Noradrenaline-induced Secretions of Pancreatic Hormones in Cold- and Heat-acclimated Rats

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Abstract Effects of noradrenaline on the portal and aortic plasma pancreatic hormone concentrations were studied in the cold- and heat-acclimated rats in order to know possible roles of these hormones in temperature acclimation. Noradrenaline (NA) infusion (2 μg/min, i.v., 30 min) effected greater elevation of colonic temperature (Tc) in the cold-acclimated rats (CA) than in the warm controls (WC), and did not influence Tc in the heat-acclimated rats (HA) under hexobarbital anesthesia. Portal and aortic glucagon levels increased in the NA-infused CA and HA, but no changes were observed in the NA-infused WC. NA-infusion did not affect the portal and aortic insulin levels in WC and CA, but increased aortic insulin level in HA. Aortic glycerol and free fatty acid (FFA) levels increased in all NA-infused groups. Portal and jugular vein FFA levels increased in NA-infused WC, but did not in NA-infused CA and HA. Neither NA infusion, nor glucagon was related to the elevation of Tc in HA. These results suggest that temperature acclimation modifies a glucagon-releasing action of NA and the NA-released glucagon could cooperate with NA to enhance nonshivering thermogenesis in the cold.

Key Words: cold acclimation, heat acclimation, glucagon, insulin, noradrenaline.

It has been well established that an enhanced thermogenesis due to cold acclimation is attained by sympathetically controlled nonshivering thermogenesis (NST) in the brown adipose tissue (BAT) (Foster and Frydman, 1979). However, it has been argued that humoral factors other than noradrenaline released from the sympathetic nerve terminals may contribute to the development and maintenance of NST (Janský, 1973; Mory et al., 1982). We have proposed by a series of experiments that pancreatic hormone, glucagon, is closely associated
with an enhanced NST in the cold-acclimated rats through its action on BAT (Kuroshima et al., 1978, 1979; Yahata et al., 1981).

It has been shown that the sympathetic nervous system influences the pancreatic hormone secretion either neurally or by the release of adrenomedullary catecholamines. Catecholamines could stimulate glucagon secretion in dogs, baboons, and men (Unger and Orci, 1981). On the other hand, glucagon has been suggested to modulate the activation of glycogenolysis by the sympathetic stimulation in the liver (Beckh et al., 1982). Glucagon and nerve stimulation are additive in an enhancement of glucose release from the liver possibly through the different mechanisms, while insulin reduces the enhancement of glucose output by the sympathetic stimulation. Recently it was estimated that the maximum glycolytic capacity of BAT in the cold-acclimated rats is compatible to that of the liver (Cooney and Newsome, 1982). These findings appear to indicate a close correlation of noradrenaline and pancreatic hormones in the regulation of NST in the cold.

In the present study the effect of noradrenaline on the secretion of pancreatic hormones was examined in order to know possible interrelation between pancreatic hormones and noradrenaline in temperature acclimation.

MATERIALS AND METHODS

Male rats of Wistar strain, weighing about 150 g, were used as experimental animals. They were divided and prepared for warm control (25±1°C, 4 to 5 wk) (WC), cold-acclimated (5±1°C, 4 to 5 wk) (CA) and heat-acclimated (34±1°C, 4 to 5 wk) (HA) rats. CA and HA were moved to the control temperature of 25°C 18 to 24 hr before the experiments. The experiments were performed immediately after the animals were anesthetized with hexobarbital dissolved in 0.01 N NaOH-saline (20 mg/100 g, i.p.). Half dose of hexobarbital was added 15 min after the first administration. L-Noradrenaline bitartrate was infused into the external jugular vein for 30 min in a dose of 2 µg as a base of noradrenaline in 0.005 ml saline/min. Saline control rats were infused with saline alone. Colonic temperature (Tc) was continuously measured with the thermistor thermometer. After 30 min of infusion the blood was obtained directly into the heparinized syringe from the external jugular vein (EJV) and portal vein (PV), or from the abdominal aorta (AA). All the experiments were done between 9:00-12:00 in the fed state.

Plasma immunoreactive glucagon (IRG) and insulin (IRI) were determined using the commercial kits (Glucagon Kit Daiichi employing glucagon–COOH–specific antiserum and Insulin Kit Daiichi employing pork-insulin antiserum, Daiichi Radioisotope Institute, Tokyo). Blood free fatty acids (FFA) were measured by the method of Itaya and Ui (1965), blood glucose by the anthrone reagent method (Roë, 1955), and plasma glycerol by the enzymatic method of.
LAURELL and TIBBLING (1966). The significance of the difference between the means was tested by the Student's t-test.

RESULTS

Changes in body weights

The initial body weight was about 150 g, being not different among the groups. The body weight at the experiment was 266±3.3 g in WC, 232±4.8 g in CA (p vs. WC <0.001) and 173±3.3 g in HA (p vs. WC <0.001). Ponderal growth was significantly smaller in both CA and HA as previously reported (KUROSHIMA et al., 1978).

Changes in $T_c$

The initial $T_c$ (°C) before saline or noradrenaline infusion was 37.4±0.08 in WC, 37.0±0.1 in CA and 36.3±0.1 in HA. It was significantly lower in CA and HA. During saline infusion $T_c$ increased by 0.2±0.05°C in WC and by 0.4±0.1°C in CA, and no significant difference in $T_c$ was observed between WC (37.5±0.07) and CA (37.4±0.13). In HA $T_c$ decreased by 0.6±0.06, being 35.7±0.18 and lower than $T_c$ in WC during saline infusion. Noradrenaline infusion brought about significant elevations of $T_c$ in both WC (0.5±0.13) and CA (2.0±0.15), and these changes were significantly greater than those in the saline-infused respec-

![Fig. 1. Effect of noradrenaline infusion (2 µg/min, 30 min, i.v.) on colonic temperature ($T_c$). WC, warm controls; CA, cold-acclimated rats; HA, heat-acclimated rats. Vertical line denotes the standard error and number is the number of animals. P vs. saline control: * <0.05, ** <0.02, *** <0.01, **** <0.001.](image-url)
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Changes in IRG and IRI (Fig. 2A and B)

IRG as well as IRI in PV were about twice as much as those in AA as seen in Fig. 2. IRG levels in PV and AA were not different between WC and CA, while IRG in HA was significantly lower in AA ($p<0.001$) and tended to be lower in CA ($p<0.001$). In HA the fall in $T_{ss}$ similar to that during saline infusion was observed after noradrenaline infusion ($0.6\pm0.1$).
in PV ($p \approx 0.05$). Noradrenaline infusion significantly elevated IRG levels in both PV and AA of CA and HA, while it did not influence the levels in both sites of WC.

IRI levels in PV and AA were not significantly different between WC and

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Fig. 3. Effect of noradrenaline infusion on plasma PV, AA, and jugular venous (EJV) blood glucose. Legends same as in Figs. 1 and 2.

Fig. 4. Effect of noradrenaline infusion on AA, PV, and EJV plasma free fatty acids (FFA). Legends same as in Figs. 1 and 2.
Changes in blood metabolites

Blood glucose levels in the saline-infused animals were significantly lower in AA ($p<0.02$) and PV ($p<0.01$) of HA, and in PV ($p<0.01$) of CA as compared with the counterparts of WC. Noradrenaline caused the elevation of blood glucose to a similar extent ($p<0.01$–0.001) in every site of all groups as shown in Fig. 3. Blood FFA levels were significantly lower in AA ($p<0.001$) and PV ($p<0.05$) of HA than those of WC (Fig. 4). Noradrenaline infusion caused significant elevations in blood FFA levels in every site of WC. In CA and HA significant elevations of FFA levels were induced in AA, but not in PV and EJV. Plasma glycerol levels were significantly lower ($p<0.01$) in CA and HA ($p<0.01$) than in WC (Fig. 5). Plasma glycerol levels were significantly elevated in AA of all groups by noradrenaline infusion, but the percent increase in CA ($100\%$) was greater than those in WC ($45\%$) and HA ($50\%$).

DISCUSSION

The present result showing more elevation of $T_e$ of CA due to noradrenaline infusion should be considered to confirm an enhanced NST mediated by nor-
adrenaline in the cold-acclimated animals (JANSKÝ, 1973). On the other hand, it is shown that noradrenaline-mediated NST is suppressed in the heat-acclimated rats (PETROVIĆ and MARKOVIĆ-GIAJA, 1973). This seems to be also the case for the present study, since \( T_c \) of HA was lower than that of WC, and it decreased during saline infusion and never rose during noradrenaline infusion. On the other hand, significant elevations were observed in \( T_c \) in WC and CA 30 min after saline infusion. The cause for these changes remains unexplained. Certain temperature-maintaining mechanisms might be developed in WC and CA against hypothermic effect of anesthetic during 30 min period of anesthesia.

Portal IRG as well as IRI could be better indices of their secretions than those in AA. Therefore, low level of portal and AA IRG in HA may result from the reduced secretion of this hormone due to heat acclimation.

It has been suggested that glucagon is attributable to an enhanced NST possibly through its action on BAT (KUROSHIMA and YAHATA, 1979; DOI and KUROSHIMA, 1982; YAHATA et al., 1981). From the present finding indicating that noradrenaline could elevate PV IRG as well as AA IRG in CA, it is inferred that glucagon released by the adrenergic transmitter is, at least in part, involved in an enhanced NST in cold-acclimated organism. It has been previously evidenced that catecholamines would stimulate glucagon secretion from pancreatic A cells through both \( \alpha \)- and \( \beta \)-adrenergic receptors (SAMOLS and WEIR, 1979). This result, therefore, suggested that cold acclimation potentiates a glucagon-releasing action of catecholamines. Stimulative action of catecholamine on glucagon secretion was also observed in HA. However, this increase in glucagon due to noradrenaline was not related to an increased NST in HA. It has been shown that calorigenic action of glucagon is suppressed in HA as is that of noradrenaline (DOI and KUROSHIMA, 1982). Accordingly, such enhanced glucagon response to noradrenaline may be compensatory for the diminished responsiveness of HA to thermogenic agents such as glucagon and noradrenaline, although the mechanisms involved remain to be clarified. It is possible that the decreased secretion of noradrenaline due to heat acclimation (PETROVIĆ et al., 1976) may increase the number of adrenergic receptors of pancreas through down-regulation mechanism (KURAHASHI and KUROSHIMA, 1981), sensitizing the pancreatic A cell-response to noradrenaline.

Insulin has been implicated to be involved in temperature acclimation. It was reported that glucose-induced insulin release was potentiated (HARADA et al., 1982) or reduced (SASAKI and TAKAHASHI, 1983) in the cold-acclimated rats and sheep, respectively. We found that both glucagon and insulin contents in BAT markedly increased in CA and decreased in HA (HABARA and KUROSHIMA, 1983). In the present study it was noted that plasma insulin levels in PV and AA significantly decreased in HA. As to this finding, it should be referred that many of the alterations such as increased glucose uptake, increased conversions of glucose to fatty acids and glyceride glycerol, \( \text{CO}_2 \), and glycogen, observed in the cold-acclimated BAT, are similarly found when BAT is incubated with insulin (STEINER
Noradrenaline is known to inhibit insulin secretion via α-receptor (Unger and Orch, 1981). However, noradrenaline did not affect PV and AA insulin levels in CA as well as WA in the present study. Aortic, but not portal, insulin level was significantly elevated by noradrenaline infusion. This suggests that peripheral metabolic clearance of insulin is modified in heat acclimation. Further studies should be required for this problem.

Lower levels of both FFA and glycerol in AA of HA suggest a suppressed lipid metabolism (reduced lipolysis) due to heat acclimation. Noradrenaline infusion caused the same extent of elevations in plasma glycerol of AA in WC and HA. Although plasma glycerol level in CA during saline infusion was lower than in WC, the increment of glycerol due to noradrenaline in CA was twice as much as that in WC, and the plasma level reached that in the noradrenaline-infused WC. The increases in AA glycerol levels were accompanied by the increases in AA FFA levels in all groups. Nevertheless, PV and EJV FFA levels were not affected by noradrenaline infusion in CA, while they increased concomitantly with AA glycerol in WC. These findings suggest that noradrenaline-mediated NST is brought about by an accelerated FFA utilization caused by cold acclimation as evidenced previously (Lafrance et al., 1980). It was reported that the capacity of gluconeogenesis from glycerol was increased in CA, providing plasma with glucose as an energy source (Burlington, 1966). Therefore, it is surmised that the lower glycerol level in the saline-infused CA results from an increased utilization of glycerol for gluconeogenesis. It is difficult to explain the fact that PV and EJV FFA levels did not change during noradrenaline infusion in HA, although AA FFA as well as AA glycerol level was significantly elevated. Apparently, this finding suggests an increased utilization of FFA in the peripheral tissues of HA, being not related to the thermogenesis as observed in the present study. FFA utilization as a form of its intermediates, ketone bodies, might be suppressed in HA (Kuroshima et al., 1982). Further study should be needed to clarify the mechanism.

Recently we observed that the adrenal demedullation plus chemical sympathectomy with 6-hydroxydopamine lowered plasma glucagon level in the rat, but this sympathectomized animal retained less, but significant, function to stimulate glucagon secretion to acute cold exposure (Habara et al., 1983). Therefore, glucagon released by noradrenaline may indeed cooperate with noradrenaline to enhance NST in the cold, but a participation of glucagon released by cold independently of sympathoadrenal system could be involved. It is quite possible that synergistic interaction of glucagon and noradrenaline promotes NST, so as to efficiently cope with cold, just as seen in the stress-induced hyperglycemia by means of concomitant increases of glucagon, adrenaline and corticosteroid secretions (Eigler et al., 1979).
REFERENCES


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