

Effects of Cold and Intracerebroventricular Injection of Glucagon on Plasma Catecholamines in Muscovy Ducklings

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Abstracts: *The aim of the present study was to understand the effect of cold and i.c.v glucagon administration on plasma catecholamine. Cold exposure (+4°C) induces a 42% increase of arterial plasma norepinephrine (NE) in thermoneutral (TN) ducklings. Thus, cold has a stimulatory effect on the sympathetic nervous system (SNS). 15min after i.c.v glucagon injection plasma epinephrine (E) increase in TN ducklings, (74%) whereas NE concentration decrease (23%) only in TN. Plasma NE remained in glucagon treated (GT). Injection of glucagon causes a decrease in heart rate (HR) in TN duckling whereas it has no effect on GT ducklings. In conclusion, large increase in E levels in TN ducklings may be due to a massive release of adrenal catecholamine in response to cold and i.c.v glucagon administration. Treatment with glucagon twice daily rendered ducks possibly insensitive to the effect of i.c.v glucagon injection.*

Keywords: Cold ; i.c.v Glucagon ; Norepinephrine ; Thermoneutral ; Sympathetic Nervous System

1. Introduction

In birds, sympathetic neurons are involved in many thermoregulatory functions by their catecholamine release in several tissues during cold exposure (K. E. Cooper, 2002). The catecholamines norepinephrine (NE) and epinephrine (E) are associated with sympathetic nerve endings and adrenal chromaffin cells in avian (Ariel Y et al., 2009).

El-Halawani evoked the mediation of nonshivering thermogenesis by catecholamines in chicks (El-Halawani M. E. et al., 1970 ; Y. Filali-Zegzouti et al., 2005). Intravenous injections of NE and E in the king penguin chick show that the metabolic action of NE is less than that to E. Thus E plays an important role in thermogenesis in the king (P. Laurberg et al., 2005).

Interaction of glucagon with catecholamines, has been suggested by previous work (A. S. H. Squalli, 2006). Indeed glucagon exerts a controlling influence on the thermoregulation in birds particularly as a possible mediator of non-shivering thermogenesis (NST) (Barré et al., 2014 ; Kristy M. et al., 2010). Intraperitoneal (ip) injection of glucagon inhibits shivering and decreases the metabolic rates (MR) in thermoneutral (TN), glucagon treated (GT) duckling exposed to cold (Barré et al., 2014). The intracerebroventricular (i.c.v) injection of glucagon suppresses shivering and induces a fall in MR in acute cold exposure (A. S. H. Squalli, 2006). It is not well understood how i.c.v glucagon inhibits shivering and influence MR.

The purpose of the study was to determine the effects of i.c.v injection of glucagon on plasma catecholamines in TN and GT duckling in a cold environment.

2. Materials and Methods

2.1 Animals and Surgery

Male Muscovy ducklings (*Cairina Moschata*, pedigree R 31, INRA, France) were obtained from a commercial mash (Genthon). For chronic treatment, the following schedule was used: from the age of 1 wk the ducklings were caged for a period of 6 wks at 25°C Ta in a constant photoperiod (8:16 light:dark) and treated with glucagon (GT ; 360µg/kg ip) twice daily at 8 A.M. and 6 P.M.

Ducklings were caged in groups of 6 for a period of 5 wks at either 25°C Ta (thermoneutral controls, TN). Stainless steel cannula 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 (A. S. H. Squalli, 2006). Amoxiciline powder (Clamoxyl, Smithkline Beecham) was used prior to stitching. After surgery, the animals were allowed to recover for one week.

2.2 Experimental Procedure

Ducks were bound in the sitting position in a quiet darkness box during daytime (between 8 A.M. and 7 P.M.).

To obtain metabolic steady state and thermal equilibrium at 25°C, the duckling was left sitting in the thermostatic chamber for an initial 120min adjustment period before the experiment was begun and also to prevent stress. At the end of the initial period the duckling was usually very quiet and after we expose them to cold (4°C).

2.3 Drug Treatment

Glucagon (Novo) was diluted less than 2min before injection, and delivered in 80µl saline solution at a dose of

$10^{-7}M$ using microsyringe and cannula. I.c.v injections were made when shivering was continued.

Heart rate:

Electrocardiogram (ECG) recordings were obtained using two subcutaneous electrodes (Stabilohmo 110, nichrom, 0.12mm diam, Johnson Matthey) in the pectoral muscle and recorded on a Racia pen polygraph (DUO 75).

2.4 Blood-sampling protocol

Polyethylene catheter (o.d. 0.96, i.d. 0.58, Biotrol) were introduced through the right carotid for blood sampling. Briefly, a length of 13cm tubing terminating near the right brachial artery, and held in place with ligature of silk.

Blood sampling was carried out, in which blood is withdrawn through the Biotrol sampling catheter, and an identical volume of saline is concomitantly infused, maintaining isovolemia.

Six blood samples were drawn in polyethylene vials containing 10 μ l heparin immersed in ice 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 (15min, 30min, 45min and 60min).

2.5 Catecholamine assay

Whole blood was collected in chilled tubes, immediately centrifuged, aliquots of plasma were frozen and stored at -80°C until biochemistry studies. After centrifugation at 1000 x g for 10min, Norepinephrine (NE), Epinephrine ϵ were

assayed simultaneously by HPLC with electro-chemical detection (Richard M. et al., 2009).

2.6 Data presentation and statistical analysis

The catecholamine levels for ducklings exposed to cold was expressed as percentages of values obtained in the group before i.c.v injection. Data are reported as the arithmetic mean \pm sem. Difference 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 $p < 0.05$.

3. Results

Effect of cold exposure on circulating catecholamine levels:

Large concentrations of NE were measured in the plasma of TN ducklings after 120min of cold exposure (i.e. 5.14 ± 0.20 vs $3.61 \pm 0.15nM$; $p < 0.01$), whereas E remains unchanged. No significant cold effect was shown in GT ducklings (Table 1).

Effect of glucagon on plasma catecholamine levels:

I.c.v injection of glucagon is followed by large increase in arterial plasma E levels from $0.42 \pm 0.04nM$ to $1.61 \pm 0.30nM$ after 15min in TN ducklings ($p < 0.05$)(Table 1 ; Fig. 1,2).

Glucagon injection did not affect significantly plasma E and NE concentrations in GT ducks

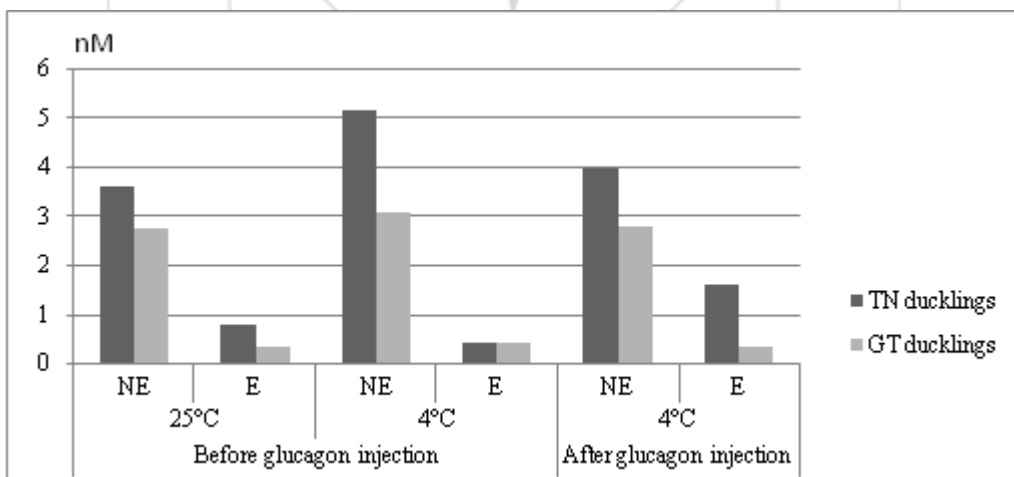


Figure 1: Effect of cold and i.c.v injection of glucagon on catecholamines arterial plasma of ducklings

Value are means \pm s.e.m. ; n = 6 in each group of ducklings. Significantly different from before-injection value *P < 0.05 ; **P < 0.01. Comparisons are made between catecholamine values measured at 25°C with values versus 4°C, $^{\$}p < 0.05$; $^{SS}p < 0.01$.

Heart rate

At 15min after i.c.v injection, HR in TN ducklings were 20% lower that control before injection ($p < 0.05$), and thereafter heart rate decreases continuously reach a minimum after 20min (159 ± 5 Beats min^{-1} ; $p < 0.05$). GT ducks were less responsive to the action of glucagon than were controls (Table 2).

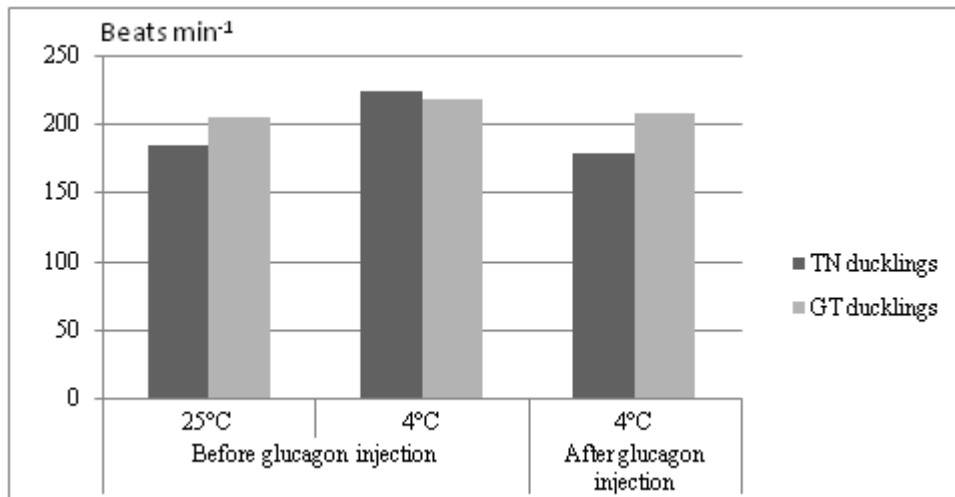


Figure 2: Effect of cold and ICV injection of glucagon on heart rate (beat/min) in duckling

Value are means \pm s.e.m. ; n = 6 in each group of ducklings. Significantly different from before-injection value *P < 0.05 ; **P < 0.01. Comparisons are made between HR values measured at 25°C with values versus 4°C, ^sp < 0.05 ; ^{ss}p < 0.01.

4. 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 ducklings under cold exposure. No changes on the levels of plasma E and NE in GT ducklings were observed.

Plasma concentrations of NE and E are composed of secretions from adrenal and extraadrenal chromaffin cells. NE and E are associated with sympathetic nerve endings and adrenal chromaffin cells in avian (Ariel Y et al., 2009). Additionally neurotransmitters which were released from peripheral nerve endings flow into the bloodstream (Ariel Y et al., 2009). It is unlikely that the collection of blood markedly affected circulating catecholamines levels because the ducks were isolated in his box, the volume of blood removed was small (6ml ; 2%) relative to total blood volume (approx. 300ml) (M.R. Hughes., 2003), the blood was withdrawn through a chronically implanted catheter and the volemia was corrected.

We found the same pattern of plasma catecholamines in TN duckling if we compared to other results for domestic ducks maintained at 20-22°C on a 12Light: 12Dark, NE: 3.61 ± 1.10 nM ; E: 1.21 ± 0.32 nM (A. Robertson, 2009).

During cold exposure, the plasma levels of NE was markedly increased in TN ducklings, without any change in the level of E. This result shows 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 (H. W. Cheng et al., 2001 ; Y. Filali-Zegzouti et al., 2005). In contrast, cold exposure failed to alter catecholamines levels in GT ducklings. The absence of a SNS activation in GT ducklings may be explained by the development of adaptative mechanism after cold acclimation or chronic treatment with glucagon. But the mechanism is unclear.

Single i.c.v injection of glucagon in TN duckling, decreased the HR and the plasma NE levels after 15min (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 i.c.v glucagon injection on the HR in the vagotomized cold-exposed ducklings.

The level of plasma E was increased in TN ducklings by about 4 fold after i.c.v glucagon injection ; this release can be attributed to the adrenals activation associated with a prolonged inhibition of the sympathetic nerves, as evidenced by the decrease in plasma NE. Chemical sympathectomy in pigeons with 6-hydroxydopamine (6-OHDA) at +6°C, impairs the thermogenesis, but the lower body temperature and oxygen consumption were stable and vasoconstriction was normal. This stability may be explained by a massive release of E from the adrenals: plasma E level increase 1.5 fold after 20min cold exposure (H. Abdelmelek et al., 2001). Propranolol, a β -adrenergic blocker impairs the chick's thermoregulation in 5-days old chick exposed at 10°C (N. Cohen et al., 2007). Propranolol, administrated during cold exposure in domestic fowl reduced Vo_2 , EMG activity and HR (Claus Bech et al., 2014).

The involvement of the SNS in plasma NE level and HR decrease may be suggested. Thus, modulation of adrenosympathetic activity may be one of the most important functions of glucagon.

No change in circulating NE levels and HR were detected following glucagon injection in CA ducklings, whereas we noted a 625% increase of the E level (p < 0.01) after 15min of glucagon i.c.v injection. This observation suggests that i.c.v glucagon can effect E secretion in CA ducklings independently of the NE activity. There is clear evidence that in the CA fowls. HR was less affected by cold exposure. Acclimation to cold accentuated the depressing effect of propranolol on Vo_2 , HR, Tb and EMG (Claus Bech et al., 2014). Cold acclimation masks shivering in male Leghorns chickens but does not destroy it as indicated by the appearance of shivering after tyramine treatment (Y. Filali-Zegzouti et al., 2005). Cold exposure increased the number

of glucagon receptors, resulting in 40% in terms of unit cell, and 260% increase per unit surface area in mammals adipocytes. (A. Morales et al., 2000). Cold acclimation might influence the responses of the sympathetic nervous system to i.c.v glucagon injection.

In GT ducklings, i.c.v glucagon did not effect plasma NE, E levels. It should be noted that GT duckling received a large dose of glucagon (GT ; 360*g/kg ip) twice daily during five weeks. Such dose is expected to induce important desensitization (M. Slimani et al., 2007 ;Eduardo C. et al., 2006). Glucagon treatment might induce a desensitization of glucagon receptors that can explain the absence of effect of this peptide on catecholamine levels.

In summary, cold increase the SNS activity in TN ducklings. I.c.v injection of glucagon induces a large increase in E level in TN and CA ducklings which are attributed to the adrenal compensatory effect. 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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