Is Glucagon Involved in Cold Acclimatization?

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Summary. Cold acclimatization in rats at 5 °C for 2 weeks caused a significant elevation of plasma glucagon concentration, accompanied by increased plasma FFA and glucose levels. Acute cold exposure at 5 °C for 5 or 60 min did not affect these parameters in plasma.

The recent development of radioimmunoassay method for glucagon has stimulated numerous studies of physiological significance of this hormone. Presently, the concept of glucagon as a hormone of energy supply is proposed, based on the observations which show that glucagon is able to promote mobilization of free fatty acids (FFA) from adipose tissue, as well as glucose from liver, in response to need increased endogenous production of energy substrates, for example, in fasting, muscular exercise, adaptation of mammals to extra-uterine life, etc.[2]. Cold acclimatization is known to require increased supply of fuels, mainly FFA, of which metabolic use is mediated via norepinephrine release from sympathetic nerve terminals[3]; and further the calorigenic effect of this neurohumor has been shown to be intensified in cold-acclimatized mammals, including men[3,4]. Recently, the authors observed an enhanced sensitivity to in vivo lipolytic action of glucagon in cold-acclimatized rats[5], suggesting an involvement of this hormone in cold acclimatization. However, there has been no report on the regulation of glucagon secretion in this connection. This study provides the preliminary result, for the first time, indicating that circulating glucagon is elevated in the course of cold acclimatization.

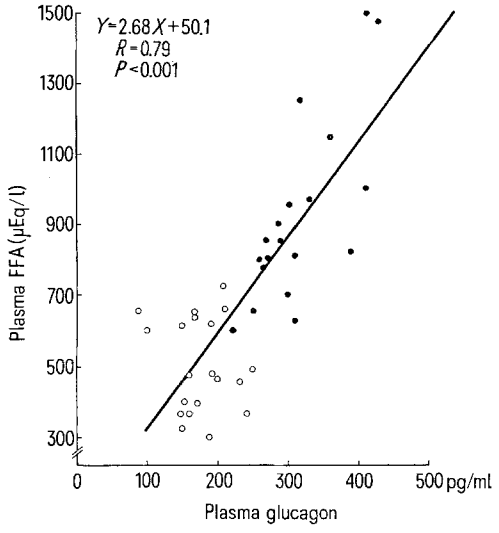

Fig. 1. Relationship between plasma glucagon and FFA concentrations in warm-acclimatized control and cold-acclimatized rats. ○, warm-acclimatized control rats; ●, cold-acclimatized rats at 5 °C for 2 weeks.

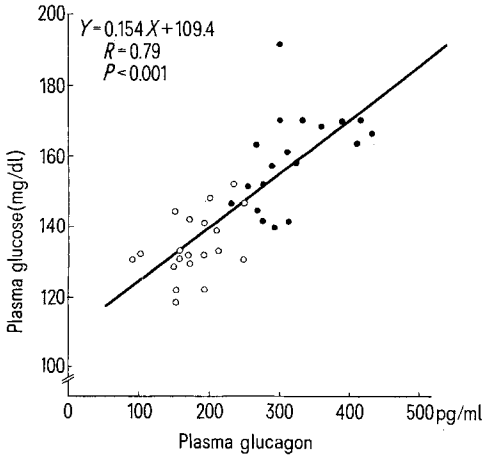

Fig. 2. Relationship between plasma glucagon and glucose concentrations in warm-acclimatized control and cold-acclimatized rats. ○, warm-acclimatized control rats; ●, cold-acclimatized rats at 5 °C for 2 weeks.

Male rats of the Wistar strain were used as experimental animals. Control warm-acclimatized rats were kept at 25 °C and cold-acclimatized ones were prepared by keeping them at 5 °C for 2 or 4 weeks in separate metal cages. It has been established that cold acclimatization is obtained in 2 to 4 weeks in rats, at whatever temperature the animals are kept[6]. Acute cold exposures were also performed at 5 °C for 5 min or 60 min in control warm-acclimatized rats. 5 ml of trunk blood was obtained by decapitation into test tube with 0.5 ml Trasylol solution (10,000 IU/ml) and 6 mg EDTA. Plasma was immediately separated at 4 °C and kept frozen at −30 °C. Plasma glucagon was determined for duplicate samples by the method of Unger et al.[7] with minor modifications[8] by the use of antipork glucagon serum K 964 (Novo Research Institute, Copenhagen, Denmark). Differences of less than 10 pg/ml for glucagon concentrations between 50 and 800 pg/ml could be discriminated with 95% confidence. The accuracy of the method for values between 0 and 800 pg/ml is 7.2 ± 1.1% (SEM) (duplicates on 25 random determinations). Plasma FFA was measured according to the method by Itaya and Ui[9] and plasma glucose according to the method by Roe[10].

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Effects of acute cold exposure and cold acclimatization on plasma glucagon, FFA and glucose concentrations

		Body weight (g) Initial	At experiment	Glucagon (pg/ml)	FFA (μEq/l)	Glucose (mg/dl)
I	Warm-acclimatized controls (10)	165 ± 1.9	233 ± 8.9	195 ± 12.6	484 ± 52.2	141.3 ± 2.29
	Cold-acclimatized rats (5 °C, 2 weeks) (8)	157 ± 5.2	169 ± 6.4[c]	328 ± 20.5[c]	911 ± 97.7[c]	153.3 ± 4.49[a]
	Acute cold exposure (6 min) (6)	—	207 ± 8.9	176 ± 19.3	516 ± 66.8	145.6 ± 7.12
	Acute cold exposure (60 min) (8)	—	215 ± 11.5	149 ± 15.4[b]	540 ± 69.5	134.2 ± 3.52
II	Warm-acclimatized controls (10)	232 ± 7.9	289 ± 5.2	153 ± 11.7	500 ± 34.3	128.4 ± 1.77
	Cold-acclimatized rats (5 °C, 2 weeks) (10)	240 ± 7.9	249 ± 13.1[b]	305 ± 18.3[c]	929 ± 75.9[c]	161.7 ± 4.13[c]
III	Warm-acclimatized controls (10)	165 ± 2.3	299 ± 3.8	203 ± 10.5	410 ± 33.4	122.0 ± 2.08
	Cold-acclimatized rats (5 °C, 4 weeks) (5)	165 ± 4.1	217 ± 18.0[c]	181 ± 15.5	443 ± 17.5	155.6 ± 4.16[c]

Mean ± SEM. Number in parenthesis indicates the number of animals. P vs warm-acclimatized control rats: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$. Values with no marks indicate no significant difference vs warm-acclimatized controls.

The results were summarized in the Table. The increment of body weight was significantly smaller in rats exposed to cold for 2 or 4 weeks, possibly due to higher metabolic rate in the cold. Plasma glucagon concentration was markedly elevated in cold-acclimatized rats for 2 weeks. This finding was confirmed by 2 different series of experiments with younger and older rats as seen in the Table (I and II). However, these elevated glucagon levels were returned to the control values after cold acclimatization for 4 weeks. Such changes observed in plasma glucagon were also the case for plasma FFA; it was increased in cold-acclimatized rats for 2 weeks, while it was not different in those for 4 weeks from that in warm-acclimatized controls. Plasma glucose level remained elevated in cold-acclimatized rats for 2 to 4 weeks. Acute cold exposure did not appreciably affect the plasma concentrations of these three parameters. Further, there were highly significant correlations between plasma glucagon and plasma FFA, or glucose concentrations as a whole in warm-acclimatized and 2 weeks cold-acclimatized rats (Figures 1 and 2).

The present results would appear to indicate that plasma glucagon plays a role in the development of cold acclimatization through its lipolytic and glycogenolytic actions. It is of interest to notice that the blood corticosterone response to histamine stress increased rapidly with the time of cold exposure, reaching a maximum after 14 days in the cold, and then declined, reaching control levels after 28 days in the cold[11]. Consequently, the changes in the plasma glucagon in the cold are comparable to those in the adrenal function in the cold. However, the normalization of plasma glucagon level under continuing cold exposure would not eliminate the possibility of a role of this hormone in cold acclimatization, since cold acclimatization might induce an enhancement of lipolytic action of glucagon as described above[5].

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Heart Rate and Locomotor Activity in Fish: Correlation and Circadian and Circannual Differences in *Cyprinus carpio* L.

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Summary. Long-term measurements of locomotor activity and heart rate in relatively free moving carps demonstrated a correlation between the two parameters examined. Under laboratory conditions, both parameters exhibit a circadian and a circannual rhythm.

Recording of heart rate in fish is an usual method for investigating the effect of exogeneous acoustical, chemical or optical stimuli[1-3]. There is also some information on the correlation of heart rate and respiratory activity under constant and varied environmental conditions[4,5].

Hitherto, we know little about the relation of heart rate and locomotor activity in poicilothermes[4,6-9], and most investigations are based on short term measurements. Therefore we have performed continuous long term measurements of heart rhythm and locomotor activity with carp (*Cyprinus carpio* L.) under laboratory conditions.

Materials and methods. 2 short silver wires were inserted near the heart of anaesthetized carps aged one summer.

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