Effects of Asphyxia on Arterial Blood Pressure, Formation of Nitric Oxide in Medulla and Blood Parameters in the Cat

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Abstract

The rostral ventrolateral medulla (RVLM) plays an important role in the integration of cardiovascular functions. We examined the effect of asphyxia on cardiovascular responses, on sympathetic vertebral nerve activity (VNA) and nitric oxide (NO) formation in the RVLM, on hemodynamics, and on plasma concentrations of catecholamines, blood gas partial pressures and carbohydrate metabolites. Using 16 anesthetized cats we found that the systemic arterial pressure (SAP), VNA, NO formation and the release of plasma catecholamine components of norepinephrine and epinephrine were increased during asphyxia. The onset of NO production was significantly earlier than that of SAP and VNA. The venous partial pressure of O_2 decreased, while the partial pressure of CO_2 increased. Furthermore, metabolism of glucose and lactate increased, as did the blood concentrations of white and red blood cells, hemoglobin and platelets. Thus, asphyxia increased SAP, VNA and NO formation. It increased the plasma catecholamines, blood gases, carbohydrate metabolites and blood cells.

Key Words: epinephrine, glucose, lactate, hemoglobin, nitric oxide, norepinephrine

Introduction

Hypoxia causes alternations in plasma catecholamines, blood gases and general metabolic rate (13). It also produces marked elevation of systemic arterial pressure (SAP) increases in plasma catecholamines (2), accumulation of lactate (LAC) and lowering of pH during cerebral ischemia (21). The rostral ventrolateral medulla (RVLM) plays an important role in cardiovascular regulation (6, 10), and the dorsomedial medulla (DM) shares this function (3). Microinjection of glutamate (Glu) into the RVLM induces pressor responses, increases in sympathetic vertebral nerve activity (VNA) (25), and increases in plasma epinephrine (Epi) and norepinephrine (NE) concentrations (4, 16). Furthermore, microinjection of NMDA produces changes in SAP and nitric oxide (NO) formation in the nucleus tractus solitarius (NTS) and RVLM (27, 28). The onset of NO production was earlier than that of SAP during bilateral common carotid occlusion (31). The aim of this study was to examine the effect of asphyxia on the SAP, on VNA and NO formation in the RVLM and on changes in plasma catecholamine, blood gas, carbohydrate metabolites and blood cells.

Materials and Methods

General Procedures

Sixteen adult cats, weighing 2.5 ~ 4.0 kg, were anesthetized intraperitoneally with urethane (400 mg/

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kg) and α-chloralose (40 mg/kg). All experimental procedures were approved by the Committee of Animal Care and Use of the Institute of Biomedical Sciences Academia Sinica, under the guidelines of the National Science Council. The general procedures have been described (28). Briefly, the SAP, mean SAP (MSAP) and heart rate (HR) were monitored. Artificial ventilation and asphyxia was achieved *via* tracheal intubation.

Brain Stimulation

The head of each cat was fixed in a David-Kopf stereotaxic apparatus. The dorsal surface of the brain stem was exposed and the obex was used as the reference point. A micropipette (outside tip diameter 20-30 μm) was glued together with a NO electrode. The distance between the tips of the micropipette and electrode was 150-250 μm. This pipette-electrode, inclined at 34° from the stereotaxic frame, was inserted into the RVLM (3.8-4.5 mm anterior to the obex, 3.7-4.2 mm lateral to the midline and 3.5-4.5 mm ventral to the dorsal surface of the medulla). The micropipette, containing glutamate (Glu, 0.1 M) was connected to a pneumatic pressure system (PPS-2, PPM-2, Medical Systems Corp., Great Neck, NY, USA) for chemical microinjection. The Glu was dissolved in saline with 0.01% Pontamine sky blue marker dye. The injection volume was measured by monitoring the movement of the fluid meniscus in the micropipette through a stereomicroscope. At the end of each experiment, the animal was euthanized with an overdose of pentobarbital. The brain was removed and immersed in 10% formalin saline for 8 h. After fixation, frozen transverse sections (50 μm thick) of the brain were stained with Cresyl violet to identify the injection sites.

Nerve Recordings

The left sympathetic vertebral nerve was isolated, cut at its distal end, desheathed and placed on a bipolar platinum electrode. Nerve activities were measured using a preamplifier (WPI, DAM 60; bandpass frequency 3 Hz to 3 kHz), monitored with an oscilloscope (Gold 4050), rectified and integrated (Gold, integrator) with a reset time of 1 s as described previously (25, 26).

NO Measurement

Extracellular NO concentration was monitored using a voltammetric measurement system (IVEC-10, Medical Systems Co., Greenvale, NY, USA) as described (28-30). A miniature Ag/AgCl reference electrode was inserted into the cerebrum. The working electrode consisted of a double carbon fiber filament (each 30 μm in diameter), coated with nafion (5%) solution at 65 °C (9) and then electropolymerized with 2 mM Ni-meso-tetra (*N*-methyl-4-pyridyl) porphyrine-tetratosylate in 0.1 M NaOH (19) and 5 mM *o*-phenylenediamine solution in 0.1 M phosphate buffer solution (PBS; pH 7.4) at +0.9 V for 25-50 min (8). This was inserted into the RVLM. Detection and calibration of NO concentrations were carried out using 1.0-3.0 μM S-nitroso-*N*-acetyl-DL-penicillamine (SNAP), NE (2 μM), dopamine (2 μM), tyrosine (2 μM) and nitrite (2 μM) in 0.1 mM PBS. One micromole per liter of SNAP produces 1 nmol/l NO *in vitro* (7).

Plasma Measurements

Blood samples (2 mL each), were collected from the right femoral vein through a polyethylene (PE 90) at baseline and ~3 min after the start of asphyxia. Asphyxia was produced by turning off the ventilator and clamping both endotracheal tubes. Blood gas and pH were measured at 37.5°C using an automated blood gas analyzer (ABL-300, Radiometer Copenhagen). Blood concentrations of glucose and lactate were measured using a Glucose/L-Lactate analyzer (2300 STAT). White blood cell (WBC), red blood cell (RBC), hemoglobin (HB) and platelet (PLT) counts were measured using Haematology (Cobas Minos). Blood was centrifuged immediately after withdrawal; the plasma was drawn off, and 4N perchloric acid (1/10 of plasma volume of the sample) was added. After storage overnight at -70°C, the plasma was thawed and then centrifuged at 12000 rpm (Kuba TA KR-1500 centrifuge) for 10 min. Catecholamines were extracted using the method as previously described (4). Plasma catecholamine concentrations were analyzed by high performance liquid chromatography (HPLC; Waters 717 plus) with electrochemical detection (Waters 464), using 3, 4-dihydroxy benzylamine as an internal standard following the standard procedure of Millipore (Millipore, Water Chromatography, Milford, MA, USA).

Data Analysis

All data are presented as means ± standard error of mean (SEM). Percent changes in parameters in response to asphyxia were calculated using the following formula: (Response value – Control value) / (Control value) × 100%. Changes in all data were analyzed using Student's paired *t*-tests with statistical significance set at $P < 0.05$.

Results

Sixteen RVLM sites from 16 cats were recorded

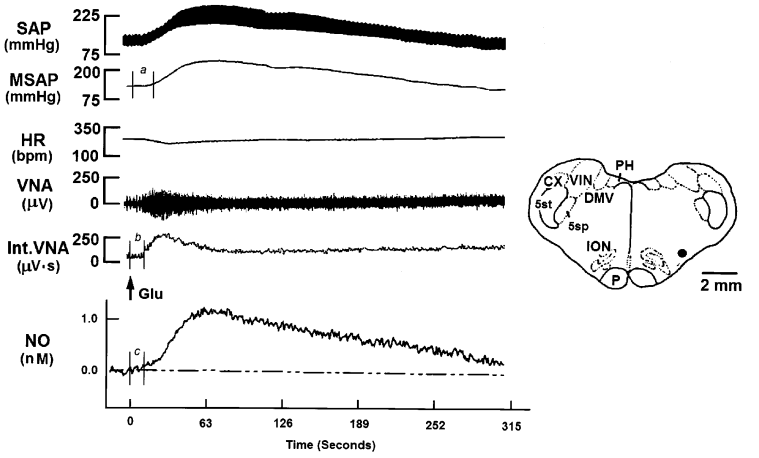

Fig. 1. Effects of glutamate (Glu) on systemic arterial pressure (SAP), mean systemic arterial pressure (MSAP), heart rate (HR), vertebral nerve activity (VNA) and nitric oxide (NO) in the rostral ventrolateral medulla (RVLM), shown from top to bottom, respectively. Microinjection of Glu (5 nmol in 50 nl) produced increases in MSAP by 80 mmHg, a decrease in the HR by 35 bpm and an increase in integrated VNA (Int.VNA) by 260%, and these were associated with an increase in NO concentration of 1.1 nM. The latency of onset was different: MSAP (a) 16.2 s; Int.VNA (b) 12.1 s; NO (c) 11.6 s.

using NO electrodes in this study. The electrodes were sensitive to SNAP (2 μM), but not to NE (2 μM), dopamine (2 μM), tyrosine (2 μM) and nitrite (2 μM). A stable background was monitored for about 5 min after the electrode was implanted in the RVLM. Asphyxia-evoked NO release (ΔNO) from the brain was measured by comparing the peak NO value before and after asphyxia. Microinjection of Glu (5 nmol in 50 nl) into the RVLM produced increases in SAP and NO, as shown in Figure 1. This produced an increase of 55% in MSAP (from 113.3 ± 10.5 to 175.6 ± 14.5 mmHg, $P < 0.05$), a reduction of 17.5% in HR (from 192.3 ± 7.4 to 158.6 ± 7.8 bpm, $P < 0.05$), an increase of 139% in integrated VNA (Int.VNA: from 78.4 ± 12.43 to 187.6 ± 19.6 μv·s, $P < 0.01$) and a ΔNO of 1.1 ± 0.3 nM. The mean onset times for changes in MSAP, Int.VNA and NO levels were 14.6 ± 2.4 s, 10.2 ± 2.1 s and 8.6 ± 1.2 s, respectively. Thirty minutes after the first injection of Glu into the RVLM, changes in MSAP, Int. VNA and peak NO signals induced by the second dose of Glu were essentially not altered (85 ± 7%, 91 ± 9% and 88 ± 6% of control; n = 5). This suggests that the sensitivity of RVLM was not altered when Glu was injected at an interval of 30 min.

Asphyxia also produced increases in MSAP, VNA and NO release in the RVLM. Typical SAP, HR, VNA and NO response curves during asphyxia are shown in Figure 2. MSAP increased 65% (from 122.5 ± 13.2 to 202.1 ± 16.2 mmHg, $P < 0.05$), HR increased 45% (from 197.1 ± 8.4 to 285.8 ± 14.6 bpm, $P < 0.05$), Int.VNA increased 150% (from 82.5 ± 13.2 to 206.3 ± 19.2 μV·s, $P < 0.01$) and the ΔNO was 4.5 ± 0.5 nM (Fig. 3). The mean onset times for

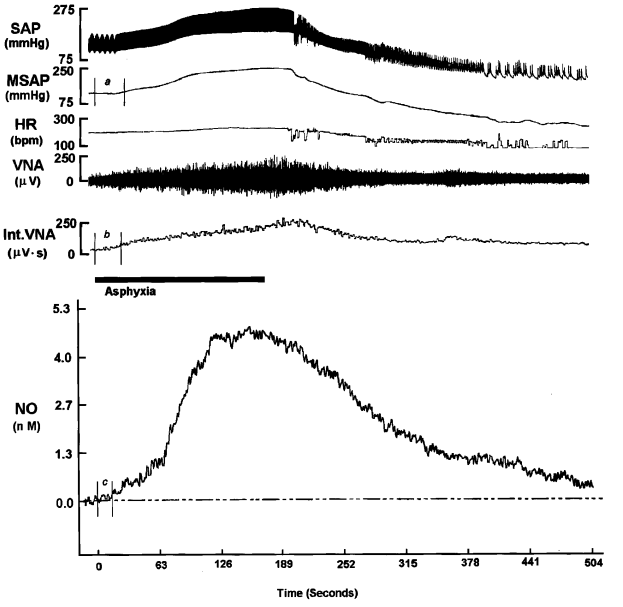

Fig. 2. Effects of asphyxia on systemic arterial pressure (SAP), mean systemic arterial pressure (MSAP), heart rate (HR), vertebral nerve activity (VNA) and nitric oxide (NO) in the rostral ventrolateral medulla (RVLM), shown from top to bottom, respectively. Asphyxia produced increases in MSAP (100 mmHg), in HR (35 bpm), in Int.VNA (320%) and in NO (4.5 nM). The latency of onset of change in MSAP was 25.3 s (a); in Int.VNA was 22.3 s (b), and in NO level was 11.6 s (c). The bar (–) indicates the time course of asphyxia.

changes in MSAP, Int.VNA and NO levels were 23.5 ± 4.2 s, 21.2 ± 3.5 s and 9.7 ± 1.7 s, respectively.

Plasma catecholamines, blood gas partial pressures and hemodynamic parameters were also

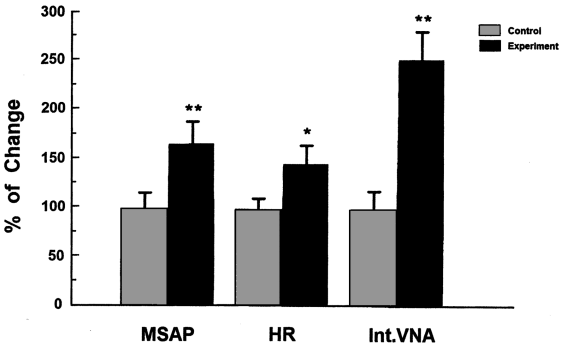

Fig. 3. Effects of asphyxia on cardiovascular responses and sympathetic nerve activity. Bar graphs show the changes in MSAP, HR and Int.VNA before (▒, n = 16) and after (■, n = 16) asphyxia. Asphyxia produced increases in MSAP, HR and Int.VNA. Values are means ± SEM. *$P < 0.05$; **$P < 0.01$ compared with control.

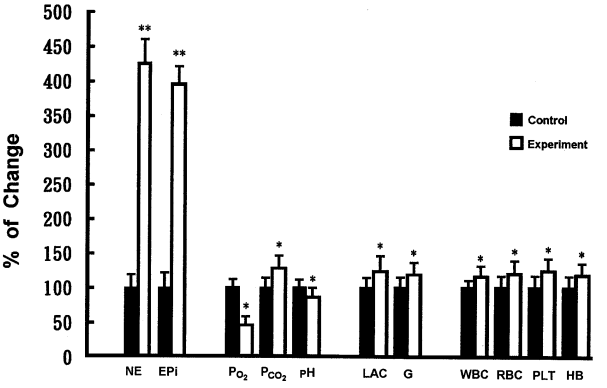

Fig. 4. Effects of asphyxia on plasma catecholamines, metabolism, blood gas, and hemodynamic parameters. Bar graphs show the percentage changes in plasma catecholamines, blood gas, metabolism and hemodynamic measures before (■, n = 16) and after (□, n = 16) asphyxia. Asphyxia produced marked increases in plasma NE and Epi concentrations, increases in the metabolism of G and LAC, increases in WBC and RBC counts and HB and an increase in PCO_2, while it decreased pH and PO_2. Abbreviations: Epi, epinephrine; NE, norepinephrine; LAC, lactate; G, glucose; HB, hemoglobin; WBC, white blood cells; RBC, red blood cells; PLT, platelets; PCO_2, partial pressure of CO_2; PO_2, partial pressure of oxygen. Values are means ± SEM. *$P < 0.05$; **$P < 0.01$ compared with control.

changed after asphyxia (Fig. 4). Blood samples were collected from the femoral vein when the SAP increase had reached its plateau (about 3 min after beginning asphyxia). For the plasma catecholamines, there was a 425% increase in NE (from 2.8 ± 0.5 to 14.7 ± 1.5 ng/ml, $P < 0.01$) and a 395% increase in Epi (from 1.85 ± 0.2 to 9.2 ± 0.8 ng/ml, $P < 0.01$). For the blood parameters, there was a 54% decrease in PO_2 (from 45.5 ± 3.2 to 20.8 ± 2.5 mmHg, $P < 0.05$), a 27% increase in PCO_2 (from 37.6 ± 3.3 to 47.9 ± 4.7 mmHg, $P < 0.05$) and a 7.1% decrease in pH (from 7.34 ± 0.18, to 6.82 ± 0.12, $P < 0.05$). For metabolites, there was a 23% increase in LAC (from 1.2 ± 0.2 to 1.5 ± 0.3 mmol/l, $P < 0.05$) and an 18% increase in glucose (from 143.3 ± 9.8 to 169.5 ± 11.5 mg/100 ml, $P < 0.05$). For blood cell components, there was a 15% increase in WBC (from 35.3 ± 1.9 to 40.7 ± 2.3 in $10^3/mm^3$, $P < 0.05$), a 20% increase in RBC (from 6.2 ± 0.4 to 7.4 ± 0.7 in $10^3/mm^3$, $P < 0.05$), a 23% increase in PLT (from 193.4 ± 15.6 to 238.3 ± 17.4 in $10^3/mm^3$, $P < 0.05$) and a 17% increase in HB (from 12.6 ± 1.8 to 14.7 ± 2.1 in g/mm^3, $P < 0.05$).

Discussion

The present study used cats as experimented model. Cats are more tolerant than rats and rabbits to asphyxia. Besides, it tolerates better withdrawal of blood sample for analysis. In this species the responses of catecholamine release, cardiovascular responses and NO formation to asphyxia will reach its plateau after asphyxia in three minutes.

We measured NO in the RVLM stimulated by Glu and alterations of NO levels during and after asphyxia. Microinjection of Glu induced hypertension and NO release in the RVLM. In addition, asphyxia produced increases of the following parameters: SAP, VNA, NO release, plasma catecholamines, carbohydrate metabolites, blood cell counts. The blood PCO_2 was increased while the pH was decreased. The onset of NO release was earlier than that of the VNA and SAP during asphyxia.

We showed previously that microinjection of Glu into the RVLM produces increases in SAP and VNA (25). This, and the results here are consistent with our report that microinjection of NMDA into RVLM induces increases in SAP and NO formation (28). The formed NO may diffuse and excite adjacent sympatho-excitatory neurons in the RVLM to activate guanylate cyclase, causing an accumulation of cGMP and then increase the entrance of Ca^{2+} and Na^{+} through their ion channels. This increased Ca^{2+} influx may activate NMDA receptors in the RVLM resulting in an increase in SAP. In addition, the onset of changes in NO resulting from Glu injection was 5 s earlier than that of SAP. These results were consistent with a report that the onset of NO production was about 5 s earlier than the change in SAP in a study of bilateral common carotid occlusion, in which the recorded area was in the NTS (31). NO levels increase immediately after the onset of asphyxia have been reported by others (18, 20). Similar to carotid occlusion, asphyxia produced hypertension and an increase in NO concentration in the RVLM, proportional to the increase of SAP. In the meantime, the onset of changes in NO levels was 14 s and 11 s earlier respectively than those of the Int. VNA and SAP.

In other word, during sympathetic neurons discharge by whatever means of activation, i.e., Glu, carotid occlusion or asphyxia, NO production appeared to be the first response. This is logical as the NO electrode was directly placed in the RVLM. It recorded instantaneously the release of NO (9.7 s). The onset of VNA increase came second (21.2 s), while the SAP appeared last (23.5 s). This is reasonable as when the RVLM neurons are activated, the increased firing takes time descending to the sympathetic nerve including the vertebral nerve that we recorded. When the nerve activity reached a critical level, then the SAP rose.

The magnitude of the asphyxia responses was different. The NO release in asphyxia was about four times higher than that of Glu stimulation (4.5 v.s. 1.1 nM), as asphyxia activates all structures containing sympathetic and parasympathetic neurons in the brain, whereas Glu injection only stimulates local sympathetic neurons. This is understandable, because the effect of asphyxia simultaneously stimulates all systems in the body and involves a large number of mechanisms, i.e., respiratory, circulatory, metabolic and nervous systems at the same time. It is worthwhile to note, however, the amplitude of changes in SAP induced by Glu or asphyxia were similar, because SAP increase activates baroreceptor reflex and thus reduces the magnitude of SAP increase.

The increase of NO release during asphyxia is interesting. NO is a potent vasodilator. Thus its release during asphyxia may help to increase brain blood flow to effect protection. However, the elevation of NO may induce cell damage because NO can react with the oxygen radical superoxide to form peroxynitrite (1, 22, 23). Asphyxia might also activate pressure-sensitive neurons of the brain and produces hypoxia of the brainstem, stimulation of the vagus nerve, and induction of baroreceptor reflex (15). It has been known that NO is an important neurotransmitter involved in the baroreceptor reflex (5, 12). An elevated SAP will activate afferent vagal neurons in NTS to release Glu that stimulates the local production of NO (24, 32). This leads to a decrease of sympathetic outflow (6).

During asphyxia, the pressor neurons in RVLM are activated. This increases plasma concentrations of Epi, NE and dopamine (2, 4). The latter event is mediated through sympathetic neural inputs from the splanchic nerve and adrenal medulla. These catecholamines, which released from the sympathetic nerve endings and the adrenal medulla, eventurally enter the general circulation. This also provides a mechanism for supplementing sympathetic nerve activity.

Blood pH and PO_2 were decreased by asphyxia while PCO_2 was increased. Blood pH is a factor involved in mediating the rise of plasma catecholamines during hypoxia (14). In addition, asphyxia increased glucose and LAC concentrations. This is consistent with observations that the metabolisms of glucose and LAC are increased during asphyxia (11).

Sympatho-adrenal mechanisms play an important role in cardiovascular responses to asphyxia in mammals. The spleen, serving partly as a blood reservoir, is innervated by the sympathetic component of the splenic nerve. The spleen plays many important functions including filtration, phagocytosis and destruction of erythrocytes, storage of viable blood cells, antigen uptake, lymphocyte production, antibody formation and potential hemopoiesis etc. (17). Theoretically, sympathetic activation will cause contraction of the spleen and release of the stored blood cells and HB. This will assist the redistribution of blood flow toward the brain and muscle beds during asphyxia. A number of studies have reported an accumulation of LAC and lowering of blood pH during cerebral ischemia (21). Hypoxia causes alternations in plasma catecholamines, blood gases and metabolic rate (13, 16).

In conclusion, we found that asphyxia in anesthetized cats produced increases in SAP, VNA and NO formation as well as increases in plasma catecholamine, carbohydrate metabolites, blood cell parameters and PCO_2, but decreases in PO_2 and pH. In addition, NO production occurred significantly earlier than the changes in SAP and VNA. The precise correlation of NO with the above physiological changes require further study.

Acknowledgments

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