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Hyperglycemic response to hemorrhage is modulated by baroreceptors unloading but not by peripheral chemoreceptors activation

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Abstract

The aim of this study was to assess the relative participation of carotid baro- and chemoreceptors on plasma glucose and lactate level in response to hemorrhagic hypotension. We also evaluated the effects of selective activation of carotid chemoreceptors.

One week before the experiments, male Wistar rats (250–300 g) were submitted to bilateral total carotid denervation (BCD-group), or to bilateral ligature of the carotid body artery (ChD-group). During the same surgical procedure, a chronic jugular catheter for blood sampling and hemorrhage (1.2 mL/100 g/2 min) and polyethylene cannula was inserted into the left femoral artery for cardiovascular monitoring. One group submitted to fictitious surgery was used as a surgical control (Sham-group). Carotid chemoreceptors were selectively activated by sodium cyanide (NaCN, 40 μg/0.1 mL i.v.) in the Sham and ChD group. The results showed that hyperglycemic response to hemorrhage in the BCD-group was reduced whereas in the ChD-group there was no significant change in this parameter compared to the Sham group (8.6±0.5 mM, Sham-hemorrhaged, $n=8$; 7.2±0.3 mM, BCD-hemorrhaged, $n=8$ and 9.4±0.6 mM, ChD, $n=8$, $p<0.05$). Increased plasma lactate levels following hemorrhage were observed in all the three experimental groups throughout the experimental period and there were no differences between the groups. Chemoreceptor stimulation by NaCN also produced hyperglycemia, as well as an increase in blood pressure and bradycardia but did not affect plasma lactate concentration. Ligature of the carotid body artery annulled the cardiovascular responses induced by NaCN, but did not change the hyperglycemic response to hypoxia. In conclusion, our data indicate that carotid chemoreceptors do not play any major role in overall metabolic response to hypoxia or hemorrhagic hypotension. Furthermore, the results suggest that carotid baroreceptors unloading play a predominant role as main source of afferent impulses leading to the hyperglycemic response to hemorrhage. In addition our data shows that the metabolic response and cardiovascular adjustment to hypoxia can be dissociated by ligature of the carotid body artery.

Keywords: Hemorrhage; Plasma glucose; Lactate; Carotid receptor

1. Introduction

Hemorrhage is a powerful stimulus that induces an increase in plasma glucose and lactate (Yamaguchi, 1992; Machado et al., 1995a,b; Silveira et al., 2003), as also seen under many other stressful conditions (Yamaguchi, 1992; Lima et al., 1998; Reis et al., 1998). Hemorrhagic hypotension induces cardiovascular adjustments that are triggered by a change in the pressure load at arterial baroreceptors located at the aortic arch and the carotid sinuses. However, these two pressure-sensitive sites are not equivalent in their ability to protect arterial blood pressure during hypotensive challenges and hypovolemia (Abdel-Rhaman, 1992; Blombery and Korner, 1979; Barazanji and Cornish, 1987; Stauss, 2002). As in the case of arterial blood pressure, the relative contribution of carotid sinus and aortic baroreceptors to the metabolic adjustments made in response to hemorrhage hypotension appear to be different. Our previous studies showing that carotid receptor (baro- and chemoreceptors) denervation markedly reduced the

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hyperglycemia induced by hemorrhagic hypotension to 30% (Silveira et al., 2003) are in agreement with this hypothesis. These studies suggest that the neural input from carotid receptors (chemo- and/or baroreceptors) are the main neuronal afferent pathway activated by hemorrhage-induced hyperglycemia. Since the aortic depressor nerve does not contain a functionally significant number of chemoreceptor-afferent fibers and does not appreciably contribute to generation of chemoreflex (Kobayashi et al., 1999), the remaining 30% of the hyperglycemic response to hemorrhage is probably due to mechanisms triggered by the unloading of aortic baroreceptors.

It is well-known that hemorrhagic hypotension, in addition to decreasing baroreceptor discharge, leads to carotid chemoreceptor stimulation as a result of stagnant anoxia induced by decreased blood flow to the chemoreceptors (McCloskey, 1975; Lahiri et al., 1980). Because the carotid denervation performed in earlier studies (Silveira et al., 2003) involved not only baro- but also chemoreceptors within the carotid body, there is a possibility that both carotid receptors could be simultaneously activated by hemorrhagic hypotension. However, in the same experiment, we observed a close correlation between the intensity of hypotension and hyperglycemia. Although it would be reasonable to assume that the carotid baroreceptors are the most relevant afferents involved in this control, the relative contribution of chemoreceptor also needs to be evaluated. It is important to point out that there is evidence that the activation of carotid chemoreceptors is involved in mechanisms of increased glucose output from the liver induced by NaCN i.v. injection (Alvarez-Buylla et al., 1997), reduction in PaO_2 (Zinker et al., 1994) or mild hypoglycemia (Koyama et al., 2000). The present experiments were therefore designed to separately assess the contribution of peripheral chemoreceptor activation and carotid baroreceptor unloading in hemorrhagic hyperglycemia and increased lactate levels.

2. Materials and methods

2.1. Animals and surgical procedure

Male Wistar rats weighing 250–300 g were housed individually, maintained on a 14:10 h light–dark cycle (0500–1900 h) and allowed free access to food and water. One week before the experiments, a silastic catheter was inserted under thiopental anesthesia (30 mg/kg body weight, i.p.) via the jugular vein into the right atrium for repeated blood sampling and bleeding (Machado et al., 1995a,b; Silveira et al., 2003). During the same surgical procedure, a polyethylene cannula was inserted into the left femoral artery for cardiovascular monitoring. The atrial and the arterial catheters were filled with heparin (25 U/mL) in normal saline which was replaced every 2 days. The animals were also implanted with arterial cannula for cardiovascular monitoring. The arterial catheter was a PE-10-tipped polyethylene cannula filled with heparin in normal saline that was inserted into the abdominal aorta through the left femoral artery. The free ends of both cannulae were tunneled subcutaneously and exteriorized in the cervical dorsal area. The arterial cannula was attached to a 40 cm PE-50 tube during the recording period, allowing the rat to move freely within the cage. Arterial pressure was recorded by connecting the arterial cannula to a pressure transducer coupled to a MP100 System Guide (Biopac Systems Inc., Santa Barbara Ca. model MP100-CE series 198122765).

To denervate both carotid receptors (baro- and chemoreceptors, BCD-rats), a 2 to 3 cm midline incision was made in the ventral neck and the sternocleidomastoid muscles were reflected laterally, exposing the carotid sheath of the cervical fascia. The left and right common carotid arteries were stripped of connective tissue and a 10% phenol solution in saline was gently applied over the surface of the carotid bifurcation region. The chemoreflex was tested 6 days after surgery to confirm chemoreceptor denervation with i.v. injection of 40 μg/0.1 mL NaCN.

To selectively denervate the carotid chemoreceptors (ChD-rats) the same surgical approach described above was used to reach the carotid bifurcation. Bilateral ligature of the carotid body artery was performed, leading to denervation due to ischemia. In this group, the chemoreflex was also tested using an i.v. injection of NaCN (40 μg/0.1 mL i.v.).

One group submitted to fictitious surgery was used as a surgical control (Sham group). Before being submitted to the experiments, all rats were allowed to recover from surgery for 5 to 7 days and all exceeded their respective preoperative weights.

All experiments were approved by the Ethics Committee for the Care and Use of Laboratory Animals of the Federal University of Minas Gerais and were carried out in accordance with the regulation described in the Committee's Guiding Principles Manual.

2.2. General procedure

On the day of the experiment each rat in each of three groups (BCD, ChD and Sham group) had its venous catheter connected to a PE-50 tube (40 cm) filled with heparin (25 U/mL) in normal saline (0.15 M NaCl). The same procedure was carried out with the arterial catheter to allow recording of arterial pressure. The animals were then allowed to rest for 1 h before the experiment (time zero). At time zero hemorrhage was induced (1.2 mL/100 g body wt./2 min of hemorrhage), or NaCN (20, 25, 30, 40, or 60 μg) diluted in 0.1 mL of saline or vehicle (0.15 M NaCl) was injected into the vein catheter.

Three different protocols were used:

Protocol 1: At time zero, animals were bled rapidly. Blood samples (0.3 mL) were collected at 0 min