Effects of carbon monoxide on the brain may be mediated by nitric oxide

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Meilin, S., G. G. Rogatsky, S. R. Thom, N. Zarchin, E. Guggenheimer-Furman, and A. Mayevsky. Effects of carbon monoxide on the brain may be mediated by nitric oxide. *J. Appl. Physiol.* 81(3): 1078–1083, 1996.—Carbon monoxide (CO) is known to be a toxic molecule due to the high affinity of hemoglobin for it. However, it has recently been shown that low doses of CO may play a physiological role. The aim of the present study was to examine processes occurring in the brain during exposure to 1,000 parts per million CO that result in an increase in cerebral blood flow (CBF) but are not accompanied by changes in oxidative metabolism. This study was carried out in awake rats with the multiprobe assembly developed in this laboratory for the simultaneous continuous measurement of CBF, intramitochondrial NADH redox levels, direct current potential, and extracellular concentrations of K^+, Ca^{2+}, and H^+ as well as the electrocorticogram. Exposure to 1,000 parts per million CO in air resulted in an increased CBF without any concomitant changes in any of the other metabolic or ionic parameters measured. This indicates that tissue hypoxia was not the trigger for this vasodilation. Injection of N^{ω}-nitro-L-arginine (L-NNA), a nitric oxide synthase inhibitor, before exposure to CO effectively blocked the increase in CBF that was observed when the animal was exposed to CO without prior injection of L-NNA. Furthermore, electrocorticographic depression was observed after the combined treatment of L-NNA and CO. In conclusion, exposure to relatively low doses of CO apparently does not have a deleterious effect on oxidative metabolism because the increase in CBF after this exposure is sufficient to prevent changes in oxidative metabolism, as indicated by the fact that NADH levels remained constant. This protective autoregulatory effect may be mediated by nitric oxide.

N^{ω}-nitro-L-arginine; cerebral blood flow; mitochondrial redox state; extracellular ions; electrocortical activity

THE AFFINITY OF HEMOGLOBIN for carbon monoxide (CO) is 200-fold greater than its affinity for O_2 (29). Thus the presence of CO in the blood will interfere with O_2 delivery to the tissues, and a high concentration of CO is known to be toxic to vital organs such as the heart and brain (11, 28). Increases in cerebral blood flow (CBF) (16) have been reported in both hemispheres after exposure to CO (14). It has been shown that this increased blood flow is proportional to the CO concentration (17). This increase in CBF has been found to maintain adequate O_2 supply to the cerebral tissue after exposure to relatively low CO concentrations (12).

Evidence has recently accumulated suggesting that CO may play a physiological role in the brain. This has been suggested because guanylate cyclase and guanosine 3′,5′-cyclic monophosphate were found in the same location as the heme oxygenase isoenzymes that produce CO (2, 20).

Nitric oxide (NO), another heme ligand molecule, was also considered to be a toxic molecule. Inhibition of NO synthase (NOS), the enzyme that synthesizes NO, causes blood vessel contraction and is reversible when L-arginine (the precursor for NO synthesis) is added to the system (25). Furthermore, NO is responsible for what used to be called endothelium-derived relaxaing factor (10, 26, 27). NO has recently also been found to be a neurotransmitter (4), playing a role in blood flow regulation (6, 30).

Today, it is known that production and localization of both NO and CO occur in the brain and that both activate guanosine 3′,5′-cyclic monophosphate. Because >50% of the energy produced by the brain is utilized for maintaining ionic homeostasis (7), it is important to evaluate the O_2 level and energy balance during CO exposure and to correlate it with ionic homeostasis.

Most studies performed to date on the effect of CO on CBF measured only the blood flow. Very little information exists on the effects of reduced blood O_2 concentrations and on the effects of a change in blood flow on the metabolic activity of the brain and on the mechanism responsible for this increase in CBF.

In our laboratory, we have developed a multiparametric monitoring system for in vivo, continuous, and simultaneous measurement of the hemodynamic, metabolic, ionic, and electrical activities of the brain at the "tissue level" (using "mini" electrodes and probes). This technology enabled us to examine events associated with CBF increases during exposure to concentrations of CO that do not produce any observable metabolic changes. The unique features of this system enable an examination of the mechanism responsible for increased CBF during exposure to 1,000 parts per million (ppm) CO.

The aim of this study was to use the multiparametric measuring system for examining the processes occurring in the brain that result in increased CBF after exposure to 1,000 ppm CO.

MATERIALS AND METHODS

Multiprobe assembly (MPA). This approach was developed several years ago by Mayevsky (21) and Mayevsky et al. (23) and has been used successfully in rats and gerbils. The MPA used in this study contained a fiber-optic (1.8 mm in diameter) probe connected to two instruments, a fluorometer/reflectometer for monitoring NADH and a laser-Doppler flowmeter (LDF) for monitoring CBF. For the LDF, one 50-μm

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input fiber and two 100-μm output fibers were glued in a triangular shape (0.7-mm separation). For measuring NADH redox state, 10 excitation and 10 emission 200-μm fibers (General Fiber, Cedar Grove, NY) were randomly mixed between and around the fiber used for the LDF (i.e., each excitation fiber was adjacent to an emission fiber). This fiber configuration enabled the monitoring of CBF and NADH redox state from nearly the same tissue volume. The MPA also contained three ion-selective electrodes capable of detecting millimolar changes in extracellular ion concentrations of K^+, Ca^{2+}, and H^+. Details of the preparation of these electrodes have been published (9, 22). Direct-current (DC) potential was measured by a highly stable Ag-AgCl electrode located around the K^+, Ca^{2+}, and H^+ electrodes. A reference electrode (also Ag-AgCl) was located below the skin in the animal's neck. Two polished stainless steel rods or platinum wires were inserted into the MPA for electrocorticograph (ECoG) measurement. Brain temperature was measured with a thermistor probe (Yellow Springs Instrument) located inside the MPA (made of Plexiglas) and connected to the thermometer. The MPA was constructed to hold the electrodes to the cannula (with epoxy glue) and was therefore suitable for use in the awake state (Fig. 1).

Animal preparation. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Bar-Ilan University (Ramat-Gan, Israel).

The rats were anesthetized by an intraperitoneal injection (0.3 ml/100 g) of Equithesin [each milliliter contains 9.72 mg pentobarbital sodium, 42.51 mg chloral hydrate, 21.25 mg magnesium sulfate, 44.34% (wt/vol) propylene glycol, 11.5% alcohol, and water]. The skull was exposed by a midline incision in the skin, and a 6-mm hole was drilled in the parietal bone. The dura mater was gently removed, and the MPA was located on the parietal cortex with a micromanipulator so that undue pressure on the brain was avoided. The MPA cannula as well as four stainless steel screws located at different locations on the skull were cemented to the skull with dental acrylic cement.

N^{ω}-nitro-L-arginine (L-NNA) preparation and CO exposure. One hundred milligrams of L-NNA (Sigma Chemical) were dissolved in 6.7 ml of a mixture containing 6.5 ml saline and 0.2 ml 0.7 N HCl. The drug was injected intraperitoneally into the awake animals (50 mg/kg). A mixture of either 1,000 ppm CO in air or pure air was flushed through the sealed cage containing the rat.

Experimental protocol. L-NNA was injected intraperitoneally 1.5 h after completion of the surgery when the animals were awake. The animals were placed in a sealed cage, and air was flushed through the cage for 30 min. A mixture of 1,000 ppm CO in air was then flushed into the cage. After 60 min of CO exposure, air was again flushed into the cage. Animals not exposed to CO but that did receive a L-NNA injection were treated identically but were exposed to air instead of to CO. CO-exposed animals underwent the same procedure only without an L-NNA injection. Control animals were saline injected, air was flushed into the cage, and the rats were monitored for an equivalent period of time, i.e., 3 h.

Experimental period. Four different groups of rats (7 animals each) were used in this study: *group A*, untreated animals; *group B*, animals injected intraperitoneally with L-NNA; *group C*, animals exposed to CO; and *group D*, animals exposed to CO after an intraperitoneal injection of L-NNA. The animals were awake during treatment, and all parameters were monitored continuously for the entire experimental period.

Statistical analysis. Statistical analysis was performed with Student's *t*-test. Values of $P < 0.05$ were considered significant.

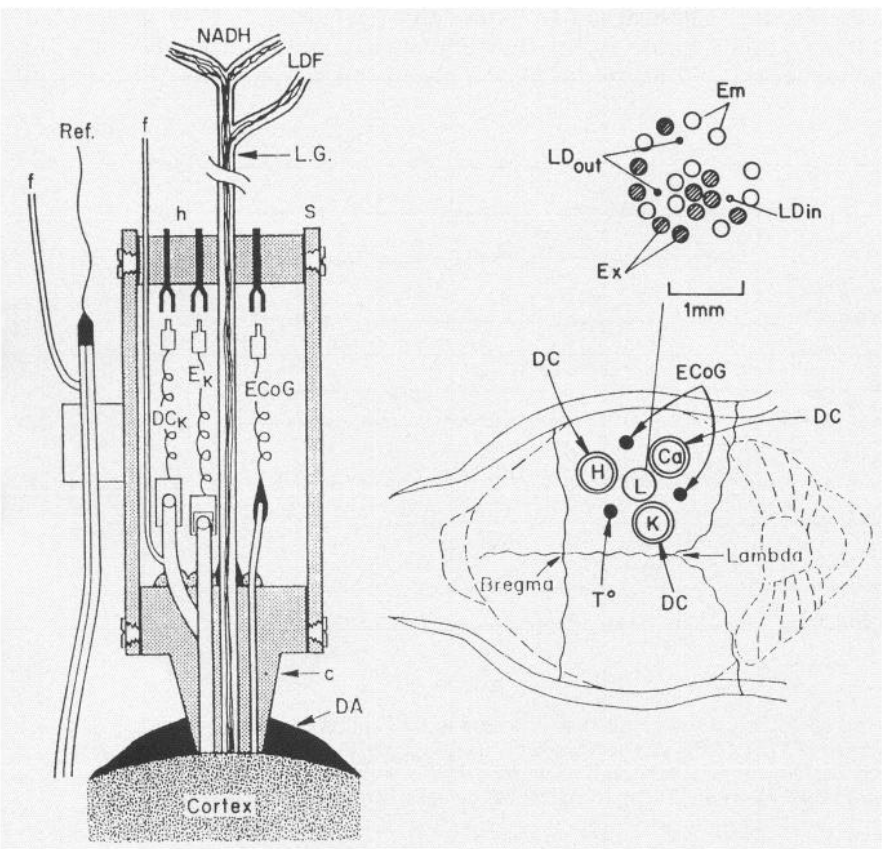

Fig. 1. Multiprobe assembly used in awake rat to monitor hemodynamic, metabolic, ionic, and electrical activities in brain. *Left*: longitudinal section. *Right*: location of MPA and skull. Ref, reference electrode; f, feeling tube of reference or other direct current (DC) electrode; NADH, 2 arms of NADH monitoring light guide; LDF, laser-Doppler flowmeter; L.G., light guide; h, connector holders; s, aluminum sleeve; DC_K and E_K, direct current and ion-specific electrodes, respectively, for K; ECoG, electrocortical electrodes; c, Plexiglas probe holder; DA, dental acrylic cement; Ex and Em, excitation and emission fibers, respectively, for NADH monitoring; LD_{in} and LD_{out}, optical fibers for monitoring blood flow and volume, respectively; DC, area of direct current steady-potential monitoring; K, Ca, and H, ion-specific electrodes; T°, thermistor for local temperature measurement.

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RESULTS

In this study, animals were exposed to CO with or without prior injection of L-NNA. Control animals were exposed to air or injected with L-NNA. When the animals were allowed to breathe air without any additional treatment (*group A*), no changes were recorded in the mitochondrial redox state (NADH), CBF, extracellular ion concentrations, or electrical activity of the cerebral cortex.

Exposure of animals to a mixture of 1,000 ppm CO in air for 1 h led to a transient increase in CBF while all other parameters (NADH, extracellular ion concentrations, and electrical DC potential) remained unchanged. The CBF increase began a few minutes after the onset of CO exposure and persisted until exposure to CO was stopped and the animal was allowed to breath fresh air (Fig. 2). Throughout the exposure to CO, the CBF continued to increase (Figs. 2 and 3*B*). At the end of the exposure, the CBF was 54% higher ($154 \pm 24\%$; $n = 7$; $P < 0.05$) than during the control period (before the CO exposure) in the same rat. CBF began decreasing immediately on cessation of CO exposure. After 15 min, the CBF was not significantly different from the control value (Fig. 3*B*). The CBF of control animals exposed to air only increased to 110% after an equivalent period of time (Fig. 3*A*).

After injection of the NOS inhibitor L-NNA (Fig. 3*C*), a slight decrease in CBF was observed, such that 15 min after injection CBF had decreased to 87% of the control value (a decrease of 13%; $n = 7$; $P < 0.05$), whereas 60 min later it had decreased to 93% (still significantly lower than the control level; $P < 0.05$). Injection of L-NNA before CO (Fig. 3*D*) also resulted in a decrease in CBF ($79.2 \pm 5.8\%$; $n = 7$; $P < 0.05$). In this case, exposure to CO did not lead to the expected elevation in CBF. On the contrary, CBF levels continued to remain lower than the control level. Approximately 150 min after L-NNA injection, CBF levels began increasing, perhaps as a result of its degradation because this increase was not related to the gases present in the respiratory mixture (CO or air).

Here, too, changes in CBF were not followed by changes in the NADH redox state (as recorded from corrected fluorescence), extracellular ion concentrations (K^+, Ca^{2+}, and H^+), or DC potentials. Table 1 summarizes the values of the intramitochondrial NADH redox levels, the extracellular K^+ and Ca^{2+} concentrations, and the pH before and after 60 min of the various treatments.

Exposure to CO after L-NNA injection caused a depression in spontaneous electrical activity (ECoG; Fig. 4). In five out of seven animals, ECoG depression was reversible after the CO mixture was replaced by air. In the two remaining animals, the ECoG depression was very severe and was not reversible even after flushing O_2 into the cage (Fig. 5). No pronounced changes in the ECoG were recorded in the control animals, animals exposed to CO but not injected with L-NNA, or animals treated with L-NNA but not exposed to CO.

DISCUSSION

In this study, we have found that exposure to 1,000 ppm CO results in an increase in CBF without affecting any of the other metabolic, ionic, or electrical parameters measured. This is consistent with our previous findings (24). Under these conditions, CBF is elevated to an extent that apparently enables the preservation of adequate O_2 supply to the tissue. This elevation in

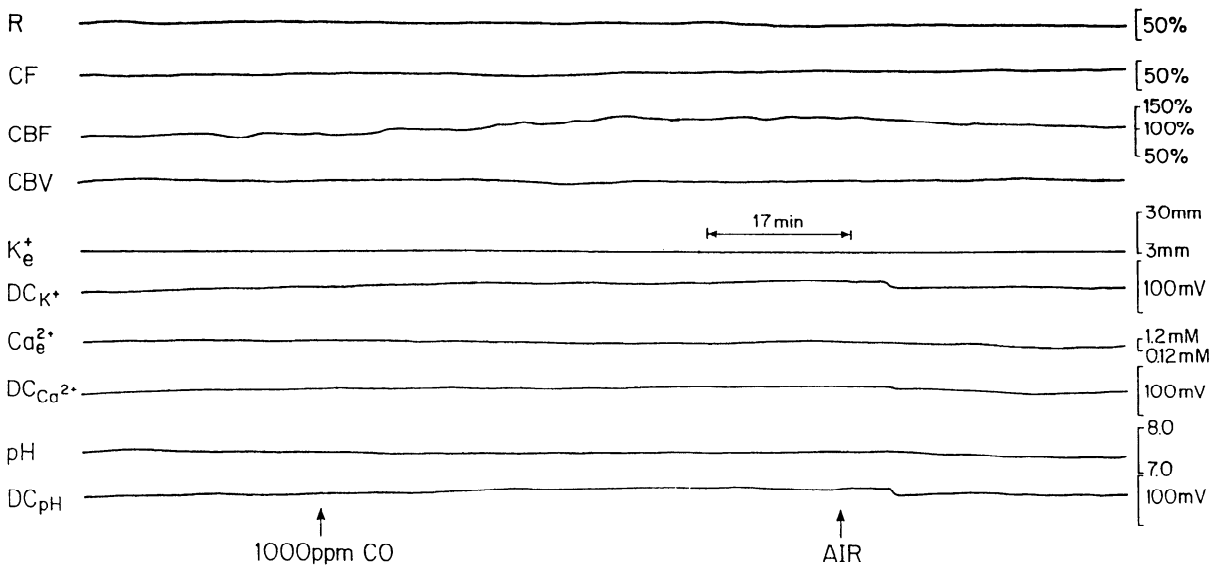

Fig. 2. Polygraph tracing of all measured parameters in 1 rat before, during, and after exposure to 1,000 parts per million (ppm) CO. R, reflectance; CF, corrected fluorescence (NADH); CBF, cerebral blood flow; CBV, cerebral blood volume; K_e^+, extracellular K^+ concentration; DC_{K^+}, electrical DC potential around K^+ electrode; Ca_e^{2+}, extracellular Ca^{2+} concentration; $DC_{Ca^{2+}}$, electrical DC potential around Ca^{2+} electrode; DC_{pH}, electrical DC potential around pH electrode.

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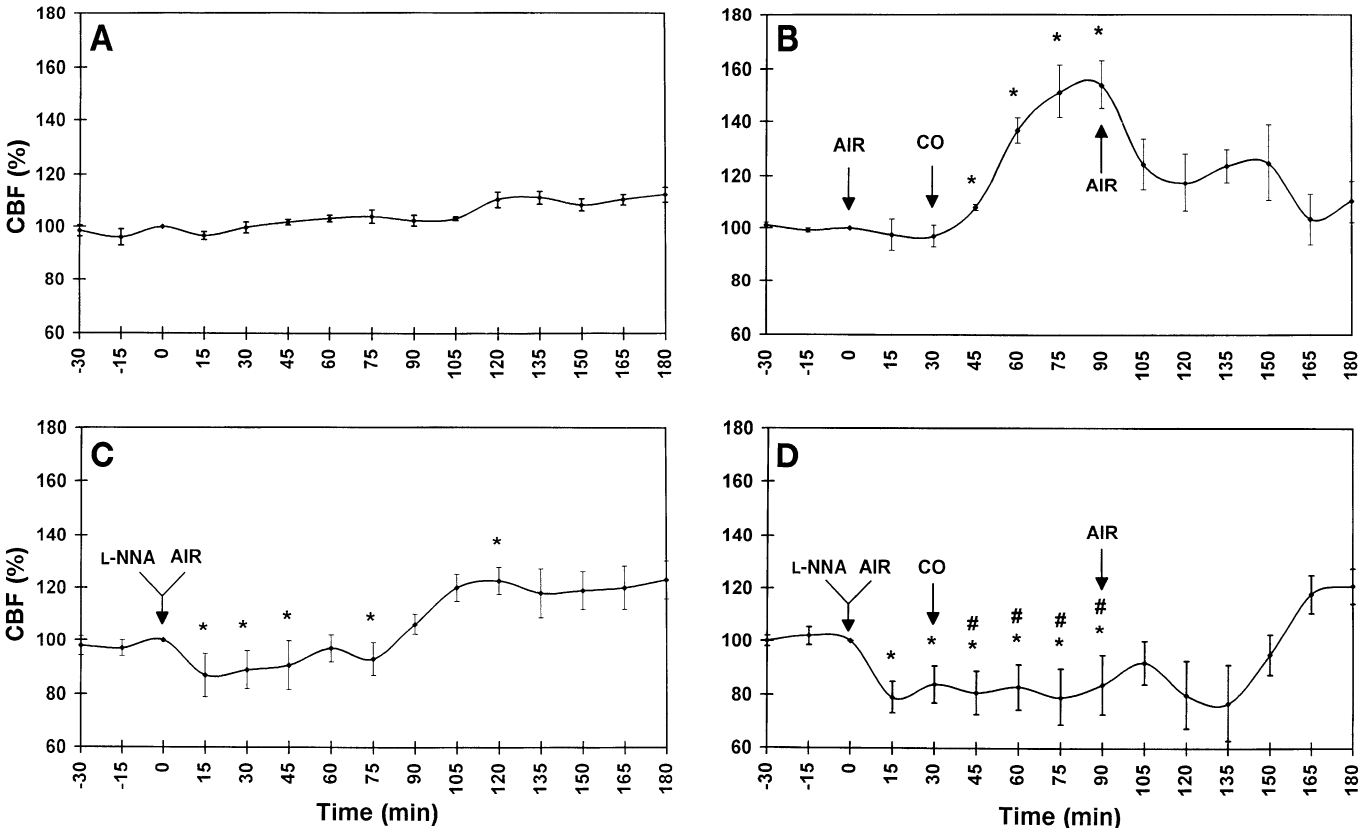

Fig. 3. Changes in CBF after exposure to CO. *A*: control animals (n = 7). *B*: animals exposed to 1,000 ppm CO (n = 7). *C*: animals injected with nitric oxide synthase inhibitor nitro-L-arginine (L-NNA; n = 7). *D*: animals injected with L-NNA and exposed to 1,000 ppm CO (n = 7). *$P < 0.05$ compared with baseline of same animal. #$P < 0.05$ compared with CO-exposed animals (*B*) at same time. Data are means ± SE.

CBF (after exposure to CO) was not preceded by changes in intramitochondrial NADH levels.

Thus the suggestion raised by Koehler and colleagues (12, 13) that the CO-induced increase in CBF is related to changes in tissue O_2 balance does not apply to this model. Ludbrook et al. (15) also does not support this hypothesis. However, they do not suggest any alternatives.

Because no changes were observed in the NADH redox state at the time of the increase in blood flow, we searched for an explanation for this increase. MacMillan (18) suggested that acidosis may be the reason for CBF increase during exposure to high concentrations of CO. In the present study, pH remained stable, possibly because the rats were exposed to only 1,000 ppm CO.

It has been reported that CO does not have a direct effect on isolated smooth muscles of brain vessels (3). This led to the assumption that the increase in CBF during CO exposure is mediated by some other component such as NO. Reports have recently been published on the involvement of NO in CBF regulation (1, 22, 24).

To determine whether the effect of CO on CBF may be mediated by NO, we injected the frequently used NOS inhibitor L-NNA (5, 8, 19) before exposure of the rats to 1,000 ppm CO. A decrease in CBF was observed after NO synthesis was inhibited by an injection of the NOS-inhibitor L-NNA (Fig. 3*C*). When L-NNA was injected before the exposure to CO, the typical increase in CBF that occurs after exposure to CO was blocked (Fig. 3*D*). These results demonstrate that NO plays a

Table 1. *Changes in intramitochondrial NADH redox levels, extracellular K^+ and Ca^{2+} concentrations, and pH before and after 60 min of various treatments*

	Group A		*Group B*		*Group C*		*Group D*	
	Before	After	Before	After	Before	After	Before	After
NADH, %	100	103 ± 3	100	102 ± 2	100	101 ± 1.5	100	101 ± 3.4
K^+, mM	3.14 ± 0.7	3.26 ± 0.6	2.98 ± 0.4	3.15 ± 0.6	4.7 ± 0.7	4.4 ± 10.6	2.47 ± 0.4	2.57 ± 0.4
Ca^{2+}, mM	1.16 ± 0.02	1.12 ± 0.05	0.92 ± 0.03	0.91 ± 0.03	1.02 ± 0.09	1.01 ± 0.09	0.97 ± 0.04	0.94 ± 0.04
pH	7.7 ± 0.06	7.5 ± 0.07	7.7 ± 0.13	7.6 ± 0.16	7.22 ± 0.11	7.2 ± 0.1	7.7 ± 0.06	7.6 ± 0.08

Values are means ± SE; n = 7 rats/group. *Group A*: control animals that breathed air for entire period. *Group B*: animals exposed to CO for 60 min. *Group C*: animals injected with N^{ω}-nitro-L-arginine that breathed air for entire period. *Group D*: animals injected with N^{ω}-nitro-L-arginine and exposed to CO for 60 min. Measurements were taken immediately after CO was replaced by air.

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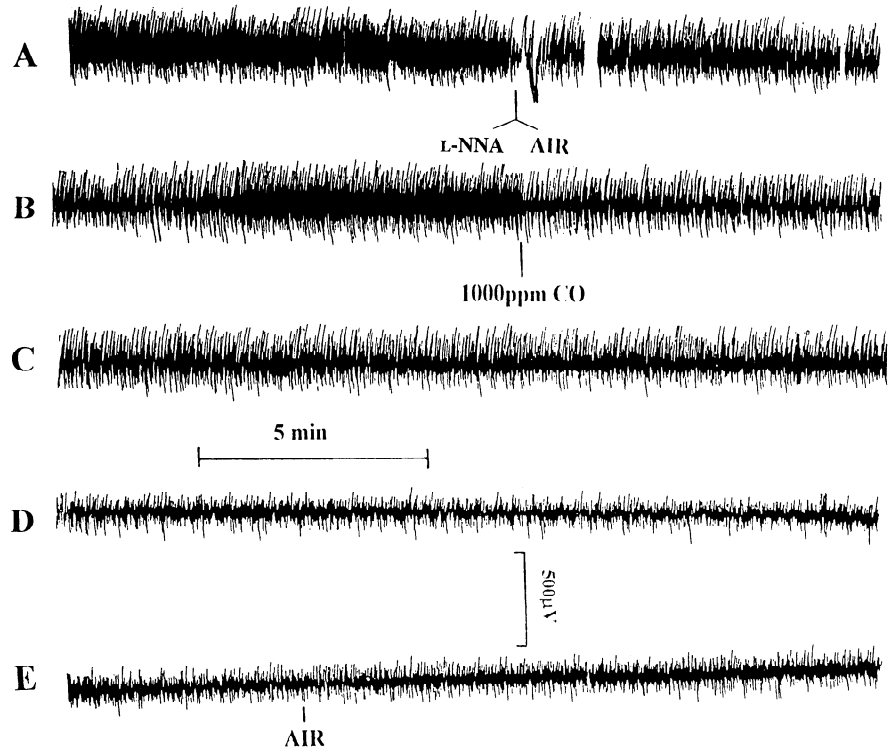

Fig. 4. Polygraph tracing of electrocorticogram (ECoG) in an animal injected with L-NNA and exposed to 1,000 ppm CO for 60 min. *A–E*, order of ECoG recordings.

major role in the regulation of CBF during exposure to 1,000 ppm CO.

Thom (31) has demonstrated that CO exposure results in increased blood NO levels. However, he found that NO production in platelets taken from CO-exposed rats was unchanged. He therefore suggested that because the heme-containing proteins, which are known to scavenge NO, are already bound to CO, they cannot also bind NO and the level of circulating NO increases. Under physiological conditions, heme-containing proteins are known to bind circulating NO. Our work supports this assumption and suggests that the elevation in CBF occurs before the onset of the tissue demand for O_2 and, at this stage, is not related to tissue energy metabolism but rather to an increase in circulating NO levels.

In this study, changes in electrical activity (ECoG) were recorded in rats exposed to CO after injection of L-NNA. The fact that the ECoG was not affected in the two control groups (CO alone or L-NNA alone) suggests a unique mechanism of activity. The only recorded change was a reduction in CBF in the group exposed to CO after L-NNA injection. Nevertheless, it is hard to relate the ECoG response to an energy failure of the brain because the mitochondrial redox state was unchanged in all four groups of rats (Table 1). Therefore, further elucidation of ECoG depression under these conditions (perhaps via NO) is necessary. Such experi-

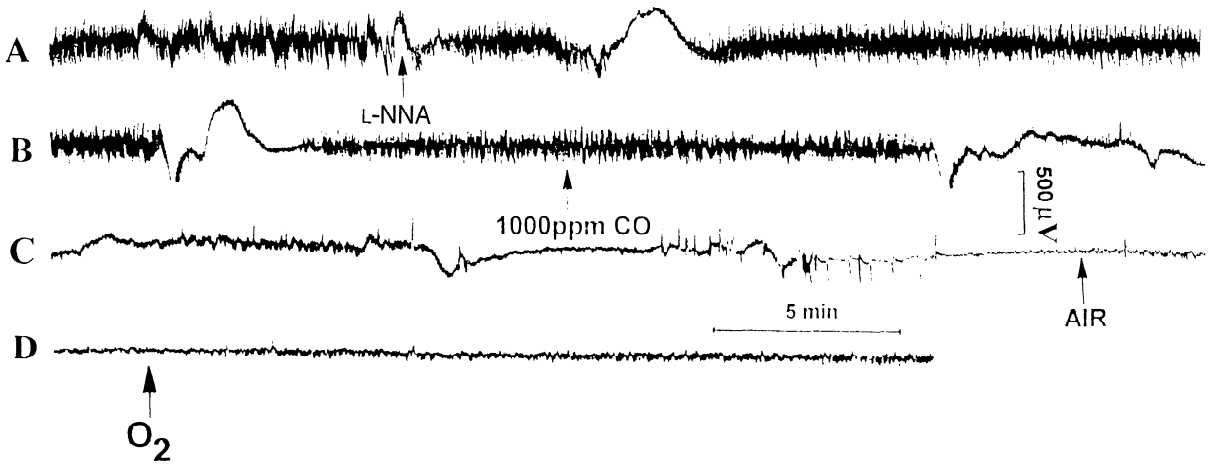

Fig. 5. Polygraph tracing of ECoG of an animal injected with L-NNA and exposed to 1,000 ppm CO in which ECoG depression was not reversible even after O_2 was flushed into cage. *A–D*, order of ECoG recordings.

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ments are now being carried out with another NOS inhibitor nitro-L-arginine methyl ester as well as various levels of CO.

In conclusion, exposure to 1,000 ppm CO apparently does not have a deleterious effect on oxidative metabolism. It is possible that relatively low doses of CO are not detrimental to the brain due to cerebral vessel vasodilation mediated by NO release after exposure to CO, which compensates for the reduction in the O_2 content of the cerebral blood vessels, as indicated by the fact that NADH redox levels as well as intracellular K^+ concentrations remained unchanged.

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