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Effect of Nitric Oxide Blockade by N^G-Nitro-L-Arginine on Cerebral Blood Flow Response to Changes in Carbon Dioxide Tension

Qiong Wang, *Olaf B. Paulson, and Niels A. Lassen

*Department of Clinical Physiology/Nuclear Medicine, Bispebjerg Hospital, and *Department of Neurology, Rigshospital, Copenhagen, Denmark*

Summary: The importance of nitric oxide (NO) for CBF variations associated with arterial carbon dioxide changes was investigated in halothane-anesthetized rats by using an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine (NOLAG). CBF was measured by intracarotid injection of ^{133}Xe. In normocapnia, intracarotid infusion of 1.5, or 7.5, or 30 mg/kg NOLAG induced a dose-dependent increase of arterial blood pressure and a decrease of normocapnic CBF from 85 ± 10 to 78 ± 6, 64 ± 5, and 52 ± 5 ml $100g^{-1}$ min^{-1}, respectively. This effect lasted for at least 2 h. Raising P_aCO_2 from a control level of 40 to 68 mm Hg increased CBF to 230 ± 27 ml $100g^{-1}$ min^{-1}, corresponding to a percentage CBF response (CO_2 reactivity) of 3.7 ± 0.6%/mm Hg P_aCO_2 in saline-treated rats. NOLAG attenuated this reactivity by 32, 49, and 51% at the three-dose levels. Hypercapnia combined with angiotensin to raise blood pressure to the same level as the highest dose of NOLAG did not affect the CBF response to hypercapnia. L-Arginine significantly prevented the effect of NOLAG on normocapnic CBF as well as blood pressure and also abolished its inhibitory effect on hypercapnic CBF. D-Arginine had no such effect. Decreasing P_aCO_2 to 20 mm Hg reduced control CBF to 46 ± 3 ml $100g^{-1}$ min^{-1} with no further reduction after NOLAG. Furthermore, NOLAG did not change the percentage CBF response to an extracellular acidosis induced by acetazolamide (50 mg/kg). The results suggest that NO or a closely related compound is involved in the regulation of CBF in normocapnia and even more so in hypercapnia.
Key Words: Acetazolamide—Carbon dioxide—Cerebral blood flow—Hypercapnic cerebral vasodilatation—Hypocapnia—Nitric oxide—N^G-Nitro-L-arginine—Normocapnia.

The endothelium-derived relaxing factor nitric oxide (NO) or a closely related NO-containing compound (Ignarro et al., 1987; Palmer et al., 1987) is currently being studied intensively. In vitro studies have shown that the amino-nitrogen of the guanidino group of L-arginine is the precursor (Palmer et al., 1988*a;* Sakuma et al., 1988; Schmidt et al., 1988). NO synthase is also located in the brain tissue as well as in perivascular nerves (Bredt et al., 1990) where it forms NO from L-arginine in a very similar manner as in the endothelium (Knowles et al., 1989; Moncada et al., 1989). With use of suitable doses of modified forms of L-arginine, in which the active group is no longer chemically active in the reaction, it is possible to block NO synthesis. N^G-Monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (NOLAG) have been found to act in this way in endothelium (Palmer et al., 1988*b;* Rees et al., 1989*a;* Moore et al., 1990) as well as in brain tissue (Bredt and Snyder, 1989; Garthwaite et al., 1989*a,b;* Knowles et al., 1989; Förstermann et al., 1990; Murphy et al., 1990). They both block NO synthase reversibly, an effect that can be counteracted competitively by administering large doses of L-arginine, while D-arginine has no effect (Palmer et al., 1988*b;* Garthwaite et al., 1989*b;* Rees et al., 1989*a;* Moore et al., 1990).

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Address correspondence and reprint requests to Dr. N. A. Lassen at Department of Clinical Physiology/Nuclear Medicine, Bispebjerg Hospital, DK-2400, Copenhagen NV, Denmark.

These results have been presented in preliminary form at the Second International Meeting, Biology of Nitric Oxide, London (Wang et al., 1991).

Abbreviations used: BBB, blood–brain barrier; L-NMMA, N^G-monomethyl-L-arginine; NOLAG, N^G-nitro-L-arginine.

We here report studies of the effect of NOLAG on the CBF in hypo-, normo-, and hypercapnia. NOLAG was found to reduce normocapnic CBF and even more markedly to attenuate the hypercapnic CBF increase, but it had no effect on hypocapnia or on the CBF response to extracellular acidosis induced by acetazolamide.

MATERIALS AND METHODS

Animal preparation

The experiments were performed on 117 male Wistar rats weighing 300–350 g (Møllegaard Ltd., Denmark). Anesthesia was induced with 4% halothane and maintained with 0.7% halothane in 30% oxygen/70% N_2O during the operation and with 0.6% halothane in O_2/N_2O after the operation. The animals were tracheostomized, immobilized with suxamethonium (40 mg/kg), and artificially ventilated by a rodent respirator. The scalp over the right hemisphere of the brain was removed, and the right temporal muscle was loosened from the cranium and flapped down to avoid extracranial deposition of injected ^{133}Xe within the field of view of the detector. Both femoral arteries were cannulated (PE-50) to allow continuous blood pressure recording and collection of blood samples. Both femoral veins were cannulated as well and used for drug and donor blood supplement. All extracerebral branches of the right common carotid artery were ligated, including the pterygopalatine artery, to minimize the extracerebral distribution of ^{133}Xe. The animals were heparinized (3,000 IU/kg) prior to ligation of the pterygopalatine artery. Finally, the external carotid was cannulated (PE-25) with the catheter tip close to the carotid bifurcation and used for bolus injection of ^{133}Xe and for nitro-L-arginine infusion.

Arterial P_aCO_2, P_{O_2}, and pH were recorded at each CBF measurement (ABL 30; Radiometer, Copenhagen, Denmark). Arterial P_aO_2 was kept above 100 mm Hg in all studies. In the control series, P_aCO_2 was 38–40 mm Hg and pH 7.37–7.42. All blood samples collected were replaced with donor blood from rats of the same strain. The arterial blood pressure was continuously followed during the study by using a conventional capacitance manometer. Body temperature was maintained close to 37°C by a rectal thermostatic controller connected to a heating pad.

For measurement of CBF, the intracarotid ^{133}Xe method as adapted for small rodents (Hertz et al., 1977) was used. CBF was determined from the 10- to 15-s initially steepest slope of the semilogarithmic clearance curve recorded from the ratemeter analog output after injection of a 10- to 60-μl bolus of ^{133}Xe dissolved in saline (10 mCi/ml; Amersham).

Protocol

In one group of rats, we measured the effect of NOLAG on the basal and CO_2-stimulated CBF. CBF was first measured at normocapnia 12 min after ending the intracarotid infusion of saline, 0.6 ml over 6 min. Then hypercapnia was induced by adding 5% CO_2 to the inspired gas, and CBF was measured after steady state had been reached. Thereafter, CO_2 breathing was stopped and a dose of 1.5 or 7.5 mg/kg NOLAG was infused intracarotidly at a rate of 0.1 ml/min for 6 min. CBF was studied 12 min later in normocapnia and subsequently in hypercapnia. In another six rats, a dose of 30 mg/kg NOLAG was given intracarotidly with a speed of 0.1 ml/min for 25 min (7.5 mg/kg NOLAG is the maximal dose to be dissolved in 0.6 ml saline ultrasonically) to test the effect of a high dose of NOLAG on normocapnia and hypercapnia. As NOLAG increases systemic blood pressure quite markedly, we also included a second control group of five rats in which blood pressure was elevated by an infusion of angiotensin ($2–3 \times 10^{-5}$ mg/min) to reach the same pressure level as with the highest dose of NOLAG.

In second group of rats, hypocapnia was induced by hyperventilating the animals to a P_aCO_2 of 20 mm Hg. After a stable hypocapnic level had been reached, control CBF was measured. Thereafter 7.5 mg/kg NOLAG was infused intracarotidly at a rate of 0.1 ml/min over 6 min during hypocapnia. CBF was then measured again 12 min after infusion.

In two separate groups of rats, an intravenous bolus of L- or D-arginine (150 mg/kg) was given followed by a 100-mg/kg/h (0.05-ml/min) infusion before the application of 7.5 mg/kg NOLAG. This was done to test whether the inhibitory effect of NOLAG on normo- and hypercapnic CBF could be antagonized by L-arginine and D-arginine.

In a fifth group of rats, the effect of NOLAG on an acetazolamide-induced CBF increase (Severinghaus and Cotev, 1968; Vorstrup et al., 1984) was tested. After 7.5 mg/kg NOLAG (control animals were treated by saline), a 50-mg/kg dose of acetazolamide was injected subcutaneously, and the animals were hyperventilated to keep measured P_aCO_2 at the normocapnic level. CBF was recorded 15–20 min after acetazolamide injection.

In a sixth group of rats, mild hypercapnia (P_aCO_2 = 52 mm Hg) was induced with the purpose of obtaining the same CBF level as that during acetazolamide administration. A dose of 7.5 mg/kg NOLAG was given intracarotidly to test its effect on this hypercapnic CBF level for comparison with the acetazolamide-treated animals.

At end of most experiments, in both saline- and NOLAG-treated animals, 2.5% Evans Blue was given intravenously (2.5 ml/kg) to delineate possible major lesions of the blood–brain barrier (BBB). Five minutes later, rats were decapitated and brains were removed and examined for extravasation of the dye.

Drugs

NOLAG [N^5-(nitroamidino)-L-2,5-diaminopentanoic acid] was purchased from Aldrich Chemistry Co., acetazolamide from Lederle, and angiotensin from Ciba-Geigy. All other compounds were obtained from Sigma. The drugs were dissolved in saline and the NOLAG solution was ultrasonicated for 5–10 min to dissolve it completely in the saline. This solution had a calculated osmolarity of 328 mOsmol and a measured pH of 7.2.

Statistics and calculations

The Mann–Whitney test was used for comparing values under control conditions and during interventions. All values are presented as means ± SD. $p < 0.05$ was considered significant.

The CO_2 reactivity was calculated as $\Delta \ln CBF/\Delta P_aCO_2 \times 100$ in each rat since exponential curves give the best fit

of the relation between CBF and P_aCO_2 (Olesen et al., 1971).

RESULTS

Effect of NOLAG at normocapnia (P_aCO_2 = 40 mm Hg)

As illustrated in Table 1 and Fig. 1, NOLAG caused a dose-dependent decrease in normocapnic CBF by 9 ± 7, 23 ± 8, and 32 ± 6% and an increase in blood pressure by 10 ± 6, 38 ± 10, and 52 ± 18% at the dose levels of 1.5, 7.5, and 30 mg/kg, respectively. In a separate group of four animals, a time course study was performed showing that the effects lasted for at least 2 h.

Effect of NOLAG at hypercapnia (P_aCO_2 adjusted to 68 mm Hg)

During hypercapnia, NOLAG caused an even more marked decrease of CBF relative to the saline-treated controls. With saline infusion, hypercapnia induced almost a threefold increase in CBF from a normocapnic control level, and the calculated CO_2 reactivity of CBF was 3.7 ± 0.6%/mm Hg P_aCO_2. After NOLAG infusion, the CBF increase was much less (see Table 2 and Fig. 2), and the calculated CO_2 reactivity of CBF was only 2.5 ± 0.5, 1.9 ± 0.4, and 1.8 ± 0.4%/mm Hg P_aCO_2 at the three dose levels. This means that NOLAG reduced the CO_2 reactivity by 32, 49, and 51%, respectively.

As a check on the validity of these data, two additional studies were carried out. In three animals, hypercapnia was repeated several times in the saline-infused control state. No attenuation of CBF increase or calculated CO_2 reactivity was found. In the five saline-infused animals in which blood pressure was raised to ~150 mm Hg by angiotensin, hypercapnic CBF increased to the same high levels as in the normotensive controls and the calculated CO_2 reactivity even rose slightly to 4.2 ± 1%/mm Hg P_aCO_2 (see Table 2).

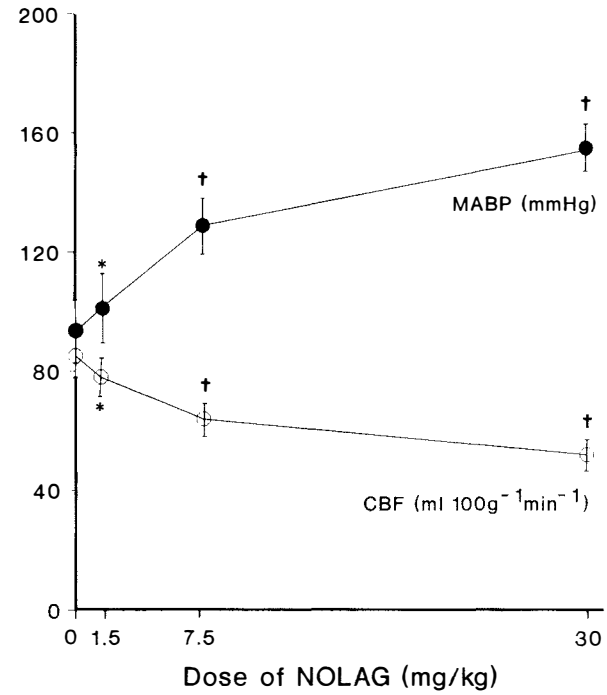

FIG. 1. The dose-dependent CBF decrease and MABP increase caused by N^G-nitro-L-arginine (NOLAG) at three dose levels, 1.5, 7.5, and 30 mg/kg, during normocapnia. *p < 0.05, †p < 0.001 versus saline-treated rats.

Effect of NOLAG during hypocapnia (P_aCO_2 = 20 mm Hg)

As shown in Fig. 2, reducing P_aCO_2 to 20 mm Hg caused a reduction of CBF to 46 ± 3 ml/100 g/min (n = 5). This was not further reduced by 7.5 mg/kg NOLAG because at this dose hypocapnic CBF averaged 45 ± 3 ml/100 g/min (n = 5).

Effect of NOLAG when combined with acetazolamide and comparison with mild hypercapnia (P_aCO_2 = 52 mm Hg)

When hyperventilation was induced to keep the measured P_aCO_2 constant, the inhibitor of carbonic

TABLE 1. *CBF and physiological parameters in all normocapnic groups of rats*

Group	CBF (ml/100 g/min)	MABP (mm Hg)	pH	P_aCO_2 (mm Hg)	P_aO_2 (mm Hg)
Saline (n = 21)	85 ± 10	93 ± 7	7.41 ± 0.03	39.7 ± 1.1	147 ± 17
1.5 mg/kg NOLAG (n = 24)	78 ± 6[a]	102 ± 11[a]	7.40 ± 0.02	39.3 ± 0.8	140 ± 15
7.5 mg NOLAG (n = 14)	64 ± 5[b]	129 ± 9[b]	7.40 ± 0.03	39.3 ± 0.9	145 ± 18
30 mg NOLAG (n = 6)	52 ± 5[b]	154 ± 6[b]	7.38 ± 0.01	39.1 ± 0.9	150 ± 20
Angiotensin (n = 5)	79 ± 10	148 ± 7[b]	7.42 ± 0.01	40.2 ± 0.7	143 ± 22
7.5 mg/kg NOLAG + L-Arg (n = 9)	75 ± 12[c]	109 ± 12[d]	7.38 ± 0.05	39.1 ± 1.1	151 ± 16
7.5 mg/kg NOLAG + D-Arg (n = 8)	65 ± 4	145 ± 15[c]	7.38 ± 0.02	39.2 ± 0.5	140 ± 12

Values are expressed as means ± SD; n = no. of rats. NOLAG, N^G-nitro-L-arginine.
[a] $p < 0.05$, [b] $p < 0.001$ vs. saline-treated animals.
[c] $p < 0.05$, [d] $p < 0.01$ vs. 7.5 mg/kg NOLAG–treated animals.

TABLE 2. *CBF, calculated CO_2 reactivity, and physiological parameters in all hypercapnic groups of rats*

Group	CBF (ml/100 g/min)	CO_2 reactivity (%/mm Hg)	MABP (mm Hg)	pH	P_aCO_2 (mm Hg)	P_aO_2 (mm Hg)
Saline (n = 14)	230 ± 27	3.7 ± 0.6	112 ± 13	7.23 ± 0.02	68.8 ± 2.7	159 ± 9
1.5 mg/kg NOLAG (n = 6)	172 ± 38[b]	2.5 ± 0.5[b]	116 ± 18	7.20 ± 0.02	69.9 ± 3.8	165 ± 11
7.5 mg NOLAG (n = 10)	104 ± 21[c]	1.9 ± 0.5[c]	120 ± 15	7.22 ± 0.03	68.0 ± 3.1	160 ± 22
30 mg NOLAG (n = 6)	89 ± 9[c]	1.8 ± 0.4[c]	142 ± 8[a]	7.20 ± 0.03	68.9 ± 1.3	161 ± 22
Angiotensin (n = 5)	251 ± 48	4.2 ± 1.0	143 ± 7[a]	7.24 ± 0.03	68.3 ± 3.9	156 ± 19
7.5 mg/kg NOLAG + L-Arg (n = 9)	195 ± 45[d]	3.6 ± 0.5[e]	110 ± 13	7.19 ± 0.03	68.9 ± 1.6	169 ± 23
7.5 mg/kg NOLAG + D-Arg (n = 8)	122 ± 21	2.2 ± 0.5	132 ± 10	7.20 ± 0.01	66.3 ± 2.4	153 ± 13

Values are means ± SD; n = no. of rats. CO_2 reactivity was calculated as $\Delta \ln CBF/\Delta P_aCO_2 \times 100$ (%/mm Hg). NOLAG, N^G-nitro-L-arginine.
[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ vs. saline-treated animals.
[d] $p < 0.01$, [e] $p < 0.001$ vs. 7.5 mg/kg NOLAG–treated animals.

anhydrase acetazolamide raised CBF from a control level of 81 ± 14 to 130 ± 19 ml/100 g/min, corresponding to a 61 ± 16% increase in CBF when comparing individual control values. With 7.5 mg/kg NOLAG, the basal and acetazolamide-induced CBF values were both reduced proportionally (see Table 3). Thus, relative to the lowered basal CBF level, acetazolamide still elicited the same percentage increase in CBF of 62 ± 17%; i.e., the acetazolamide CBF response was unchanged.

Elevating P_aCO_2 to 52 mm Hg was found to increase CBF to about the same level as acetazolamide in saline-infused controls. When giving NOLAG at 7.5 mg/kg at this level of hypercapnia, CBF decreased much more than after acetazolamide as the percentage increase in CBF was only 30% (see Table 3).

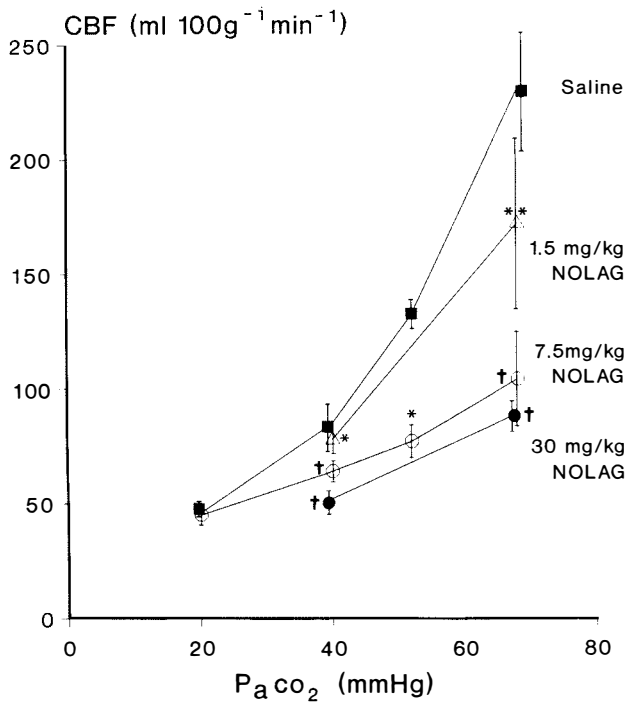

FIG. 2. Effect of N^G-nitro-L-arginine (NOLAG) on CBF-P_aCO_2 response. *p < 0.05, **p < 0.01, †p < 0.001 versus saline-treated animals.

Effect of L- and D-arginine on NOLAG (Fig. 3)

The preapplication of L-arginine significantly prevented the effect of 7.5 mg/kg NOLAG in normocapnia as blood pressure and CBF were close to control levels (Table 1). It also completely preserved the response of cerebral vessels to increase P_aCO_2 as hypercapnia increased CBF to 222 ± 45 ml $100g^{-1}$ min^{-1} and CO_2 reactivity was brought back to 3.6 ± 0.5%, very similar to that of the saline-infused controls (3.7 ± 0.6%). In contrast, the decrease in hypercapnic CBF in response to NOLAG was not significantly changed by preapplication of D-arginine. D-Arginine significantly potentiated the effect of NOLAG on MABP, increasing it from 129 ± 9 to 145 ± 15 mm Hg at normocapnia.

Effect of NOLAG on BBB

There was no evidence of damage to the BBB caused by NOLAG, as judged by the lack of visible extravasation of Evans Blue in the brains studied.

DISCUSSION

The method using intracarotid NOLAG infusion was designed to maximize local relative to systemic effects. The effect of NOLAG is, however, almost as marked on the contralateral hemisphere (data not shown). In a few studies infusing NOLAG intravenously at a dose of 30 mg/kg, essentially the same effects on normo- and hypercapnic CBF were produced as by the intracarotid route (data not shown).

TABLE 3. *Effect of 7.5 mg/kg N^G-nitro-L-arginine (NOLAG) combined with acetazolamide and mild hypercapnia*

Group		CBF (ml/100 g/min)	% CBF increase	MABP (mm Hg)	pH	P_aCO_2 (mm Hg)	P_aO_2 (mm Hg)
Acetazolamide	Saline	130 ± 19	61 ± 16	99 ± 10	7.39 ± 0.02	40.2 ± 1.2	206 ± 16
(n = 4)	NOLAG	103 ± 14	62 ± 17	115 ± 15	7.37 ± 0.02	40.5 ± 1.0	205 ± 6
Mild hypercapnia	Saline	132 ± 7	72 ± 15	105 ± 13	7.30 ± 0.03	51.8 ± 0.9	144 ± 8
(n = 5)	NOLAG	77 ± 7[a]	30 ± 9[a]	129 ± 4[a]	7.29 ± 0.03	52.0 ± 0.09	141 ± 6

Values are means ± SD; n = no. of rats. % CBF increase; average values of percentage increase of CBF in each rat.
[a] $p < 0.05$ vs. saline-treated animals.

We saw a clear-cut rise in MABP with NOLAG. This agrees with other studies using L-arginine analogues blocking NO synthase, such as L-NMMA (Aisaka et al., 1989; Rees et al., 1989*b*; Koźniewska et al., 1992) and NOLAG (Beckman et al., 1991). Our finding of enhanced pressor response when D-arginine was combined with NOLAG was unexpected. It seems to be a nonspecific effect caused by the volume of vehicle injected along with the D-arginine, reaching 2.2 ml at the time the effect of NOLAG was studied, because injecting the same volume of vehicle with NOLAG caused the same accentuated hypertension.

At normocapnic P_aCO_2 levels, we found a 23 ± 8 and 32 ± 6% decrease of CBF after intracarotid infusion of 7.5 and 30 mg/kg NOLAG. This is consistent with Beckman et al.'s findings (1991) in normocapnic rats infused intravenously with 30 mg/kg of NOLAG. The decreased basal CBF was also observed by using another inhibitor of NO, L-NMMA, in different species (Greenberg et al., 1991; Tanaka et al., 1991; Koźniewska et al., 1992). The reduced CBF in the face of a clear-cut rise of systemic blood pressure implies a sharp increase in cerebrovascular resistance; thus, the arterioles must have been contracted to a considerable degree. Apparently continuous NO production is a necessity for keeping the cerebral vessels at the relatively low tone characteristic of the normocapnic state.

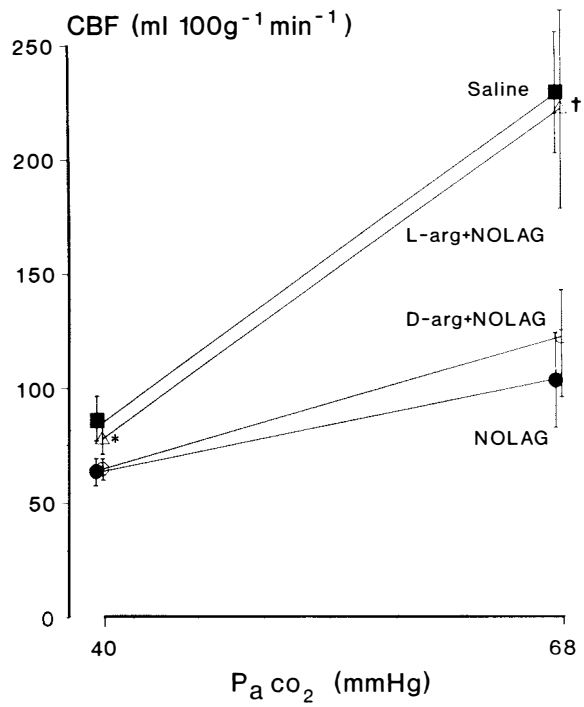

FIG. 3. Effect of L-arginine and D-arginine on N^G-nitro-L-arginine (NOLAG) as compared with rats given only NOLAG (7.5 mg/kg) and with saline controls. *$p < 0.05$, †$p < 0.001$ versus 7.5 mg/kg NOLAG–treated animals.

During hypercapnia, CBF increased in the saline-infused control state to the expected very high level. This was markedly attenuated by NOLAG in a dose-dependent manner. The inhibitory effect of NOLAG was, as expected, almost completely abolished by high doses of L-arginine, whereas D-arginine had no effect. The depressed CO_2 reactivity caused by NOLAG could not be explained by the associated rise in blood pressure, because in angiotensin-infused animals in which the same level of hypertension was reached, the normal CBF response to CO_2 was preserved. This is also in agreement with early studies in humans showing that the response of the CBF to CO_2 in elderly hypertensive patients is same as in normal young men (Fazekas et al., 1953; Novack et al., 1953). The intact BBB after NOLAG administration also suggests that its effects are not related to barrier damage.

Nevertheless, one could argue that the markedly depressed CO_2 reactivity caused by NOLAG might be secondary to a depression of tissue metabolism. Barbiturates, to give a well-known example, depress the metabolic level of the brain and secondarily, so it appears, reduce CBF. The fact that NO is produced and exerts effects in the brain tissue should here be mentioned (Knowles et al., 1989; Garthwaite et al., 1989*a,b*, 1991; Moncada et al., 1989; Gally et al., 1990; Murphy et al., 1990). However, studies using L-NMMA (Greenberg et al., 1991; Koźniewska et al., 1992) and NOLAG (Q. Wang et al., unpublished data) lend no support to this hypothesis as the $CMRO_2$ was found not to be

reduced. Moreover, a preserved autoregulation of CBF after NOLAG (Wang et al., 1992) also suggests that the depressed CO_2 response caused by NOLAG is not related to interference with cerebral autoregulation.

A possible nonspecific effect of NOLAG on hypercapnia should be mentioned. In this hypothesis, the vasodilatation induced by hypercapnia is not mediated by NO, but depends on other mechanisms. Yet, only when NO is present, reducing cerebrovascular tone, will the CO_2-induced effects have their normal magnitude. Extrapolating, by the biochemical "surgery" of a false substrate, the NO component might result in a decreased CO_2 responsiveness without NO necessarily being directly involved in the CO_2 effect. But, if this were true, we would expect that inhibition of NO should also suppress other cerebral vasodilator responses, such as the one seen with hypoxia. However, Koźniewska et al.'s recent study (1992) showed a preserved hypoxic cerebral response after NO inhibition, tending to disprove this hypothesis of a nonspecific effect of NOLAG in our study. Thus, it could be concluded that the vasodilation induced by hypercapnia, and already seen at normocapnia when compared with hypocapnia, is at least in part mediated by NO.

If, indeed, the NO concentration of the vessel wall increases during hypercapnia, where does this NO come from? Recent studies suggest that endothelium-derived factors are not involved as damage of the endothelium in vitro or in vivo was found not to influence the central vasodilator response to CO_2 (Gotoh et al., 1987; Toda et al., 1989). However, the pioneering work of Bredt et al. (1990) has demonstrated NO synthase immunoreactivity in neurons and also in perivascular nerves contacting smooth muscles (but not in the smooth muscle cells). An astrocyte-derived vasorelaxing factor with similar properties to those of NO has also been described (Murphy et al., 1990). These observations imply that other sources of NO must be considered.

Hypercanic central vasodilatation is undoubtedly related to CO_2-induced pH changes in and around the smooth muscle cells (Gotoh et al., 1961; Lassen, 1968; Wahl et al., 1970). NO would seem, at least in part, to be involved in the mechanisms by which these pH changes influence the contractile elements of the vessel wall. With the aim of possibly clarifying this possibility, the acetazolamide experiments were performed. Acetazolamide results in clear-cut extracellular acidosis in brain, both in spontaneously breathing humans and in hyperventilated animals, whereas intracellular pH (pH_i) is unchanged (Bickler et al., 1988; Vorstrup et al., 1989). In contrast, hypercapnia causes acidosis in both compartments (Loeschcke and Ahmad, 1980; Jensen et al., 1988). After NOLAG infusion, acetazolamide did not increase CBF as much as in the saline-infused controls, but the percentage increase in CBF remained unchanged. By contrast, in hypercapnia the CBF reduction after NOLAG was much more marked (see Table 3). This suggests that the increase in NO hypothesized above to occur with CO_2 should be expected to act on the pH_i-sensitive component of the CO_2 responsiveness. This hypothesis fits with the interesting in vitro study of Heinzel et al. (1992) showing that NO snythase activity from the brain tissue (cerebellum) is quite markedly influenced by pH in that acid pH increases NO production. With acetazolamide, pH_i is essentially unchanged, while with CO_2, pH_i becomes acid and NO synthesis might therefore be increased.

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