_2}$ among the experimental groups. Saline injection produced no significant increase in $\dot{V}_{O_2}$ from the resting level up to 60 min. Glucagon injection caused a significant increase in $\dot{V}_{O_2}$ from the resting level at 15 to 25 min after the injection of 40 $\mu\text{g}/100\text{ g}$ ($p<0.05$) and at 10 to 60 min after the injection of 100 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.001). The increase in $\dot{V}_{O_2}$ in a dose of 100 $\mu\text{g}/100\text{ g}$ was significantly higher only at 20 min ($p<0.05$).

The resting $\dot{V}_{O_2}$ in 8 WA DHS mice was $2.6\pm0.091\text{ ml}/(\text{g}\cdot\text{hr})$, being significantly higher than that ($2.23\pm0.068\text{ ml}/(\text{g}\cdot\text{hr})$) in 8 WA ddN ones ($p<0.01$). Glucagon injection in a dose of 100 $\mu\text{g}/100\text{ g}$ produced a slight, but not significant increase in $\dot{V}_{O_2}$ (0.10 to 0.23 $\text{ml}/(\text{g}\cdot\text{hr})$) in 10 WA DHS strain mice.

In ddN mice, NE also caused a significant increase in $\dot{V}_{O_2}$ from the resting level at 20 to 40 min after injection in a dose of 40 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.001) and at 20 to 50 min after the injection of 100 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.001). The increase in $\dot{V}_{O_2}$ in a dose of 100 $\mu\text{g}/100\text{ g}$ was significantly higher at 20 to 25 min after injection ($p<0.01$). NE produced a more prominent elevation in $\dot{V}_{O_2}$ than glucagon at 15 to 40 min after the injection of 100 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.01).

Increase of $\dot{V}_{O_2}$ after glucagon and NE injections in CA mice

As shown in Table 3, there was no significant difference in the resting levels of $\dot{V}_{O_2}$ among the five experimental groups in CA ddN strain mice. Each resting level in CA mice was significantly higher than its corresponding resting level in WA ones ($p<0.01$) (Table 2). Saline injection produced no significant changes in $\dot{V}_{O_2}$ throughout the experimental period. Glucagon caused a significant increase in $\dot{V}_{O_2}$ from the resting level at 15 to 40 min after the injection of 40 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.001) and at 10 to 40 min after the injection of 100 $\mu\text{g}/100\text{ g}$ ($p<0.01$ –0.001). The response of CA mice to glucagon was significantly greater than that of WA ones at 15 to 25 min after the injection of 40 $\mu\text{g}/100\text{ g}$ ($p<0.05$ –0.01) and at 20 to 40 min after the injection of 100 $\mu\text{g}/100\text{ g}$ ($p<0.01$ –0.001). The increase in $\dot{V}_{O_2}$ in a dose of 100 $\mu\text{g}/100\text{ g}$ was significantly higher at 15 to 25 min after injection ($p<0.05$).

NE also caused a significant increase in $\dot{V}_{O_2}$ from the resting level at 10 to

Table 2. Increase of oxygen consumption after injection of saline, glucagon, and norepinephrine in WA ddN mice (ml/(g·hr)).

	Time after injection of saline, glucagon, and norepinephrine (min)									
	Resting	10	15	20	25	30	40	50	60	
Saline (8)	2.27±0.077	0.23±0.136	0.33±0.217	0.33±0.213	0.34±0.160	0.30±0.168	0.14±0.131	0.26±0.122	0.07±0.037	
Glucagon										
40 µg/100 g (5)	2.30±0.098	0.33±0.124	0.53±0.118	0.62±0.181	0.57±0.190	0.74±0.280	0.33±0.145	0.27±0.126	0.17±0.158	
<i>p</i> vs. resting	—	—	*	*	*	—	—	—	—	
<i>p</i> vs. saline	—	—	—	—	—	—	—	—	—	
100 µg/100 g (9)	2.23±0.068	0.51±0.108	0.63±0.186	1.18±0.186	1.09±0.224	0.83±0.143	0.26±0.101	0.19±0.088	0.31±0.137	
<i>p</i> vs. resting	—	***	**	***	***	***	*	*	*	
<i>p</i> vs. saline	—	—	—	*	*	*	—	—	—	
<i>p</i> vs. glucagon 40 µg	—	—	—	*	—	—	—	—	—	
Norepinephrine										
40 µg/100 g (5)	2.26±0.088	0.30±0.163	0.49±0.285	1.10±0.203	1.00±0.178	0.85±0.123	0.53±0.154	0.35±0.179	0.35±0.193	
<i>p</i> vs. resting	—	—	—	***	***	***	*	—	—	
<i>p</i> vs. saline	—	—	—	*	*	*	—	—	—	
<i>p</i> vs. glucagon 40 µg	—	—	—	—	—	—	—	—	—	
100 µg/100 g (7)	2.23±0.053	0.37±0.173	0.55±0.321	2.07±0.171	1.80±0.162	1.36±0.212	0.96±0.200	0.51±0.184	0.33±0.184	
<i>p</i> vs. resting	—	—	—	***	***	***	***	*	—	
<i>p</i> vs. saline	—	—	***	***	**	**	**	—	—	
<i>p</i> vs. norepinephrine 40 µg	—	—	—	**	**	—	—	—	—	
<i>p</i> vs. glucagon 100 µg	—	—	**	*	*	**	**	—	—	

Legends are the same as in Table 1. —: N.S. *, **, and ***: $p < 0.05$, 0.01, and 0.001, respectively.

Table 3. Increase of oxygen consumption after injection of saline, glucagon, and norepinephrine in CA ddN mice (ml/(g·hr)).

	Time after injection of saline, glucagon, and norepinephrine (min)									
	Resting	10	15	20	25	30	40	50	60	
Saline (8)	2.83±0.128	0.22±0.161	0.22±0.161	0.25±0.199	0.19±0.104	0.16±0.151	0.19±0.138	0.17±0.175	0.16±0.093	
<i>p</i> vs. resting	—	—	—	—	—	—	—	—	—	
<i>p</i> vs. WA saline	**	—	—	—	—	—	—	—	—	
Glucagon										
40 µg/100 g (5)	2.77±0.120	0.53±0.190	1.33±0.196	1.56±0.183	1.33±0.200	0.96±0.254	0.66±0.198	0.34±0.213	0.09±0.110	
<i>p</i> vs. resting	—	—	**	***	**	*	*	—	—	
<i>p</i> vs. WA glucagon 40 µg	**	—	**	*	*	—	—	—	—	
100 µg/100g (10)	2.75±0.135	0.82±0.815	2.64±0.420	2.64±0.420	2.34±0.455	1.74±0.410	0.72±0.175	0.33±0.210	0.24±0.120	
<i>p</i> vs. resting	—	***	***	***	***	***	**	—	—	
<i>p</i> vs. saline	—	*	***	***	***	***	*	—	—	
<i>p</i> vs. glucagon 40 µg	—	—	*	*	*	—	—	—	—	
<i>p</i> vs. WA 100 µg	**	—	—	**	*	*	*	—	—	
Norepinephrine										
40 µg/100 g(5)	2.76±0.132	0.90±0.203	2.56±0.199	2.69±0.205	2.60±0.210	1.70±0.185	0.53±0.100	0.10±0.109	0.11±0.099	
<i>p</i> vs. resting	—	*	***	***	***	*	*	—	—	
<i>p</i> vs. saline	—	*	***	***	***	***	—	—	—	
<i>p</i> vs. WA 40 µg	**	*	***	***	***	**	—	—	—	
100 µg/100 g (6)	2.80±0.128	1.52±0.370	3.80±0.560	3.83±0.392	3.73±0.458	1.78±0.262	0.32±0.234	0.10±0.210	0.08±0.081	
<i>p</i> vs. resting	—	***	***	***	***	***	—	—	—	
<i>p</i> vs. saline	—	***	***	***	***	***	—	—	—	
<i>p</i> vs. WA 100 µg	**	***	***	***	**	—	—	—	—	
<i>p</i> vs. norepinephrine 40 µg	—	—	*	*	*	—	—	—	—	
<i>p</i> vs. glucagon 100 µg	—	—	—	*	—	—	—	—	—	

Legends are the same as in Tables 1 and 2.

Fig. 1. Relation between $\dot{V}_{O_2}$ and BAT weight after administration of glucagon. WA, warm-acclimated ($\circ$); CA, cold-acclimated ($\bullet$); BAT, interscapular brown adipose tissue.

Fig. 2. Relation between $\dot{V}_{O_2}$ and BAT weight after administration of NE. Legends are the same as in Fig. 1.

40 min after the injection of 40 μ g/100 g ($p<0.05-0.001$) and at 10 to 40 min after the injection of 100 μ g/100 g ($p<0.05-0.001$). The increase in $\dot{V}_{O_2}$ from the resting level due to NE in CA mice was significantly greater than that in WA ones at 10 to 30 min after the injection of 40 μ g/100 g ($p<0.05-0.001$) and at 10 to 25 min after the injection of 100 μ g/100 g. The time taken to reach the peak value was 20 min after the injection of glucagon in both CA and WA mice. The difference in the increases of $\dot{V}_{O_2}$ due to glucagon and NE in doses of 100 μ g/100 g was significant only at 20 min after injection.

Correlation between increases in $\dot{V}_{O_2}$ and weights of interscapular BAT

As shown in Fig. 1, a significant positive correlation was observed between the increases in $\dot{V}_{O_2}$ at 20 min after the injection of glucagon and weight of BAT in WA mice ($p < 0.05$) and in CA ones ($p < 0.01$). A similar relationship was obtained in WA and CA mice respectively after the injection of NE (Fig. 2, $p < 0.05$).

DISCUSSION

In the present study (Tables 2, 3), an injection of glucagon caused a sustained increase of $\dot{V}_{O_2}$ in mice. Moreover, the dose-related increase in $\dot{V}_{O_2}$ was obtained after the injection of glucagon in a dose of 40 and 100 $\mu\text{g}/100\text{ g}$ in WA and CA mice (Tables 2, 3). Glucagon promotes oxidation of free fatty acids and ketone bodies (MCGARRY and FOSTER, 1977). Furthermore, the glucagon-induced rise of BAT temperature (DOI and KUROSHIMA, 1982) may be due to enhanced lipolysis in BAT (KUROSHIMA *et al.*, 1977a, b) in rats. KUROSHIMA and YAHATA (1979) reported that in isolated brown-fat cells glucagon induces a marked thermogenesis which is comparable to that elicited by NE. Therefore, there is a possibility that the calorogenic response to glucagon in mice as seen in the present study might be attributable to the effect of this hormone on BAT. This assumption may be supported by the significant positive correlation existing between the increment of $\dot{V}_{O_2}$ and the weights of BAT (Fig. 1). HEIM and HULL (1966) reported that an injection of glucagon caused an increase in blood flow through BAT as well as of whole body metabolism. Therefore, it is also likely that the increase of $\dot{V}_{O_2}$ due to glucagon is enforced by the glucagon-induced increase in blood flow through BAT. Another possibility is that glucagon produces its calorogenic effect indirectly through the release of catecholamines (SARCIONE *et al.*, 1963; SMITH *et al.*, 1962). However, glucagon stimulates $\dot{V}_{O_2}$ (HEIM and HULL, 1966) and lipolysis in BAT (KUROSHIMA *et al.*, 1977a, b) after the administration of propranolol, a β -adrenergic antagonist, in rats. White adipose tissue decreased in weight markedly after cold acclimation, but the mass of white adipose tissue did not show a weight decrease in chronically glucagon-injected rats (YAHATA *et al.*, 1981). Therefore, glucagon might exert its effect on metabolic responses through different route(s) stimulated by NE. However, the possibility that increased heat production due to glucagon may be attributable to released catecholamines cannot be completely excluded in the present study.

Contrary to our findings, MILLER and KRAKE (1963) claimed that glucagon did not increase the metabolic rate in mice. The discrepancy between the work of Miller and Krake and ours remains unresolved, but might be due to the difference in the dose of glucagon injected and the time at which the metabolic rate was measured. The dose of hormone injected in their study was 3.8 mg/kg, being about ten and four times greater than the 40 and 100 $\mu\text{g}/100\text{ g}$ used in our study, respectively. It has been shown that glucagon releases insulin from the

pancreas (SUSSMAN *et al.*, 1967). Therefore, it is possible that insulin released by glucagon inhibits lipolysis of BAT and that its effect would mask any calorogenic effect of glucagon. Secondly, Miller and Krake injected glucagon 40 min before the beginning of $\dot{V}_{O_2}$ measurements. As indicated in Tables 2 and 3, the increased $\dot{V}_{O_2}$ due to glucagon occurred at 10 min, reached a peak value at 20 min and declined sharply towards the preinjection level around 40 min after the injection. In their study, metabolic rate was measured at the phase of decline towards the resting level. However, according to KRIMPHOVE and OPITZ (1975), the same dose of glucagon as used in our study did not produce a significant increase in $\dot{V}_{O_2}$ over the period of 5 to 45 min after injection. The results obtained with ddN strain mice were in good agreement with those reported in rats. On the other hand, in DHS strain mice as shown in RESULTS, the doses of glucagon used in ddN mice did not cause any significant rise in $\dot{V}_{O_2}$. Therefore, it is likely that there is a strain difference in $\dot{V}_{O_2}$ response to glucagon in mice. The reason(s) for this difference remains to be studied.

Like glucagon, NE stimulated heat production in WA and CA mice. It has been established that animals with BAT increase their nonshivering thermogenesis in response to NE (JANSKÝ, 1973; HIMMS-HAGEN, 1972). From the present study, it might be concluded that glucagon is also involved in nonshivering thermogenesis in mice, possibly by regulating BAT function. This point of view appears to be supported by the results in CA mice. As Table 3 shows, CA mice responded to glucagon by producing a greater rise in $\dot{V}_{O_2}$ than WA ones. In this context, reference should be made to a series of reports from our laboratory that glucagon is closely associated with cold acclimation through its lipolytic as well as glycogenolytic action in rats (KUROSHIMA and DOI, 1976; KUROSHIMA *et al.*, 1978, 1979; KUROSHIMA and YAHATA, 1979) and stimulates lipolysis in BAT *in vivo* (KUROSHIMA *et al.*, 1977a, b). In rats, glucagon-induced elevation of BAT temperature *in vivo* was significantly potentiated in cold-acclimated rats (DOI and KUROSHIMA, 1982). A significant positive correlation was also obtained between the increase in $\dot{V}_{O_2}$ and the weights of BAT in CA as well as in WA mice (Figs. 1, 2). This result indicates the close relationship between the potentiated calorogenic action of glucagon and the enhanced response of BAT to this hormone in CA mice, as in the case of NE. Thus, the enhanced response to glucagon in CA mice may reflect increased nonshivering thermogenesis through BAT.

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