A POSSIBLE INTERACTION BETWEEN CATECHOLAMINE AND GLUCAGON TO INDUCED THERMOGENESIS IN DUCKLING (*CARINA MOSCHATA*): A HPLC STUDY

M. Slimani[1], S. El Antri[1], M. El Haddad[2], H. Barré[3], T. Fechtali[1*]

[1]Laboratoire de Physiologie et Ecophysiologie ; Faculté des Sciences et Techniques, B.P: 146, 20800 Mohammédia Morocco;
[2]Faculté Polydisciplinaire, Déppartement Sciences de la Matricce, Université Cadi Ayyad, BP 4162, 46000 Safi, Morocco
[3] Physiologie-U.F.R. Médecine Lyon-Nord 8, avenue Rockfeller 69373 Lyon cedex, France.

*Corresponding author: toufiqr@yahoo.com, Fax : 212 (0) 23 315 353

Received: 09/07/2007
Accepted after revision: 15/09/2007

Abstract: The aim of the present study was to investigate whether the thermoregulation in avian results from a direct action of glucagon or requires the participation of other agents such as catecholamines. We focused our study on the effects of central glucagon on plasma catecholamine and heart rate on thermoneutral (TN) and glucagon treated (GT) ducklings in cold environment. Our results showed that cold exposure (4 °C) induced an increase of circulating Norepinephrine (NE) in TN (42%) but not significantly in GT, while Epinephrine (E) decreased only in TN (-45%). After glucagon injection, we found that circulating E increase in TN 280%, whereas NE concentrations decreased only in thermoneutral ducklings (-23%). Central glucagon injection causes a decrease in heart rate in thermoneutral duckling whereas it has no effect on glucagon treated ducklings. The increase in E levels in thermoneutral ducklings may be due

to a massive release of adrenal catecholamine in response to cold. Treatment with glucagon twice daily rendered probably ducks insensitive to the effect of intracerebroventricular (ICV) glucagon injection.

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

INTRODUCTION

The catecholamines norepinephrine (NE) and epinephrine (E) are associated with sympathetic nerve endings and adrenal chromaffin cells in avian. In birds, sympathetic neurons are involved in many thermoregulatory functions by their catecholamine release in several tissues during cold exposure [1, 2]. Previous studies have shown that glucagon is a potential mediator of nonshivering thermogenesis (NST) in ducklings [2, 3]; they 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 [4 - 6]. In our laboratory, large thermogenic responses to glucagon have been reported to occur in penguin chicks and Muscovy ducklings [4 - 7].

Moreover, the plasma glucagon concentration rises during cold exposure [3]. Chronic glucagon treatment (360 $\mu g.kg^{-1}$, twice a day) induces physiological changes similar to those observed during cold acclimation [8]. Furthermore, a marked increase in oxygen consumption in response to exogenous glucagon was observed in vivo in growing chickens [9]. Such effects of glucagon in birds are similar to those of NE in rats [10]. As reflected by in vivo measurements of muscle blood flow and arteriovenous differences in oxygen content, muscle NST can be stimulated by exogenous glucagon [11].

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 [12] as well as in adipocytes [13] and hepatocytes [14] 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 [15] and in Muscovy ducklings [16].

The catecholamines NE and E are associated with sympathetic nerve endings and adrenal chromaffin cells in avian [17]. In birds, sympathetic neurons are involved in many thermoregulatory functions by their catecholamine release in several tissues during cold exposure [1]. In previous studies we have demonstrated that glucagon is a potential mediator of NST in ducklings [18]. Moreover Filali [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 GT ducklings and in ducklings reared at thermoneutrality (TN, 25°C).
We studied the effects of ICV injection of glucagon on plasma catecholamines and heart rate in two groups of the animals.

EXPERIMENTS

Animals

Male Muscovy ducklings (*Cairina moschata* L, pedigree R31, Institut National de 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 [7] 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.
For chronic treatment, the following schedule was used: from the age of 1 week, the ducklings were caged in groups of 6 for a period of 5 weeks at 25 °C ambient temperature in a constant photoperiod (8:16 light : dark) and treated with glucagon (GT; 360 μg/kg i.p.) twice daily at 8 AM and 6 PM.

Surgery procedure

Stainless steel cannula (0.96 x 0.58 mm, Biotrol) for ICV 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 [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 AM and 7 PM). 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 ICV glucagon

injection 0 min) and 4 samples after ICV 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. ICV injection was made when shivering was continued. Electrocardiogram (ECG) recordings were obtained using two subcutaneous electrodes (Stabilohmo 110, nichrom, 0.12 mm dia, 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 "INSA". Experiments were carried out in accordance with the Animal Welfare Division of the Ministry of Environment and Forest, Council of International Organisation of Medical Sciences (WHO/UNESCO).
The catecholamine levels for ducklings exposed to cold were expressed as percentages of values obtained in the group before ICV 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 (5.14 nM ± 0.20 vs 3.61 nM ± 0.15) but not significantly in GT, whereas E decrease only in TN (0.77 ± 0.10 vs 0.42 ± 0.04) (*$p < 0.05$) (Table 1).
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 duckling. The absence of SNS activation in GT duckling may be explained by the development of adaptation mechanisms after chronic treatment.

Table 1. *Effect of cold and ICV injection of glucagon on catecholamine arterial plasma of ducklings. 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.*

	Before glucagon injection		**After glucagon injection**
	25 °C	4 °C	4 °C
TN ducklings 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**
GT ducklings NE (nM)	2.72 ± 0.28	3.07 ± 0.34	2.78 ± 0.22
E (nM)	0.31 ± 0.05	0.42 ± 0.07	0.31 ± 0.04

Effect of glucagon on plasma catecholamine level

ICV 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), whereas NE was significantly reduced in TN duckling, (i.e. 3.95 nM ± 0.04 vs 5.14 nM ± 0.20); (*p < 0.05) (Table 1).

In contrast, glucagon injection did not affect significantly plasma E and NE concentrations in GT. The results on the table above suggest that ICV 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 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 their box, the volume of blood removed was small (6 mL) and represents 2% of total blood volume (approx. 300 mL) [21].

Heart rate

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

Table 2. *Effect of cold and ICV injection of glucagon on heart rate (beat/min) in duckling. 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.*

	Before glucagon injection		**After glucagon injection**
	25 °C	4 °C	4 °C
TN ducklings	184 ± 6	224 ± 14 *	179 ± 18*
GT ducklings	204 ± 6	218 ± 14	208 ± 15

Our findings concerning the heart rate shows that intracerebroventricular injection of glucagon in TN duckling decreased the heart rate 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. In GT ducklings, ICV 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 [24]. 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.

CONCLUSION

In conclusion, this work showed that the nonshivering thermogenesis developed by duckling treated by glucagon is resulting from a direct action of glucagon on avian thermoregulatory mechanisms and requires the participation of the catecholamines. We suggest that glucagon may play a role in this implication perhaps by the stimulation of sympathetic nervous system of bird. Future pharmacological investigation is needed to understand the mechanism.

ACKNOWLEDGEMENTS

This work was supported by DRET 90/176.
Meriem Slimani was supported by the Moroccan Ministry of high education.

REFERENCES

1. Jansky, P., Jansky, L.: Sites and cellular mechanisms of human adrenergic thermogenesis, A review*, J Thermal Biol.* **2002**, **27**, 269.
2. Freeman, B.M., Adrenal glands, in: Freeman, B.M. (ed): *Physiology and biochemistry of the domestic fowl,* Academic, London, **1984**, 191.
3. Barré, H., Geloen, A., Mialhé, P., Rouanet, J-L.: Effects of glucagon on bird thermogenesis, in: Assenmacher, I., Boisson, J. (eds): *Endocrine regulations as adaptive mechanisms to the environment.* CNRS, Paris, **1986**, 395.
4. Duchamp, C., Marmonier, F., Denjean, F., Lachuer, J., Eldershaw, T., Rouanet, J.L., Roussel, D., Barré, H.: Regulatory, cellular and molecular aspects of avian muscle nonshivering thermogenesis, *Ornis Fennica*, **1999**, **76**, 151.

5. Hissa, R.: Controlling mechanisms in avian temperature regulation. *Acta Physiol Scand [Suppl 567],* **1988**, **132**, 140.
6. Jansky, L., Nonshivering thermogenesis and its thermoregulatory significance, *Biol Rev,* **1995**, **48**, 85–132
7. Barré, H., Rouanet, J.L.: Calorigenic effect of glucagon and catecholamines in king penguin chicks, *Am J Physiol,* **1983**, **244**, R758.
8. Barré, H., Cohen-Adad, F., Rouanet, J.L.: Two daily glucagon injections induce nonshivering thermogenesis in Muscovy ducklings, *Am J Physiol,* **1987**, **252**, E616.
9. Keller, J.C.: Role of glucagon in the regulation of metabolism in growing chicken, in: Mount, L.E. (ed): *Energy metabolism.* Butterworth, London, **1980**, 417.
10. Leblanc, J., Pouillot, M.: Importance of noradrenaline in cold adaptation. *Am J Physiol,* **1964**, **207**, 853–856
11. Duchamp, C., Barré, H.: Skeletal muscle as the major site of nonshivering thermogenesis in cold-acclimated ducklings. *Am J Physiol*, **1993**, **265**, R1076.
12. Montaron, A., Moyes, E., Barré, H.: Radioautographic demonstration and localisation of glucagon receptors in duck brain, *Brain Res,* **1994**, **663**, 121.
13. Oscar, T.P.: Glucagon binding to receptor on the surface of chicken adipocytes. *J Anim Sci*, **1995**, **73**, 728.
14. Padrell, E., Herberg, J.T., Monsatirsky, B., Floyd, G., Premont, R.T., Lyengar, R.: The hepatic glucagon receptor: a comparative study of the regulatory and structural properties. *Endocrinology,* **1987**, **120**, 2316.
15. Eldershaw, T., Duchamp, C., Ye, J.M., Clark, M.G., Colquhoun, E.Q.: Potential for nonshivering thermogenesis in perfused chicken (*Gallus domesticus*) muscle, *Comp Biochem Physiol,* **1997**,**117A**, 5545.
16. Marmonier, F., Duchamp, C., Eldershaw, T., Cohen-Adad, F., Barré, H.: Hormonal control of thermogenesis in perfused muscle of Muscovy ducklings. *Am J Physiol*, **1997**, **273**, R1638.
17. Akester, A.R.: The autonomic nervous system in Forms and function in birds (A. S. King and J. McLelland. eds), Academic press, London/New York, **1979**.
18. Squalli, A.S., Slimani, M., Filali, Y.Z., Benchekroun, M.N., Elantri, S., Rouanet, J.L., Barre, H., Fechtali, T.: Role of glucagon in the control of heat production in ducklings. *J Neural Transm.*, **2006**, **113**, 1417.
19. Filali-Zegzouti, Y., Abdelmalek, H., Rouanet, J.L., Cottet-Edmard, J.M., Pequignot, J.M., Barré, H.: Involvement of the catecholaminergic system in glucagons-induced thermogenesis in Muscovy ducklings (*Cairina moshata*), *Pflugers Arch – Eur J Physiol,* **2000**, **441**, 275.
20. Montaron, A., Rouanet, J.L., Barré, H.: Inhibition of shivering thermogenesis by the centrally applied glucagon in Muscovy ducklings. *Brain Res,* **1995**, **702**, 49.
21. Grouzman, E., Cavadas, C., Grand, D., Moratel, M., Aubert, J.F., Brunner, H.S., Mazzolai, L.: Blood sampling methodology is crucial for precise measurement of plasma catecholamines concentrations in mice. *Pflugers Arch-Eur J Physiol,* **2003**, **447**, 254.
22. Filali-Zegzouti, Y., Abdelmalek, H., Rouanet, J.L., Collet-Edmard, J.M., Pequignot, J.M., Barré, H: Role of catecholamines in glucagons-induced thermogenesis. *J Neural Transm,* **2005**, **112**, 481.

23. Fan, W., Voss-Andrea, A., Cao, W.H., Morrison, S.F.: Regulation of thermogenesis by the central melanocortin system. *Peptides,* **2005**, **26**, 1800.
24. Wakelam, M.J.O., Murphy, G.J., Hruby, V.J., Houslay, M.D.: Activation of two signal-transduction systems in hepatocytes by glucagon, *Nature*, **1986**, **323**, 68.