Changes in Plasma Glucagon Levels to Stressful Environmental Temperatures

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Abstract Effects of temperature stimuli on plasma glucagon levels were studied in fasted (18 hr) and shorn rats. Plasma glucagon levels rose significantly on exposures to both cold (−5°C, 60 min) (p<0.001) and heat (36°C, 60 min) (p<0.001) from that in control rats at 25°C. Cold stimulus increased blood FFA level (p<0.001) and both cold and heat stimuli increased blood glycerol levels (p<0.05-0.001). FFA/glycerol molar ratio decreased to a similar extent on both cold and heat exposures (p<0.02-0.01). It was noted that plasma glucagon, blood FFA and glycerol levels increased significantly more in cold-exposed rats (p<0.001) than in heat-exposed ones. There were positive relations of plasma glucagon levels to blood FFA and glycerol levels (p<0.001), while an inverse relation of plasma glucagon level to FFA/glycerol molar ratio was observed in all experimental rats (p<0.05). Cold exposure reduced blood glucose level (p<0.001), while heat exposure did not affect it. Cold-induced increases of plasma glucagon and blood FFA levels were diminished significantly in cold-acclimated rats as compared with those in controls (p<0.05-0.001). Blood glycerol increased and FFA/glycerol molar ratio decreased to a similar extent on cold exposure in both control and cold-acclimated rats. Correlation between plasma glucagon and FFA/glycerol molar ratio was inversely significant in controls, but not significant in cold-acclimated rats. Blood glucose level was not altered on cold exposure in cold-acclimated rats. It was concluded that acute exposures to temperature stimuli, especially to cold, stimulate glucagon release, accompanied by increased utilization and mobilization of FFA and glucose, and plasma glucagon response to cold is diminished due to cold acclimation.

Glucagon has been recognized as a hormone secreted to meet energy need, because plasma glucagon levels rise during prolonged exercise, fasting (UNGER and ORCHI, 1976) and temperature acclimation (KUROSHIMA et al., 1978). Glu-
cagon has also been shown to be rapidly released in response to various stressful conditions such as pain, fear and pyrexia which concomitantly induces hyperglycemia (BLOOM et al., 1973).

Thermal stimuli, cold and heat, are expected to act upon the organism not only as specific factors eliciting thermoregulatory responses, but also as so-called stressful agents. Hereupon, the present study dealt with the significance of glucagon in stressful temperatures by observing and comparing the changes in plasma glucagon and blood metabolites in response to acute exposures to cold and heat. Whether the response of plasma glucagon to temperature stimulus is modified with temperature acclimation was also investigated.

MATERIALS AND METHODS

Male Wistar rats weighing 150–180 g were used throughout the study. They were given the standard rat biscuit (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and tap water ad libitum. They were housed at 25±1°C at 50% relative humidity (controls) or 5±1°C (cold-acclimated rats) in the individual cages under 12 hr of artificial lighting from 7:00 to 19:00 for 4 to 5 weeks. The experiments were performed at 9:00 to 11:00 after 18 hr fasting. The cold-acclimated rats were transferred to 25°C 18 hr before the experiments. All the experimental animals were shorn immediately before exposures to warm (25°C, 60 min), cold (−5°C, 60 min) and heat (36°C, 60 min) and exposed to each temperature by placing them in the same type of cages as those in which they had been reared.

Blood samples were obtained by decapitation into the heparinized beakers. Plasma glucagon was measured by radioimmunoassay (HENQUIN et al., 1974) by the use of antiserum 30 K. Blood free fatty acid (FFA) was measured by the method of ITAYA and UI (1965), blood glucose by the anthrone reagent method (ROE, 1955), and blood glycerol by the enzymatic method of LAURELL and TIBBLING (1966).

Results were expressed by the mean±SE. The significance of the difference between the means was tested by Student’s t test.

RESULTS

Effects of cold and heat exposures on plasma glucagon and blood metabolite concentrations

As seen in Fig. 1, both cold and heat exposures induced significant increases of plasma glucagon levels from 183±14.6 pg/ml of basal level at 25°C to 1,226±117.0 pg/ml (p<0.001) and 505±60.6 pg/ml (p<0.001), respectively. However, the extent of increment was significantly greater in the cold-exposed rats (670% of basal level) than in the heat-exposed ones (280% of basal level) (p<0.001). Heat exposure in the present study appeared to be severe, since most rats died on
Fig. 1. Effects of cold and heat exposures on plasma glucagon levels in control rats. Number in the parenthesis indicates the number of animals. Vertical line denotes SE. ***p vs. 25°C-exposed rats <0.001.

heat exposure for longer than 80 min, while the animals survived well during the same period of cold exposure. Therefore, it is conceivable and probable that a large increase of plasma glucagon level in the cold-exposed rats is due to the specific response of glucagon secretion to cold.

Table 1 summarizes the changes in blood metabolites observed concurrently with those in plasma glucagon. Cold exposure caused a significant decrease in the blood glucose level as previously reported (KUROSHIMA et al., 1977), while heat exposure did not significantly affect the blood glucose level, though it tended to increase. Both blood FFA and glycerol concentrations increased significantly on exposure to cold. Blood glycerol rose significantly, but blood FFA was not changed on exposure to heat. FFA/glycerol molar ratio decreased significantly to a similar extent in both cold- and heat-exposed rats. However, the increment of blood glycerol level was significantly higher in the cold-exposed rats (280% of basal level) than in the heat-exposed ones (137% of basal level) (p<0.001).

As seen in Figs. 2 and 3, plasma glucagon levels were positively correlated

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Glucose (mg/dl)</th>
<th>FFA (μEq/liter)</th>
<th>Glycerol (mm)</th>
<th>FFA/glycerol molar ratio</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm (25°C) (8)</td>
<td>89.8±4.06</td>
<td>472±24.9</td>
<td>0.115±0.0141</td>
<td>4.36±0.353</td>
<td>41.9±0.91</td>
</tr>
<tr>
<td>Cold (−5°C) (9)</td>
<td>67.0±3.70</td>
<td>799±57.7</td>
<td>0.323±0.0177</td>
<td>2.55±0.260</td>
<td>48.6±1.51</td>
</tr>
<tr>
<td>p vs. 25°C</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heat (36°C) (8)</td>
<td>96.8±4.87</td>
<td>451±19.5</td>
<td>0.158±0.0119</td>
<td>3.08±0.275</td>
<td>43.6±1.52</td>
</tr>
<tr>
<td>p vs. 25°C</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>&lt;0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means±SE. Number in parenthesis indicates the number of the animals. NS: not significant.
with blood FFA as well as glycerol levels. Furthermore, an inverse correlation was observed between plasma glucagon level and FFA/glycerol molar ratio in all experimental rats (Fig. 4).

**Effect of cold acclimation on cold-induced rise of plasma glucagon level**

In another series of experiment the effect of acute cold exposure was examined

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in the cold-acclimated rats as well as the controls (Fig. 5). In the controls plasma glucagon level markedly increased on acute cold exposure from 168±10.6 pg/ml of basal level at 25°C to 1,410±205.1 pg/ml (839% of basal level) \( (p<0.001) \). This increment was similar to that observed in the first experiment. In the fully cold-acclimated rats plasma glucagon level at 25°C was not different from that in the controls as previously reported \( (Kurosima \ and \ Doi, 1976) \). Acute cold exposure significantly increased plasma glucagon level from 154±12.4 pg/ml of basal level at 25°C to 311±38.1 pg/ml (202% of basal level) \( (p<0.01) \) in the cold-acclimated rats. This cold-induced increment was significantly smaller \( (p<0.001) \) than that in the controls.

Blood glucose level was decreased on cold exposure in the controls as shown in Table 2, while it was not affected in the cold-acclimated rats. Both blood FFA and glycerol levels were elevated on cold exposure in both groups of rats. The rise of blood FFA was slightly greater in the controls \( (p<0.05) \), but blood glycerol level rose to a similar extent in both groups of rats. FFA/glycerol molar ratio was significantly decreased, but the extent of decrease did not differ between the groups. A significant inverse correlation between plasma glucagon and FFA/glycerol molar ratio was seen in the controls (Fig. 6A), while the correlation was not significant in the cold-acclimated rats (Fig. 6B).

Hematocrit was increased significantly on cold exposure in the controls (Tables 1 and 2), but was not changed in the cold-acclimated rats (Table 2).
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Fig. 5. Effect of cold exposure on plasma glucagon level in control and cold-acclimated rats. Legends same as in Fig. 1. **p vs. 25°C-exposed rats <0.01.

Table 2. Effect of the cold exposure (60 min) on blood metabolites in control and cold-acclimated rats.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Glucose (mg/dl)</th>
<th>FFA (μEq/liter)</th>
<th>Glycerol (mm)</th>
<th>FFA/glycerol molar ratio</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm (25°C) (10)</td>
<td>92.0±2.44</td>
<td>492±36.5</td>
<td>0.156±0.0111</td>
<td>3.23±0.257</td>
<td>41.9±0.88</td>
</tr>
<tr>
<td>Cold (−5°C) (10)</td>
<td>77.2±4.60</td>
<td>814±73.2</td>
<td>0.419±0.0168</td>
<td>1.97±0.196</td>
<td>45.1±0.68</td>
</tr>
<tr>
<td>p vs. 25°C</td>
<td>&lt;0.02</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cold-acclimated rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warm (25°C) (10)</td>
<td>86.3±2.77</td>
<td>458±27.0</td>
<td>0.154±0.0114</td>
<td>3.12±0.277</td>
<td>45.3±0.67</td>
</tr>
<tr>
<td>Cold (−5°C) (8)</td>
<td>90.0±2.48</td>
<td>634±24.7</td>
<td>0.377±0.029</td>
<td>1.76±0.161</td>
<td>47.6±0.88</td>
</tr>
<tr>
<td>p vs. 25°C</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Legends same as in Table 1.

DISCUSSION

The present study has proven that stressful stimuli of both heat and cold elicit marked elevation of plasma glucagon level, possibly resulting from increased release of this hormone. It has been reported previously that stressful stimuli such as trauma, infection and fear cause the consistent rise of plasma glucagon levels, followed by the concomitant rise of blood glucose levels (BLOOM et al., 1973; UNGER and ORCHI, 1976). These results strongly suggest that glucagon is rapidly released in response to various stressful stimuli in order to meet the energy need of stressful conditions. However, the thermal stimuli such as heat and cold do not induce hyperglycemia as was observed here, while other stressful stimuli cause hyperglycemia together with hyperglucagonemia. It is well known
that glucagon is physiologically a potent glycogenolytic as well as lipolytic agent (LEFEBVRE, 1975). However, blood glucose level is not significantly affected by heat exposure and is rather reduced by cold stimulus notwithstanding the increased plasma glucagon level. Therefore, the changes in blood glucose observed in the present study seem to indicate an increased utilization of glucose specifically in response to stressful temperature stimuli such as cold and heat. Shivering is a major mode of heat production in the cold and the fuel for shivering is mainly derived from the small reserve of glycogen and glucose in the body (DEPOCAS, 1961). Therefore, cold-induced hypoglycemia would result from the further utilization of glucose for shivering than in the heat. Heat exposure adopted in the present study elevated the colonic temperature of rats by 3.3°C (unpublished data). Such heat-induced hyperthermia may enhance glucose utilization in a passive way, leading to a nonsignificant change in the blood glucose despite the increased glucagon secretion.

Circulatory glycerol may be a good index of overall lipolysis and FFA/glycerol molar ratio may allow estimation of utilization of mobilized FFA (PORTET et al., 1974). Therefore, the increased mobilization as well as utilization of FFA on exposure to cold or heat is suggested from observations of changes in blood FFA, glycerol and FFA/glycerol molar ratio in the present study (Table 1). Although the decrease in FFA/glycerol molar ratio did not differ between the cold-

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**Fig. 6.** Relationship between plasma glucagon level and FFA/glycerol molar ratio in control (A) and cold-acclimated (B) rats. Legends same as in Fig. 2. □: 25°C-exposed rats. ■: cold-exposed rats.
and heat-exposed groups, indicating an increased utilization of FFA in both groups, blood glycerol as well as FFA level rose much higher in the former group. This finding suggests greater mobilization as well as utilization of FFA in the cold-exposed animals. Accordingly, it might be inferred that cold exposure stimulates the greater utilization of metabolic fuels such as glucose and FFA under the increased glucagon secretion in order to maintain body temperature, although heat exposure also may affect the substrate metabolism. The previous studies from our laboratories have suggested that glucagon would be involved in the temperature acclimation through its metabolic action especially on lipid metabolism (Kuroshima et al., 1978, 1979; Kuroshima and Yahata, 1979). The present study further indicated a specific role of this hormone in body temperature regulation in acute cold exposure.

In connection with suppressed response of plasma glucagon to cold in the cold-acclimated rats (Fig. 5), it is interesting to refer to the report that the cold-acclimated rats respond to cold stimulus with less increase of plasma noradrenaline level than the warm-acclimated controls (Depocas and Behrens, 1978). It is definitely established that cold acclimation effects an increased ability to produce heat by nonshivering thermogenesis characterized by an increased responsiveness to noradrenaline which is a major factor regulating nonshivering thermogenesis (Chaffee and Roberts, 1971). Less cold-induced increase of plasma noradrenaline in the cold-acclimated animals may be explained by an increased responsiveness of target tissues to this neurohormone. Less response of plasma glucagon to cold observed in the present study seems to indicate that a mechanism involved in the modified response of noradrenaline is also the case in the glucagon response. Calorigenic action of glucagon was reported to be potentiated in the white adipocytes in vitro (Kuroshima et al., 1979) and in the brown adipose tissue in vivo (Kuroshima et al., 1980) in the cold-acclimated rats. Hence, less increase of plasma glucagon level on cold exposure in the cold-acclimated rats may be attributable to their enhanced metabolic responsiveness to humoral factors such as glucagon and noradrenaline which are responsible for nonshivering thermogenesis.

It has been frequently described that an elevation of blood FFA level during cold exposure is smaller in the cold-acclimated animals (Kuroshima et al., 1974, 1976, 1977). This was also observed in the present study (Table 2). Such findings could be explained by either a decreased mobilization or an increased utilization of FFA. Since the increase of blood glycerol level during cold exposure was similar in both control and cold-acclimated rats, it is surmised that the lipolytic response to cold did not differ between the groups. Noradrenaline caused a marked thermogenesis in the cold-acclimated rats with less elevation of plasma FFA level due to a greater turnover rate of this substrate (Moriya et al., 1974). These results appear to hold the view that the lower response of blood FFA to cold is associated with greater utilization of FFA for cold-induced nonshivering
thermogenesis potentiated by cold acclimation.

As seen in Table 2 and Fig. 6, there is no significant difference in FFA/glycerol molar ratio between the controls and cold-acclimated rats and no significant correlation between plasma glucagon and FFA/glycerol ratio in the cold-acclimated animals. It is likely that these findings result from the increased utilization of glycerol as well as FFA in the cold-acclimated animals. Increased capacity of gluconeogenesis has been shown to be the major changes in carbohydrate metabolism associated with cold acclimation (CHAFFEE and ROBERTS, 1971) and the rate of gluconeogenesis from glycerol in the kidney has been proven to be considerably accelerated in the cold-acclimated rats. Therefore, blood level of glycerol may not necessarily serve as a better index of lipolysis than blood FFA during cold exposure in the cold-acclimated animals, since an enhanced utilization of glycerol would lower its blood level. Moreover, FFA/glycerol molar ratio also would not give a valid estimate of FFA utilization, inasmuch as concomitantly increased utilization of both FFA and glycerol would result in less elevations of these substrates during cold exposure in the cold-acclimated animals.

Reduced blood glucose level on cold exposure in the warm-acclimated rats may result from an accelerated utilization and small reserve of glucose in the body. Such cold-induced reduction of blood glucose has never been observed in the cold-acclimated rats (KUROSHIMA et al., 1974, 1976, 1977). The reason for this difference is possibly due to an enhanced gluconeogenesis which would compensate for an accelerated use of glucose on exposure to cold in the cold-acclimated rats (CHAFFEE and ROBERTS, 1971).

REFERENCES


