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Involvement of the Catecholamine in Glucagon-Induced Thermogenesis in Duckling (Carina Moschata)

[Ördek Yavrularında (Carina Moschata) Gözlenen Glucagon ile İndüklenmiş Termogenezde Katekolamin İlişkisi]

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ABSTRACT

Physiological studies have shown that glucagon is a potential mediator of nonshivering thermogenesis in birds. The present work was undertaken in order to investigate whether the observed thermogenesis results from a direct action of glucagon on avian thermoregulatory mechanisms or in fact requires the participation of the catecholamines.

We focused our study on the effects of central glucagon on plasma catecholamine and heart rate on cold acclimated and glucagon treated ducklings in cold environment.

Our results showed that cold exposure (4 °C) induced an increase of circulating norepinephrine in thermoneutrality (42 %) and cold acclimated (43 %) but not significantly in glucagon treated, while epinephrine decreased only in thermoneutrality (-45 %). After glucagon injection, circulating epinephrine increased in thermoneutrality (280 %) and CA (516 %), whereas norepinephrine concentrations decreased only in thermoneutral ducklings (-23 %). Plasma norepinephrine and epinephrine remained unchanged in glucagon treated ducklings. Injection of glucagon caused a decrease in heart rate in thermoneutral duckling whereas it had no effect on cold acclimated and glucagon treated ducklings.

The large increase in epinephrine levels in cold acclimated and thermoneutral ducklings may be due to a massive release of adrenal catecholamine in response to the conditions. Treatment with glucagon twice daily rendered probably ducks insensitive to the effect of intra-cerebroventricular glucagon injection.

Key Words: Glucagon; Norepinephrine, Epinephrine, Cold, Ducklings.

ÖZET

Fizyolojik çalışmalar glukagonun kuşlarda görülen titremesiz termogenezde potansiyel bir etken olduğunu göstermiştir. Bu çalışmada gözlenen termogenezin uçucu kuşların ısı regülasyon mekanizması üzerine gulukagonun doğrudan etkisi veya bu etkide katekolaminlerin de rolü olması durumu araştırılmıştır. Çalışmalarımızı merkezi glukagonun soğuğa alıştırılmış ve glukagon verilmiş soğuk ortamda bulunan ördek yavrularında plazma katekolamin ve kalp hızı üzerine olan etkisi üstüne yoğunlaştırdık. Sonuçlarımız 4 °C'de soğuğa maruz kalmanın dolaşımdaki norepinefrin düzeyinde doğal sıcaklıktaki (% 42) ve soğuğa alıştırılmış (% 43) yavrularda artışa neden olup, glukagon verilmiş ördek yavrularında belirgin bir artış yaratmadığını, sadece doğal sıcaklıktaki yavrularda epinefrin düzeyinde düşüşe (- % 45) sebep olduğunu göstermiştir. Glukagon enjeksiyonunu takiben dolaşımdaki epinefrin doğal sıcaklıktaki ve soğuğa alıştırılmış yavrularda sırasıyla % 280 ve % 516 artmış, norepinefrin düzeyleri ise sadece doğal sıcaklıktaki ördek yavrularında (- % 23) düşmüştür. Plazma norepinefrin ve epinefrin düzeyleri glukagon verilmiş ördek yavrularında değişmemiştir. Glukagon enjeksiyonu doğal sıcaklıktaki ördek yavrularında kalp hızında düşüşe sebep olurken soğuğa alıştırılmış ve glukagon verilmiş ördek yavrularında bir değişiklik yaratmamıştır.

Soğuğa alıştırılmış ve doğal sıcaklıktaki ördek yavrularında epinefrin düzeyinde gözlenen yüksek artışa uygulanan şartlar sonucu büyük bir adrenal katekolamin salgılanması sebep olabilir. Günde iki kere glukagon verilmesi ördekleri intra-serebroventriküler glukagon enjeksiyonlarına duyarsız kılmış olabilir.

Anahtar Kelimeler: Glukagon, Norepinefrin, Epinefrin, Soğuk, Ördek Yavrusu.

INTRODUCTION

Glucagon is known to be strongly lipolytic and glycogenolytic in birds (1, 2). Freeman (2) suggested that glucagon may play a role in avian thermoregulation, and in particular may mediate nonshivering thermogenesis (NST). On the other hand, glucagon appears to be a more potent thermogenic agent in birds than in mammals (3, 4, 5). In our laboratory, large thermogenic responses to glucagon have been reported to occur in penguin chicks and Muscovy ducklings (6, 3).

Moreover, the plasma glucagon concentration rises during cold exposure (1). Chronic glucagon treatment (360 µg/kg–1, twice a day) induces physiological changes similar to those observed during cold acclimation (7). Furthermore, a marked increase in oxygen consumption in response to exogenous glucagon was observed in vivo in growing chickens (8). Such effects of glucagon in birds are similar to those of norepinephrine (NE) in rats (9). As reflected by in vivo measurements of muscle blood flow and arteriovenous differences in oxygen content, muscle NST can be stimulated by exogenous glucagon (10).

Nevertheless, such experiments are unable to distinguish whether the action of this hormone is direct or indirect. Specific high-affinity glucagon binding sites were found in duck brain (11) as well as in adipocytes (12) and hepatocytes (13) of chicks. However, the presence of glucagon receptors has not been demonstrated in the skeletal muscle of birds, nor has any direct effect of glucagon in myocytes been observed.

Besides the action of glucagon, other hormones such as catecholamines may play a role in the stimulation of avian thermogenesis. In recent studies, the use of in vitro perfused muscle preparations showed that catecholamines increase muscle oxygen consumption in the chicken (14) and in Muscovy ducklings (15).

The catecholamines NE and E are associated with sympathetic nerve endings and adrenal chromaffin cells in avian (16). In birds, sympathetic neurons are involved in many thermoregulatory functions by their catecholamine release in several tissues during cold exposure (17). In previous studies we have demonstrated that glucagon is a potential mediator of NST in ducklings (18). Moreover Filali et al. (19) have suggested the involvement of the cathecholaminergic system in glucagon induced thermogenesis in ducklings.

The aim of this study was to investigate the putative involvement of catecholamines in central glucagon-induced thermogenesis in cold-acclimated (CA), glucagon treated (GT) ducklings and in ducklings reared at thermoneutrality (TN, 25 °C).

We studied the effects of intra-cerebro-ventricular (i.c.v) injection of glucagon on plasma catecholamines and heart rate in all groups of the animals.

MATERIALS and METHODS

Animals:

Male Muscovy ducklings (Cairina moschata L, pedigree R31, Institut National Recherche Agronomique, France) were obtained from a commercial stockbreeder (Ets Grimaud, France). They had free access to water and commercial mash (Aliment Genthon, France).

The cold acclimation schedule previously described by Barré et al. (6) was used. Briefly, newly hatched ducklings were kept at thermoneutrality for the first week (35 °C, 12:12 h light/dark cycle), then six ducklings were kept for 5 weeks at TN, and six cold-acclimated ducklings were exposed to cold (4 °C, CA) for 6 weeks.

For chronic treatment, the following schedule was used: from age of 1 wk, the ducklings were caged in groups of 6 for a period of 5 wks at 25 °C ambient temperature (Ta) in a constant photoperiod (8:16 light: dark) and treated with glucagon (GT; 360 µg/kg i.p) twice daily at 8 A.M. and 6 P.M.

Surgery procedure:

Stainless steel cannula (0.96 x 0.58 mm, Biotrol) for i.c.v administration of drugs was stereotaxically implanted under general anesthesia with halothane in the right lateral ventricle of the animals according to the procedure previously described by Montaron et al, 1995 (20). The canula was inserted at point 1 mm anterior to lambda, 2 mm lateral to the midline and 5 mm bellow the skull. A polyethylene catheter (0.96 x 0.58 mm, Biotrol) was fitted with a Silastic tip of about 1 cm, and subsequently inserted into the right carotid for blood sampling. A length of 10 cm tubing terminating near the right brachial artery was held in place with a silk ligature. The catheter was flushed with heparinized saline twice a day to prevent clotting.

Amoxiciline powder (Clamoxyl, Smithkline Beecham) was used prior to stitching. After surgery, the animals were allowed to recover for one week.

Experimental procedure:

Ducks were bound in the sitting position in a quiet darkness during daytime (between 8 A.M. and 7 P.M.). To obtain metabolic steady state and thermal equilibrium at 25 °C, the ducklings were left silting in the thermostatic chamber for initial 120 min, adjustment period, before the experiment begun and also to prevent stress. At the end of the initial period ducklings were usually very quiet and after that we exposed them to cold (4 °C). Six blood samples were drawn in polyethylene vials (containing 10 ml heparin) immersed in ice-cold water: two controls (25 °C and 4 °C just before i.c.v glucagon injection 0 min) and 4 samples after i.c.v glucagon injection (15 min, 30 min, 45 min and 60 min). Whole blood was collected in chilled tubes, immediately centrifuged aliquots of plasma were frozen and stored at – 80 °C for biochemistry studies. After centrifugation at 1000 x g

for 10 min, NE and E were simultaneously assayed by high-performance liquid chromatography coupled with electro-chemical detection.

Glucagon injection and heart rate: the glucagon solution (1 $mg.ml^{-1}$) was prepared in saline just before injection (Porcine glucagon, Novo-Industrie Pharmaceutique, France) and was delivered in 80 µl saline solution of 10^{-7} using micro syringe and cannula. ICV injection was co-timed with the shivering of ducklings. Electrocardiogram (ECG) recordings were obtained using two subcutaneous electrodes (Stabilohmo 110, nichrom, 0.12 mm diam, Johnson Mattey) in the pectoral muscle and recorded on a Racia pen polygraph (DUO 75).

Ethics and statistical analysis: All animals received human care according to the criteria outlined in the "Guide for the care and use of laboratory Animals". Experiments were carried out in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC). The catecholamine levels for ducklings exposed to cold were expressed as percentages of values obtained in the group before i.c.v injection. Data are reported as the arithmetic mean ± S.E.M. Different means were evaluated by the analysis of variance (ANOVA). Statistical significance of the differences between means was assessed by Fisher tests. The level of significance was set at $p < 0.05$.

RESULTS

Effect of cold exposure on plasma catecholamine level

Cold exposure (4 °C) induced an increase of circulating NE in TN and CA (i.e. 5.14 nM ± 0.20 v.s 3.61 nM ± 0.15 and 5.15 ± 1.08 vs 3.6 ± 0.60); but not significantly in GT, whereas E decreased only in TN (0.77 ± 0.10 vs 0.42 ± 0.04) (*p <0.05) (Table 1).

Effect of glucagon on plasma catecholamine level

Intracerebroventricular injection of glucagon is followed by large increases in arterial plasma E levels in TN ducklings (0.42 nM ± 0.04 to 1.6 nM ± 0.30) (**p <0.01) and from (0.4 nM ± 0.10 to 2.57 nM ± 0.46) in CA ducklings (**p <0.01)). Whereas NE was significantly reduced in TN ducklings, (i.e. 3.95 nM ± 0.04 v.s 5.14 nM ± 0.20); (*p <0.05) (Table 1).

In contrast, arterial plasma NE concentrations in CA ducklings were unchanged from the values before i.c.v injection (Table 1). Glucagon injection did not affect plasma E and NE concentrations significantly in GT.

Heart rate

Cold exposure induced in TN duckling an increase of heart rate (H.R) (224 ± 14 beat/min v.s 184 beat/min ± 6 *p <0.05), our results showed that cold did not affect significantly the heart rate of the GT and CA ducklings. After i.c.v glucagon injection, heart rate decreased in TN ducklings and reached a minimum after 20 min (179 ± 5 Beats min-1; *p <0.05). CA and GT ducks were less responsive to the action of glucagon than were controls (Table 2).

DISCUSSION

Our findings suggest that intracerebroventricular injection of glucagon has an inhibitory effect on plasma NE in TN ducklings, whereas it has a stimulatory effect on E in TN and CA ducklings under cold exposure. No changes on the level of plasma E and NE in GT ducklings were observed. It is unlikely that the collection of blood markedly affected circulating catecholamines levels because the ducks were isolated in a box, the volume of blood drawn was small (6 ml) and represents 2 % of total blood volume (approx. 300 ml) (21). During cold exposure, the plasma level of NE was markedly increased in TN ducklings without any change in the level of E.

This result showed a stimulatory effect of cold on the sympathetic nervous system (SNS) activity. The role of SNS has been recognized by a great change of catecholamine release during cold exposure of birds (22; 23). In contrast, cold exposure failed to alter catecholamines level in GT and CA duckling. The absence of SNS activation in GT and CA duckling may be explained by the development of adaptation mechanisms after cold acclimation or chronic treatment.

Single intracerebroventricular injection of glucagon in TN duckling decreased the HR and the plasma NE levels after 15 min (20 %, 76 % respectively). Glucagon shows a depressive effect on the sympathetic nervous system (SNS). This bradycardia might be involved by an inhibition of sympathetic activity or a stimulation of the parasympathetic system evoked by glucagon. The present result will be confirmed by the study of the action of intracerebroventricular glucagon injection on the HR in the vagotomized cold-exposed ducklings. No change in circulating NE level and HR were detected following glucagon injection in CA ducklings, whereas we noted a 516 % increase of the E level ($p < 0.05$) after 15 min of glucagon i.c.v injection. This observation suggests that i.c.v glucagon can affect E secretion in CA ducklings independently of the NE activity. There is a clear evidence that in the CA ducklings, HR was less affected by cold exposure. Acclimation to cold accentuated the depressing effect of propranolol on the volume of oxygen, heart rate and body temperature (24). In GT ducklings, i.c.v. glucagon injection did not affect plasma NE and E levels. It should be noted that GT ducklings received a large dose of glucagon (GT; 360 µg/kg) twice daily during five weeks. Such dose is expected to induce important desensitization (25). Glucagon treatment might induce a desensitization of glucagon receptors that can explain the absence of effect of this peptide on catecholamine

levels. This suggests a possible role of the adrenal in the mechanism of heat production.

The inhibition of NE in TN ducklings may be at least explained by a depressive action of glucagon on SNS activity during cold. Chronic treatment probably rendered ducks insensitive to glucagon.

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