CO₂ asphyxia increases plasma norepinephrine in rats via sympathetic nerves

VICKY BOROVSKY, MIKE HERMAN, GAIL DUNPHY, ANN CAPLEA, AND DANIEL ELY
Department of Biology, University of Akron, Akron, Ohio 44325-3908

CO₂ asphyxia increases plasma norepinephrine in rats via sympathetic nerves. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R19–R22, 1998.—The objective of this study was to determine whether the plasma norepinephrine (NE) increase in rats exposed to CO₂ asphyxia was due to adrenal gland release or sympathetic nerve ending (SNS) release. Plasma NE was measured by high-performance liquid chromatography in hypertensive and normotensive rats using the following protocol: control session, CO₂ exposure, N₂ exposure, reserpine + CO₂, and adrenalectomy + CO₂. Four strains of male and female rats were used: spontaneously hypertensive rats, Wistar-Kyoto rats, and two congenic strains with different Y chromosomes. The same rats were used throughout the experiment (n = 80). Blood pressure measured by aortic telemetry increased by 50–60 mmHg in response to CO₂ in all strains. CO₂ increased NE 6–10× in all strains and both genders. N₂ produced a significant increase in NE (73% of CO₂ response). Reserpine significantly decreased (67%) plasma NE after CO₂. Adrenalectomy did not significantly reduce the NE response to CO₂. In conclusion, the increase in plasma NE after CO₂ was associated with SNS release and not adrenal medullary release, was mainly due to hypoxia, and was not a specific response to CO₂.

OUR INITIAL INTEREST in plasma norepinephrine (NE) levels and CO₂ exposure began with the observation in our laboratory that CO₂ euthanasia, which is quick and inexpensive, produced high levels of plasma NE as determined from blood taken at termination. This led to a literature search, which showed that plasma NE increases in association with hypoxia during the birth process (10). Also patients with respiratory distress showed increased tidal volume CO₂, which led to increased blood pressure and increased plasma NE as a result (1, 11, 15). Likewise, plasma NE increased in patients with sleep apnea syndrome (2), which induces hypoxemia and periodic hypertension. Also, plasma NE increased after Sevoflurane anesthesia and other hypoxic conditions in rats (6, 8, 9, 13, 19) and in fetal sheep (14).

Plasma NE is released primarily by either the adrenal medulla or by spillover from peripheral sympathetic nerve endings. Therefore, our objective was to investigate the specific source of the NE in rats as they responded to CO₂ and to establish whether the increase was due to CO₂ specifically or asphyxia in general.

METHODS AND MATERIALS

The first experiment was designed to measure the relationship between time of CO₂ exposure and plasma NE in order to set standard time for CO₂ exposure. Nine groups of male adult spontaneously hypertensive rats (SHR) were used (5/group) and were exposed to CO₂ for 5–75 s; blood samples were taken for NE. The second experiment was designed to measure plasma NE different treatments. Baseline plasma NE levels were measured in four strains of rats were measured in both sexes (n = 10/group): normal blood pressure or Wistar-Kyoto (WKY) strain, SHR, normal rats with a Y chromosome from an SHR father (SHR/y), and hypertensive rats with a Y chromosome from a normotensive father (SHR/a) (5, 18). Sodium brevital (50 mg/kg ip; Eli Lilly, Indianapolis, IN) was given, and blood was collected retroorbitally (16). Samples were centrifuged (5,000 g), and plasma was transferred to a separate test tube and frozen at −70°C for later NE measurement by high-performance liquid chromatography with electrochemical detection (Waters, Milford, MA) using the technique of Foti et al. (7). The number of animals for each group that had an adequate blood sample volume for NE measurement ranged from 6 to 10.

One week after this baseline procedure, we determined the NE response after the rats were exposed to 100% CO₂ (asphyxia) in a closed chamber for ~30 s. Blood was collected immediately afterward and processed for NE.

The next step in our study was to identify the cause of the NE increase and determine whether it was asphyxia in general or CO₂ specifically that affected the NE level. In order to do that, 1 wk later we exposed the rats to 100% N₂ gas in a closed chamber for 30 s, and blood was collected and processed for NE as before.

After this, we determined whether blocking the sympathetic nerve release of NE with the use of reserpine, which depletes the sympathetic nerve endings of NE, would affect the response to CO₂ exposure. If sympathetic nerves were the major source of NE release in response to CO₂, then the NE level should not increase significantly. The rats were administered reserpine (1.7 g/l, a dose that will lower blood pressure in hypertensive rats; Sigma, St. Louis, MO) in their drinking water for 14 days and then they were exposed to CO₂. Blood was collected for NE as before.

Finally, we performed bilateral adrenalectomies on all animals and evaluated plasma NE levels in response to CO₂. If the adrenal glands were a major source of NE in response to CO₂ asphyxia, the NE level should not significantly increase in adrenalectomized animals after CO₂ exposure. The rats were anesthetized with sodium brevital (50 mg/kg ip); the abdominal area was shaved and disinfected with an iodine solution. A midline incision was used, and each adrenal gland was removed. Ethicon (4–0) was used to suture the peritoneum, and surgical staples closed the skin. Animals were placed on 0.9% saline, final exposure to CO₂ was conducted 2 days later, and blood was collected for NE analysis. In another group of adult males of the same four strains [SHR, SHR/y, SHR/a, and WKY (n = 5–6/group)], aortic telemetry transmitters were implanted (Data Sciences, St. Paul, MN) (4), and, after recovery (1 wk), systolic blood pressure responses to CO₂ were measured. The following statistics were performed: one-way and two-way analysis of variance, followed by Newman-Keuls t-tests where appropriate, Pearson correlation (Sigma Stat, Jandel Scientific, San Rafael, CA), and significance was assumed if P < 0.05.
RESULTS

Figure 1 shows the significant positive correlation between CO₂ exposure time and plasma NE \((r = 0.93, P < 0.001)\) in the group of SHR males.

Figure 2 represents plasma NE responses for both males and females of the SHR/y and WKY strains and of the SHR/a and SHR strains. For the NE level after CO₂ exposure, NE increased 6- to 12-fold compared with baseline (significant in all strains and both genders, \(P < 0.001\)). The results for the NE level after N₂ exposure ranged from 59 to 94% of the NE response to asphyxia with the average N₂ response being 73% of the asphyxia response. The values for the NE level after asphyxia exposure in rats treated with reserpine were about the same as baseline level (67% decrease, \(P < 0.01–0.001\) compared with CO₂ response for all strains and both genders). The NE response to asphyxia in the adrenalectomized group was about the same as in the rats with an adrenal gland: the range of NE increase was 91–107% of the baseline values (\(P < 0.01–0.001\) compared with baseline for all groups and both genders).

DISCUSSION

Our results showed a 6- to 10-fold increase in plasma NE after asphyxia and a 6.8-fold increase to N₂ gas exposure, which suggests a generalized response to the lack of oxygen, not specifically because of CO₂ or gender. Both genders were examined since we have seen gender effects in blood pressure and NE responses.
to acute stress. Similar results were found where increased arterial CO$_2$ and a corresponding decrease in plasma pH were observed to increase plasma NE in anesthetized humans (11). Also Ohkawa et al. (13) and Yli-Hankala et al. (19) noticed an increase in NE levels in response to Sevoflurane gas anesthesia in rats. Anderson et al. (1) showed a correlation between resting end-tidal CO$_2$ concentration and blood pressure. Similarly, an increased plasma NE concentration was observed in patients suffering from sleep apnea syndrome, which supports the conclusion that it is the lack of O$_2$ and not CO$_2$ in particular that produced the elevated NE response (2). It is possible that NE influences arterial chemoreceptor sensitivity and attenuates the hypoxic excitation of the carotid body in response to general hypoxia (15), which could influence gene regulation. Indeed, Millhorn et al. (12) showed that graded hypoxia (21–5%) caused an approximate fourfold increase in tyrosine hydroxylase mRNA in PC-12 cells.

Because reserpine decreased the amount of plasma NE produced in response to asphyxia, it appears that sympathetic nerve release of NE plays a major role in increasing plasma NE. Because adrenalectomy did not reduce the NE response to asphyxia, but in seven-eighths of the groups actually increased the response, the majority of NE appeared to be released from the sympathetic nerve spillover and not from the adrenal medulla. Although there are genetic differences in ventilation rate, minute ventilation, and oxygen consumption in different rat strains (17), all of our strains of rats and both genders reacted to asphyxia in a similar way, which suggests a generalized response.

The blood pressure increase of 50–60 mmHg after CO$_2$ exposure in the males was most likely attributable to the tremendous elevation of NE, acting as a vasoconstrictor. The animals were conscious when the gases were administered and with the CO$_2$ became unconscious at the end of the 30 s, which could account for some of the rise in NE. However, studies in our laboratory using ether, which agitates the animals even more than CO$_2$, only doubled plasma NE compared with a 6–12 times increase in the current study. Therefore, the agitation and handling factor does increase NE but not to the same extent as the sympathetic nervous system (SNS) response from asphyxia. Also taking blood retroorbitally even under anesthetic will produce higher NE values than via catheter collection, but, nevertheless, our results show tremendous NE elevations over baseline values. These results have important implications for studies using CO$_2$ for euthanasia, especially when final blood samples are collected for various assays. The tremendous SNS activation for survival could mask or elevate other hormones or signals being assayed. Female blood pressure was not examined because the NE data did not show any differential response. With milder stimuli, we have shown that the strains with the Y chromosome from a hypertensive father (SHR, SHR/Y) had higher acute and chronic indexes of SNS activity and blood pressure than those with a Y chromosome and from a normotensive father (WKY, SHR/a) (3).

In conclusion, acute exposure to 100% CO$_2$ (asphyxia) increased plasma NE 6–12 fold, mainly through increased release at sympathetic nerve endings and not from adrenal medullary release. This response appears to be a generalized one that is not gender or strain specific or CO$_2$ specific, but rather is due to a lack of oxygen. This finding may be important to know when using CO$_2$ to euthanize animals, since such increased SNS arousal could interfere with other physiological or biochemical variables that may be collected at that time. Also, this response could be used as a stressor to examine maximal SNS responses without causing euthanasia (i.e., 30 s).

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Address for reprint requests: D. L. Ely, Dept. of Biology, Univ. of Akron, Akron, OH 44325-3908.

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