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Autonomic control of the cardiovascular system in the cat during hypoxemia

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ABSTRACT

This study aimed to determine the roles played by the autonomic interoreceptors, the carotid bodies (cbs) and the aortic bodies (abs) in anesthetized, paralyzed, artificially ventilated cats' response to systemic hypoxemia. Four 15 min challenges stimulated each of 15 animals: (1) hypoxic hypoxia (10%O_2 in N_2; HH) in the intact (int) cat where both abs and cbs sent neural traffic to the nucleus tractus solitarius (NTS); (2) carbon monoxide hypoxia (30%O_2 in N_2 with the addition of CO; COH) in the intact cat where only the abs sent neural traffic to the NTS; (3) HH in the cat after transection of both aortic depressor nerves, resecting the aortic bodies (HHabr), where only the cbs sent neural traffic to the NTS; (4) COH to the abr cat where neither abs nor cbs sent neural traffic to the NTS. Cardiac output (C.O.), contractility (dP/dt_{MAX}), systolic/diastolic pressures, aortic blood pressure, total peripheral resistance, pulmonary arterial pressure, and pulmonary vascular resistance (PVR) were measured. When both cbs and abs were active the maximum increases were observed except for PVR which decreased. Some variables showed the cbs to have a greater effect than the abs. The abs proved to be important during some challenges for maintaining blood pressure. The data support the critically important role for the chemoreceptor–sympathetic nervous system connection during hypoxemia for maintaining viable homeostasis, with some differences between the cbs and the abs.

1. Introduction

Studies of the autonomic regulation of the cardiovascular system have had a long history. But with the advance in our understanding of mechanisms operating at the cell and sub-cell levels of principal sensors in the autonomic nervous system a study integrating such observations at the organismal level of organization seemed appropriate. This seemed particularly so in view of the new information on the cellular and sub-cellular behavior of the much less studied chemoreceptor, the aortic bodies (Piskuric et al., 2011; Piskuric and Nurse, 2012).

The purpose of this study was to determine the regulatory role of the carotid bodies and aortic bodies on the cardiovascular response to the challenge of hypoxemia. Specifically, we wished to explore how the carotid bodies (cbs) and aortic bodies (abs) acting together, acting singly, or not at all impacted the cardiovascular component of the cardiopulmonary system charged with the delivery of oxygen to the tissues. The animal model used was the cat since it has been reported that the feline genome more closely approximates the human genome than any other mammal outside of primates. Chromosomes from the cat are highly homologous with those of the human (Watanabe, 2000).

2. Materials and methods

2.1. Animal model

Cats of either sex weighing approximately 4 kg were initially sedated with ketamine (35 mg/kg, ip), anesthetized (sodium pentobarbital, 30 mg/kg, iv), paralyzed (succinylcholine, 5 mg/kg, iv), and artificially ventilated. Anesthesia was renewed when the medial canthal reflex showed only a small response.

2.2. Preparation

1. Trachea was cannulated after a midline incision which also exposed the bilateral aortic depressor nerves running within and/or juxtaposed to the cervical vagi.
2. Catheters were inserted into the
   a. Femoral artery for measuring blood pressure and for drawing blood samples.
   b. Femoral vein for further injections of anesthetic, $NaHCO_3$, and glucose. This catheter was advanced into the right atrium for measurements of pressure (Statham pressure transducer P-23 De). After these general procedures were completed a left lateral thoracotomy was performed at the fifth interspace with the animal ventilated on 100% oxygen. The pericardium was cut and the ascending aorta was gently separated from the pulmonary artery.

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An electromagnetic flow probe (Biotronex Laboratory Inc., 6.0 mm; connected to Biotronex Flowmeter, BL 620) was placed around the root of the aorta. Tests were performed to show that this placement did not modify the aortic nerve activity. Proper calibration of the probe was made using a dog's femoral artery through which blood was pumped at differing rates and at various pressures; outflow into a graduated cylinder was timed. A straight line graph was created having a correlation coefficient of 0.99.

c. Left atrium via the appendage for left atrial pressure (P-23 De).
d. Left ventricle for pressure (P-23 Db). The first derivative of the ventricular pressure (LV dP/dt_{MAX}) was recorded with a differentiator (SGM-2).
A triangular wave signal with a known slope was substituted for the pressure signal to calibrate directly the LV dP/dt_{MAX}.
e. Pulmonary artery anchored with a small purse string on the surface of the artery for measuring pressure (P-23 Db).
All pressures were referenced to the level of the right atrium.

3. Aortic depressor nerves were isolated, covered with pledgets soaked in Krebs Ringer bicarbonate solution with a layer of mineral oil on top, and, when necessary, kept warm with a lamp.
4. Temperature was monitored and kept constant between 37 and 39 °C with a rectal probe and heating pads.

2.3. Recordings

1. Variables being recorded from the preparation were led to a polygraph (Electronics for Medicine).
2. Arterial blood samples (0.5–1.0 mL) were collected periodically during the preparation phase and during the control/challenge phases of the protocol. Partial pressures of oxygen and carbon dioxide, and hydrogen ion concentration were measured in arterial blood on the Radiometer BMS3MK2 blood gas analyzer. Oxygen saturation, hemoglobin concentration, and carboxyhemoglobin were measured with a CO-oximeter B (Instrumentation Laboratories #182).

2.4. Experimental design

1. Background studies (Fitzgerald et al., 1979; Fitzgerald and Traystman, 1980; Lahiri et al., 1981b) showed that both carotid bodies and aortic bodies increased their neural output to the nucleus tractus solitarius (NTS) in response to lowered partial pressures of oxygen in the arterial blood (P_aO_2); this also lowered arterial oxygen saturation (S_aO_2). However, the carotid bodies (cbs) did not respond to a lowering of S_aO_2 with carbon monoxide, whereas the aortic bodies (abs) did respond with increased neural output.
2. This behavior of the arterial chemoreceptors allowed a design in which the animal could be challenged with 10% O_2 in N_2 (hypoxic hypoxia, HH) and have both cbs and abs sending increased neural output to the NTS. The animal could then be challenged with carbon monoxide hypoxia (COH) with a normal P_aO_2 and only the abs would increase their output to the NTS. Then once the aortic depressor nerves were transected eliminating their connection to the NTS and the animal was again challenged with HH, only the cbs would be sending increased neural output to the NTS. Finally with COH, neither cbs, nor abs would be sending increased neural output to the NTS. In summary the design allowed having both cbs and abs (HH intact), having only cbs (HH abs transected), having only abs (COH intact), or having neither cbs or abs (COH abs transected) as the organism responded to systemic hypoxemia.

2.5. Statistical evaluation tools

1. Virtually all evaluations used the repeated measures analysis of variance (RMANOVA). If the data did not pass the Normality or Equal Variance Tests, then the Repeated Measures Analysis of Variance on Ranks (RMANOVAR) was used.
2. For the more complex analysis needed for Fig. 2 a generalized least squares with autoregressive correlation for repeated measurements was used.

2.6. Protocol

1. Before settling on the specific protocol below, several pilot experiments were performed to perfect the preparing of the animals, and for testing their tolerance to the hypoxic challenges.
2. Three hours were allowed for the preparation to recover from the surgical procedures.
3. The experimental procedure started with a control period of normoxia, normocapnia. Measurements of aortic flow, cardiac contractility, heart rate, right and left atrial pressure, arterial systolic and diastolic pressures, and pulmonary artery pressures were made (Control; time period 0′). Subsequently total peripheral resistance and pulmonary vascular resistances were calculated.
4. A 15 minute challenge of hypoxic hypoxia (HH; 10% O_2 in nitrogen) ensued in which all variables were measured continuously with blood samples taken at the 7 and 15 minute time period.
5. A one hour time period under normoxic/normocapnic conditions followed.
6. After a second control period the animal was then ventilated for 15′ on carbon monoxide (COH; 2% in air for the first 2 min [reducing S_aO_2 to50–60%]and then 0.1% for the last 13 min [reducing S_aO_2 to 40–45%]). Blood samples were taken as above. This completed the INTACT phase of the experiment.
7. Aortic nerve transection (i.e., aortic body resection) followed while the animal was moderately hyperventilated on 95%O_2/5%CO_2 for 1 h or more to facilitate the elimination of carbon monoxide (CO).
8. When S_aO_2 had returned to control values, the animal was ventilated on room air (21%O_2 in N_2) for 15–20 min.
9. Then steps 4, 5, 6 above were repeated in the abr animals. This concluded the experiment.
10. Animals were sacrificed with an i.v. injection of Na Pentobarbital (50 mg/kg). An absence of heart beat for 5 min was the end point. Preparation of the animal and steps in the protocol were approved by the University's Animal Care and Use Committee which follows the National Institutes of Health's norms and guidelines for the care and use of animals.

3. Results

I. Stimulus
  A. Blood gas data are found in Table 1.
II. Cardiac responses
  A. Cardiac output (C.O.)
    1. C.O. (Fig. 1) rises during HHint (*cbs/abs*) to a maximum at 5′ of 428.7 mL/min, which significantly exceeds control by 56.7%. During COHint (*abs*) the increase is more gradual but significantly exceeds control if only by 39.3%. In the HHabr (*cbs*) cats C.O. rose to a maximum increase at 7′ of 381.4 mL/min which significantly exceeds control by 43.2%. During COHabr (*none*) C.O.'s control was 289.8 mL/min; this actually decreased, and then returned to 292.9 mL/min at 13′. No significant change.
    2. We then sought to determine if there was a difference in C.O. during a hypoxic challenge when both cbs and abs are acting compared to when just one or the other acts. Fig. 2 shows the four C.O. responses over the 15′ of hypoxic challenges. Comparisons will proceed from top to bottom of the plot. It is clear that C.O. during HHint (*cbs/abs*) is significantly higher across the 15′ challenge than C.O. during the COHabr (*none*) challenge over the same time course. Next, using a generalized least squares method with autoregressive correlation for repeated measurements, we found that C.O. during HHint (*cbs/abs*) differed significantly from C.O. during HHabr