Short Comunications

INSULIN IN THE CAROTID SINUS INCREASES SUPRAHEPATIC AND ARTERIAL GLUCOSE LEVELS

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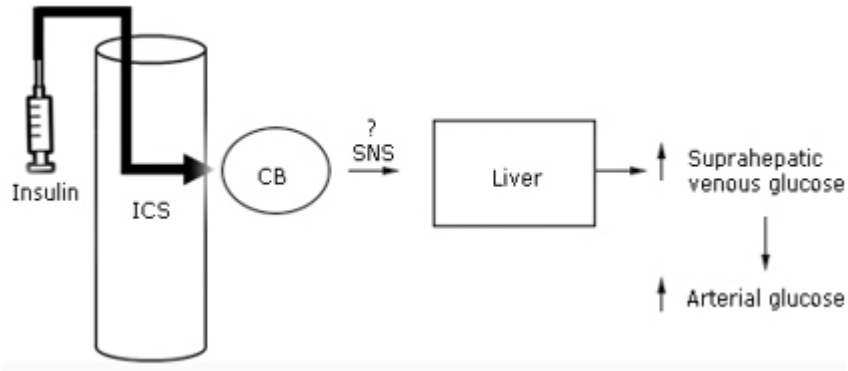
Graphical Abstract



ICS, isolated carotid sinus; CB, carotid body; SNS, sistema nervioso simpático

ABSTRACT

Objective: Considering that insulin is a clue hormone in glucose homeostasis, the aim of this study was to analyze the effects of this hormone infused into the isolated carotid sinus (ICS), on suprahepatic and arterial glucose levels. *Methods:* All procedures were carried out in accordance with the United States National Institutes of Health. Ten male Wistar rats 280-300 g anesthetized with sodium pentobarbital (5 mg /100 g i.p.) after 12 h fasting were randomly divided into control and experimental groups. Saline (100µL) or insulin (15 mIU/rat in 100 µL saline) were injected into the ICS. Blood was collected from catheters inserted in the suprahepatic vein (SHV), starting in the jugular vein and into the femoral artery (FA) were placed. Glucose levels were determined at -10 and -5 min before saline or insulin were injected in the ICS; and 1, 5, 10, 20, and 40 min after the above injection. *Results:*Insulin injection significantly increased glucose levels in the SHV from 128.8 ± 5.2 mg/dL to 207.4 ± 10.6 mg/dL (p = 0.00005), while in the FA they increase from 123.4 ± 6.7 mg/dL to 199.8 ± 9.6 mg/dL (p = 0.0008) at 40 min after insulin injection. Control group with saline did not show significant changes (p = 0.97 in FA) and (p = 0.34 in SHV). The comparison between both groups was significant on arterial (p = 0.007) and venous (p = 0.003) blood glucose levels. *Conclusion:* As other studies report overactivation of the carotid bodies and sympathetic activity increase after insulin injections in the carotid artery, we assume that insulin in the carotid bodies (CBs)) activates hepatic glycogenolysis to increase blood glucose levels (hyperglycemia).

Keywords: carotid body chemoreceptors; insulin; isolated carotid sinus; glycemia

INTRODUCTION

The carotid bodies (CBs), located bilaterally in the bifurcation of the common carotid detect changes in pO2, pCO2, pH (Alvarez et al. Buylla, 1951; Eyzaguirre and Zapata, 1984; Pardal, et

al., 2007) and glucose (Álvarez-Buylla and Álvarez-Buylla, 1988, Álvarez-Buylla and Roces de Álvarez-Buylla, 1994, Koyama et al., 2000,Pardal and López-Barneo, 2002).The CBs are innervated by the carotid sinus nerve, a descending branch of the glossopharyngeal nerve, which runs from the CBs, passing through the petrosal ganglion,to the tractus solitarius nucleus (NTS) (Finley and Katz, 1992). The CBs are integrated into the brainstem and produce respiratory and cardiovascular reflexes through the adrenal sympathetic pathway (González et al., 1994, Marshall, 1994, Álvarez-Buylla et al., 1997), to activate glycogenolysis (Cao and Morrison, 2001). In recent years it is suggested that CBs are also sensitive to insulin due to the presence of insulin receptors (Ribeiro et al., 2013). Intravenous infusion of insulin triggers the action of CB (Conde et al., 2018) and increases ventilation (Bin-Jaliah et al., 2004); likewise, hypercaloric diets cause overactivation of these organs and induce insulin resistance, hyperinsulinemia and hypertension in rats (Ribeiro et al., 2013). That is to say, that insulin stimulates CBs and produces sympathoadrenal activation with a hyperglycemic response secondary to hepatic gluconeogenesis increase and/or glycogenolysis. In this work, insulin injection was performed in the isolated carotid sinus from the circulation (ICS), to analyze the glycemic levels in the suprahepatic vein (glucose output from the liver) and in the femoral artery in healthy Wistar rats.

MATERIAL AND METHODS

All procedures were performed on male Wistar rats with strict adherence to the Guide for the Care and Use of Laboratory Animals. The experiments were carried out in rats of 280-300 g of body weight (n = 10). The rats were housed individually in light-dark conditions 12h /12h, and temperature of 22-24 °C, with water and food (Teklad Global Diet) at free demand. Two groups were randomly conformed: a) control, with saline injection into the ICS (n = 5), and b) experimental, with insulin injection into the ICS (n = 5). The rats were anesthetized with sodium pentobarbital (3 mg/100 g, i.p., Anestesal, Pfizer, Mexico), the level of anesthesia was kept constant throughout the experiment (0.063 mg/min, i.p.). Before the surgical procedure, buprenorphine was injected for analgesia (0.03 mg/kg, i.m., Temgesic, Schering-Plow, Mexico).

Rats were kept under artificial respiration with a small species respirator (Stoelting-Ugo Basile, Italy) connected to an endotracheal tube. To catheterize the SH vein, the right external jugular vein was dissected and catheterized through a silastic tube (Dow Corning 602-155) to the inferior cava vein, at the exit of the suprahepatic vein, in order to collect the blood that drains from the liver to determine the concentrations of glucose that leaves this organ. To catheterize the abdominal aorta, an incision was made in the inguinal fold, superior and medial to the right, once visualized the vasculo-nervous bundle, the femoral artery was dissected to introduce a polyethylene tube PE- 10 (Clay Adams, Division of Becton, Dickinson & Co., Parsippany, NJ,

USA) until reaching the abdominal aorta (1 cm below the renal arteries). All catheterizations were made with heparinized cannulas (500 U/mL). Saline or insulin injections were made into the ICS following the technique of Álvarez-Buylla and Álvarez-Buylla, (1988). Glucose concentrations was measured in the Accu-Chek Sensor glucometer (Roche, Mannheim, Germany). The blood was collected at: t = -10 min -5 min (before the injections in the ICS), and t = 1 min, 5 min, 10 min, 20 min and 40 min after, which is considered as t = 0 min). After collecting the last blood sample the rats were sacrificed under anesthesia and the site of catheters tip was checked. The chemical substances used are the following:

Sodium Pentobarbital (Pfizer) (3 mg / 100 g) and (1.8 mg / 100 mL saline) (Álvarez-Buylla and Álvarez-Buylla, 1988).

Buprenorphine (Schering Plow, Temgesic, Mexico, 0.005 mg / 100 g i.m.) (Cowan et al., 1977).

Saline solution (Pisa) at 0.9% (100 µL) (Yarkov et al., 2001; Lemus et al., 2008; Lemus et al., 2011).

Insulin Lispro (Sigma) (15 mUI / rat in 100 µL of saline) (Ribeiro et al., 2013).

For the statistical analysis, the mean and standard error of the values obtained from the dependent variables in each group (determination of glucose concentrations) were determined. Student's t-test was used, paired *t*-test to compare the averages of the dependent variables, before and after the application of the substances in the SCA, and two-sample *t*-test when the glucose levels were compared between both groups (insulin *vs* saline). The confidence interval was set at 95%, with a level of significance $p < 0.05$.

RESULTS

In the control group, saline infusion in ICS did not produce significant increases in the FA glucose concentration or SH venous glycaemia (Figure 1A) compared to their respective basal levels. In the experimental group, the infusion of insulin in the ICS produced significant increases in both, arterial and suprahepatic venous glycaemia compared with their respective basal levels (Figure 1B). The comparison between the mean concentration of FA and the SH vein glycemias was significant ($p < 0.001$). When control and experimental arterial blood glucose levels groups were compared, a significant difference was observed from min 1 to min 40 after ICS insulin injection (Figure 2A). Similarly, the increase in SH venous glycaemia after insulin infusion in ICS was significant compared to SH venous glycaemia after saline in ICS (Figure 2B). No significant changes were observed between the SH vein and the FA (calculated as the suprahepatic blood glucose minus the blood glucose of the femoral artery), both, in control rats with saline in the ICS or in the experimental group with insulin in the ICS with respect to its baseline, nor with respect to the comparison between both groups with their respective times

(Figure 3). However, the comparison of total differences between the two groups was significant ($p = 0.005$) (Figure 3), which means that insulin increased the glycemia in the SH vein and decreased blood glucose levels.

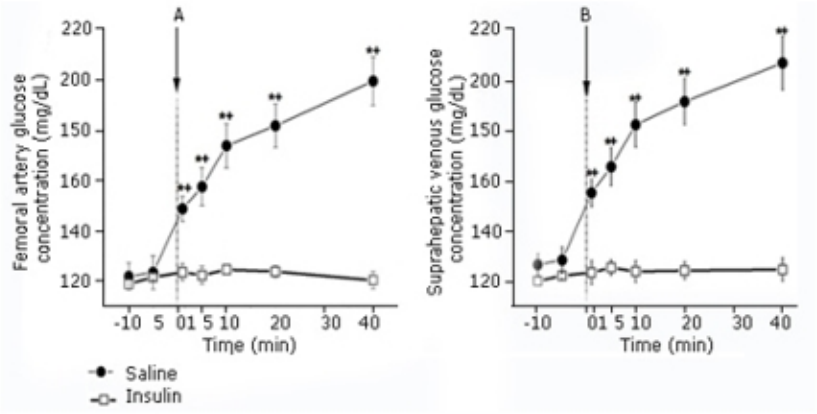


Fig. 1.- Concentration of suprahepatic arterial and venous glucose in healthy control rats with an injection of saline (A) or insulin (B) in the carotid sinus isolated, compared with its basal. The values are means ± standard error, * $p < 0.05$ compared to its basal (paired *t*–test).

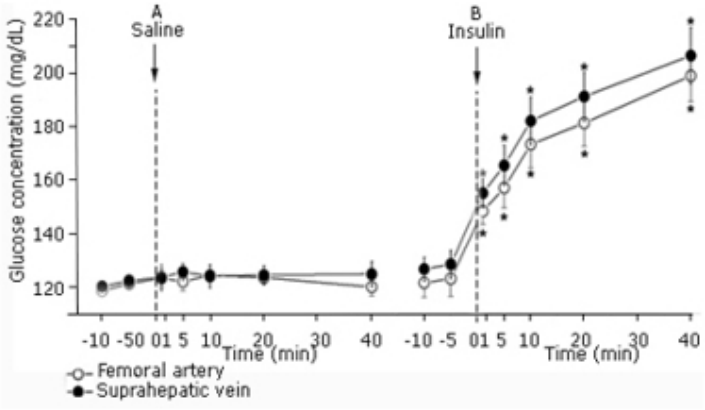


Fig. 2.- (A) femory artery and (B) suprahepatic venous glucose in healthy control rats (saline in the isolated carotid sinus) and in experimental rats (insulin in the isolated carotid sinus). Insulin produced a significant increase in both, arterial glycemia and suprahepatic vein; means ± standard error * $p < 0.05$ compared to baseline (paired *t*–test); + $p < 0.05$ compared to saline (two-sample *t*–test).

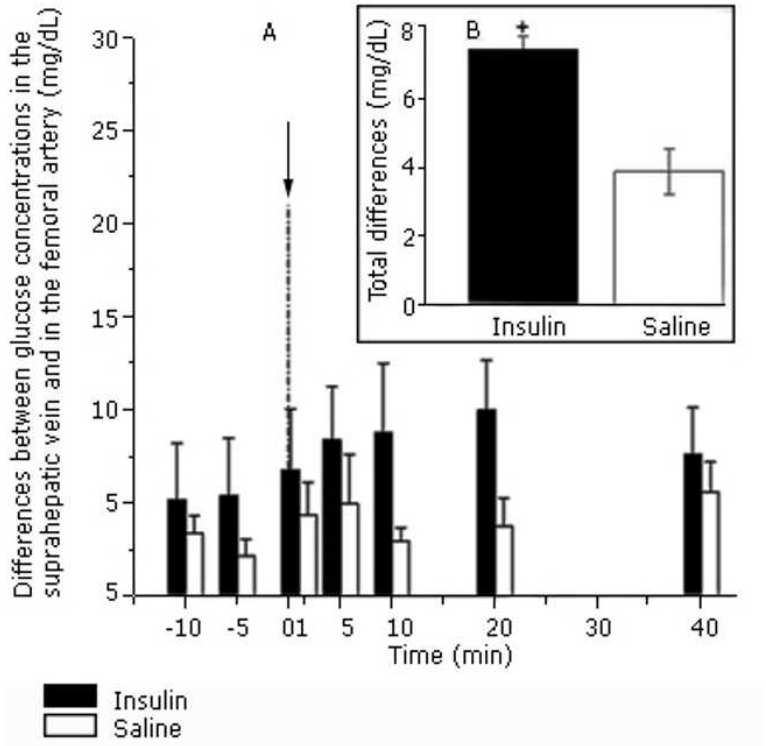


Fig. 3. A) Differences between glucose concentrations in the suprahepatic vein and in the femoral artery in healthy rats with saline or insulin injection in the isolated carotid sinus. B) Comparison of the total differences in all the studied times between both groups: p = 0.005; means ± standard error, two-sample *t*–test.

DISCUSSION

These results suggest that the increases in the glucose levels found in the SH vein and in FA are due to CBs insulin stimulation. The participation of CBs in the metabolic functions initiated by insulin is already reported (Prabhakhar, and Joyner, 2015, Conde et al., 2018). In this work CB stimulation by insulin induces hyperglycemia, contrary to the classic insulin hypoglycemic effect. The activation of CB by hypercaloric diet induces hyperinsulinemia and arterial hypertension (components of the metabolic syndrome) (Ribeiro et al., 2013). Intravenous insulin on CB augments ventilation (Prabhakar and Joyner, 2015). CB stimulation activates the sympathoadrenal pathway with hepatic glycogenolysis (Álvarez-Buylla et al., 1997). Therefore, hyperinsulinemia detected by CB induces hyperglycemic reflex (paradoxical effect) establishing a vicious circuit that leads to a greater hyperinsulinemia due to sympathoadrenal activity (Álvarez-Buylla et al., 1997; Thompson et al., 2016).

Conclusion: Insulin in ICS stimulates the carotid body and triggers an increase in glucose output by the liver (SH glycemia) that results in FA hyperglycemia (Figure 8). Saline into ICS had no effect on blood glucose levels.

Perspectives : In order to determine whether the hyperglycaemic effect of insulin is due to a direct stimulation on CC or to the central action of this hormone, with participation of sympathetic-adrenal system counterregulation mechanism of (Thompson et al, 2016), glycemia could be quantified venous suprahepatic, arterial glucose and adrenaline levels in the blood rats in order to determine the sympathetic-adrenal effect in the hyperglycemic response, after injecting insulin into the CC or into the nucleus of the solitary tract of the brain stem, place of confluence of the afferents of the carotid sinus nerve.

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