# THE ROLE OF EPINEPHRINE AND NOREPINEPHRINE ON GLUCAGON-INDUCED THERMOGENESIS IN DUCKLING (*CARINA MOSCHATA*): A HPLC STUDY

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Received: 27/06/2007
Accepted after revision: 15/07/2007

**Abstract:** Physiological studies have shown that glucagon is a potential mediator of nonshivering thermogenesis (NST) 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 (CA) ducklings in cold environment.

Our results showed that cold exposure (4 °C) induced an increase of circulating norepinephrine (NE) in thermoneutral (TN) and CA (42 % and 43 % respectively), while epinephrine (E) decreased only in TN (-45%). After glucagon injection, we found that circulating E increase in TN and CA (280%, 516% respectively), whereas NE concentrations decreased only in TN ducklings (-23%). Injection of glucagon causes a decrease in heart rate in TN duckling whereas it has no effect on CA ducklings.
The large increase in E levels in CA and TN ducklings may be due to a massive release of adrenal catecholamine in response to the conditions below.

**Keywords:** *glucagon, norepinephrine, epinephrine, cold, ducklings.*

## 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 NST. On the other hand, glucagon appears to be a more potent thermogenic agent in birds than in mammals [3 – 5]. In our laboratory, large thermogenic responses to glucagon have been reported to occur in penguin chicks and Muscovy ducklings [3, 6].
Moreover, the plasma glucagon concentration rises during cold exposure [1]. Furthermore, a marked increase in oxygen consumption in response to exogenous glucagon was observed in vivo in growing chickens [7]. Such effects of glucagon in birds are similar to those of NE in rats [8]. As reflected by in vivo measurements of muscle blood flow and arteriovenous differences in oxygen content, muscle NST can be stimulated by exogenous glucagon [9].
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 [10] as well as in adipocytes [11] and hepatocytes [12] 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 [13] and in Muscovy ducklings [14].
The catecholamines NE and E are associated with sympathetic nerve endings and adrenal chromaffin cells in avian [15]. In birds, sympathetic neurons are involved in many thermoregulatory functions by their catecholamine release in several tissues during cold exposure [16]. In previous studies we have demonstrated that glucagon is a potential mediator of NST in ducklings [17]. Moreover Filali and al. [18] 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 CA ducklings and in ducklings reared at thermoneutrality (TN, 25 °C).
We study the effects of intra-cerebro-ventricular (i.c.v) injection of glucagon on plasma catecholamines and heart rate in both groups of the animals.

## EXPERIMENTAL

### Animals

Male Muscovy ducklings (*Carina 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 at this age, 12:12 h light/dark cycle), then six ducklings were kept for 5 weeks at thermoneutrality, and six CA ducklings were exposed for 6 weeks to cold (4 °C, CA).

### 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. [19]. 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 AM and 7 PM). To obtain metabolic steady state and thermal equilibrium at 25 °C, the ducklings were left sitting 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 1000g 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. I.C.V. injection was made when shivering was continued. Electrocardiogram (ECG) recordings were obtained using two subcutaneous electrodes (Stabilohmo 110, nichrom, 0.12 mm diameter, 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 AND DISCUSSION

**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), whereas E decrease only in TN ( 0.77 ± 0.10 v.s 0.42 ± 0.04) (*$p < 0.05$) (Table 1).

***Table 1.*** *Effect of cold and i.c.v. injection of glucagon on catecholamine arterial plasma of ducklings**

| | Before glucagon injection | | After glucagon injection |
|---|---|---|---|
| | **25 °C** | **4 °C** | **4 °C** |
| **TN duckling** | | | |
| NE (nM) | 3.61 ± 0.15 | 5.14 ± 0.20** | 3.95 ± 0.04** |
| E (nM) | 0.77 + 0.10 | 0.42 + 0.04** | 1.61+ 0.30*** |
| **CA duckling** | | | |
| NE (nM) | 3.60 ± 0.60 | 5.15 ± 1.08** | 6.45 ± 1.40 |
| E (nM) | 0.43 ± 0.04 | 0.40 ± 0.10 | 2.57 ± 0.46*** |

* Comparisons are made between catecholamine values measured at 25 °C with values versus 4 °C;
** $p < 0.05$;
*** $p < 0,01$.
Values are means ± S.E.M. ; n = 6 in each group of duckling.

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 [20 – 21]. In contrast, cold exposure failed to alter catecholamines level in CA ducklings. The absence of SNS activation in CA duckling may be explained by the development of adaptation mechanisms after cold acclimation.

**Effect of glucagon on plasma catecholamine level**

I.C.V. 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 duckling (**$p < 0.01$). Whereas, NE was significantly reduced in TN duckling ((i.e. 3.95 nM ± 0.04 v.s 5.14nM ± 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), as can be seen from this table the 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. 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 (6 mL) and represents 2% of total blood volume (approx. 300 mL) [22]. During cold exposure, the plasma level of NE was markedly increased in TN ducklings without any change in the level of E.

**Heart rate**

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

***Table 2.*** *Effect of cold and i.c.v injection of glucagon on Heart Rate (beat/min) in duckling*

| Beat/min | Before glucagon injection | | After glucagon injection |
|---|---|---|---|
| | 25 °C | 4 °C | 4 °C |
| TN ducklings | 184 ± 6 | 224 ± 14 * | 179 ± 18* |
| CA ducklings | 186 ± 11 | 203 ± 27 | 178 ± 18 |

Comparisons are made between catecholamine values measured at 25 °C with values versus 4 °C, *$p < 0.05$. Values are means ± S.E.M; n = 6 in each group of duckling.

Table 2 shows that the 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 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 [23].
The inhibition of NE in TN ducklings may be at least explained by a depressive action of glucagon on SNS activity during cold.

## CONCLUSION

In conclusion, the results presented above show that the concentrations of circulating catecholamines can be rapidly and markedly changed by central administration of glucagon in birds.
This work showed also that the observed thermogenesis resulting from a direct action of glucagon on avian thermoregulatory mechanisms requires the participation of the catecholamines.

## ACKOWLEDGEMENTS

This work was supported by DRET 90/176.
*Meriem Slimani* was supported by the Moroccan Ministry of High Education.

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