Dual response of cerebrocortical blood flow and arterial blood pressure to transient CO_2 stimulus after inhibition of nitric oxide synthesis in rats

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Abstract. Inhibition of nitric oxide synthase (NOS) by Nitro-L-arginine-methyl-ester (L-NAME 15 mg and 70 mg/kg i.v.) in 16 male Wistar rats anaesthetized with urethane, paralysed and artificially ventilated, increased significantly local peripheral vascular resistance in the parietal cortex (CVR) along with augmentation of the mean arterial blood pressure (MAP) and no change of the local cerebrocortical blood flow (CBF) recorded with a Laser-Doppler-Flowmeter. In 11 rats L-NAME reversed a pressor effect of brief hypercapnia induced by 10% CO_2/air mixture ($PaCO_2$ 84.1±5 mm Hg) into a depressor response, reduced CBF response proportionally to the reduction of MAP and did not influence CVR response to CO_2. In 5 rats L-NAME did not abolish the central pressor effect of a CO_2-stimulus and significantly augmented CO_2-induced vasodilatatory response in the cortex (43.4±24% before L-NAME and 137.8±38.8% after L-NAME) by a larger reduction of CVR (-11±8% before L-NAME and -47.1±7.6% after L-NAME). It is concluded that NO does not mediate the vasodilatatory effect of brief hypercapnia in the cortex. NO appears critical for the central pressor effect of CO_2. In those rats in which the central pressor effect of a CO_2-stimulus was not abolished by an NOS blocker, an increased CBF and augmented decrease in CVR was observed during brief hypercapnia. Possible mechanisms of this dual responsiveness of cortical blood flow and arterial blood pressure to CO_2, induced by inhibition of NOS, are discussed.

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INTRODUCTION

Nitric oxide (NO), which mediates vasorelaxation induced by most vasodilatatory factors and transmitters and accounts for the endothelium-dependent vasodilatatory tone (Moncada et al. 1991), is synthetized from L-arginine in the central nervous system by a constitutive NO synthase (NOS) in neurones (Bredt and Snyder 1992) and in the endothelium of the cerebral arteries (Cosentino et al. 1993), as well as by an inducible NOS in glial cells (Norman and Kitamura 1993) and in the vascular smooth muscle (Nunokawa et al. 1993). The significance of NO derived from the vascular endothelium or from extravascular intracerebral vasodilatatory nerves in the control of the cerebral vascular vasodilatatory tone has been demonstrated (Toda and Okamura 1990a,b, 1991, Faraci 1991, Persson et al. 1991, Tanaka et al. 1991, Kozniewska et al. 1992). Yet the role of NO in the mechanism of cerebral vasodilatation produced by CO_2, the most powerful physiological controller of the cerebral blood flow, is still debated.

Significant attenuation of CO_2-induced cerebral vasorelaxation by NOS blockers has been reported by most authors using various techniques (Iadecola 1992, Wang et al. 1992, Pellegrino et al. 1993, Fabricius and Lauritzen 1994, Iadecola and Zhang 1994, Reid et al. 1994, Sandor et al. 1994). On the other hand, video microscopic observations of the pial microvessels do not support the conclusion that NO mediates a vasorelaxant effect of CO_2 (Amano et al. 1993). *In vitro* measurements of cerebral arterial tension do not provide any evidence of NO release by CO_2 (Wang et al. 1993). These discrepancies may be due to different experimental methods *in vivo* or *in vitro*, to systemic *versus* local application of NOS blockers, and finally, due to the fact that CO_2, besides its local vasodilatatory effects, acts through neurogenic intracerebral vasodilatatory mechanisms (see Busija and Heistad 1984). CO_2 activates brain stem mechanisms involved in the central control of circulation (Trzebski et al. 1974, Hanna et al. 1981, Trzebski and Kubin 1981). Blockers of NO synthesis may have an effect on central neurogenic mechanisms by which CO_2 elicits an increase in the sympathetic activity, in arterial blood pressure and, possibly, also in the cerebral blood flow.

The present paper was aimed at checking if inhibition of NO synthesis influences a transient, immediate response of cerebrocortical microcirculation and a pressor response to a short-lasting CO_2 stimulus, known to produce a central sympathoexcitatory effect (Trzebski et al. 1974, Hanna et al. 1979, Trzebski and Kubin 1981, Lioy and Trzebski 1984). For this purpose, laser Doppler flowmetry (LDF), a method which provides a fast dynamic and continuous recording of the blood flow in cortical microvessels, was applied as more suitable than microsphere techniques or hydrogen and radioactive clearance methods, which require steady state conditions.

METHODS

Preparation of experimental animals

Sixteen male Wistar rats weighing 370-490 g were anaesthetized with urethane 1g/kg i.p. Occasionally additional small doses were added i.v. during the experiment to eliminate sudden elevations of arterial blood pressure and retraction of the hind paw in response to a painful pinch. The animals were paralysed with Pavulon ($2 \, \text{mg kg}^{-1}$ i.v.) and artificially ventilated (with O_2-enriched room air) through a tracheal cannula. The body temperature was continuously monitored in the rectum and maintained at $37.0 \pm 0.5^\circ$ C with a DC heating pad.

Both femoral arteries were cannulated for continuous blood pressure measurement (with ELEMA-SIEMENS equipment) and for gasometric purposes (with BLOOD GAS ANALYZER, AVL 995-Hb).

The femoral vein was also catheterized for i.v. injections. The animal was placed in a prone position and the rat's head was fixed in a head-holder.

Measurement of cortical blood flow

A cranial window was drilled and the sensory cortex exposed. The dura mater was left intact. The

cerebral blood flow was continuously recorded with a Laser-Doppler Flowmeter (ALF 2100, wavelength 780 mm, Advance Co, Tokyo). The probe (external diameter 0.8 mm) was placed on the surface of the dura mater at a site 3 mm lateral and 2 mm caudal to bregma. The position of the probe was adjusted with a micromanipulator so as to avoid any visible blood vessels. Once a suitable placement was obtained, the position was unchanged during the experiment.

Control of the gasometric parameters

Partial pressures of arterial CO_2 and O_2, along with arterial pH, were measured and maintained at P_aCO_2 38.2±3 mm Hg, P_aO_2 380±19 mm Hg, pH 7.39±0.06, by adjustment of a ventilatory pump.

Hypercapnia was introduced by application of a gas mixture consisting of 10% CO_2 and 90% O_2 into an inspiratory line of the respirator (HARVARD Rodent Ventilator, MODEL 683). Partial pressures of arterial CO_2, O_2 and pH after 1 min exposure to CO_2 were as follows: P_aCO_2 84.1±5 mm Hg, P_aO_2 398±11 mm Hg, pH 7.1±0.06. After 2 min of CO_2 exposure the values of arterial CO_2, O_2 and pH did not differ significantly from those measured after 1 min.

Experimental protocol

The response of cerebrocortical blood flow (CBF), mean arterial blood pressure (MAP) and cerebrocortical vascular resistance (CVR), estimated from the MAP divided by the CBF, during 2-min exposure to hypercapnia were recorded before and after i.v. administration of Nitro-L-arginine-methyl-ester (L-NAME, 15 mg/kg), a known L-arginine analogue, which inhibits NO formation.

In the second run of experiments L-NAME was supplemented so as to achieve a total dose of 70 mg/kg and the experimental protocol was repeated. L-arginine (600 mg/kg i.v.) was administered at the end of the experiment to reverse the effects and to test the specificity of the action of L-NAME. The administration of either L-NAME or L-arginine did not change arterial pH significantly.

Data analyses

For the statistical analysis we employed one-sided Student's t-tests, paired or unpaired as appropriate.

Before any interpretation, we checked if there is any relation between initial, control MAP, CBF and CVR values and the respective changes of these values after L-NAME administration as well as after exposure to 10% CO_2. For each group of data the best fitting straight line was approximated using the method of least squares (not shown) and correlation coefficients were calculated. This procedure was repeated for both relative and absolute changes of CBF, MAP and CVR. It was assumed that the best representation of the data was found when the slant of the regression line and the correlation coefficients were not statistically significant.

This approach allowed us to find such a representation of the data, that the results were possibly independent of the baseline values of CBF, CVR and MAP.

It was found that the most reliable comparison between responses of the analysed parameters to L-NAME and to CO_2 administration was obtained by computing absolute changes of MAP (ΔMAP) and relative changes of CBF(ΔCBF/CBF$_o$ x 100%) and of CVR(ΔR/R$_o$ x 100%).

RESULTS

Dual response of cortical blood flow to CO_2 after L-NAME administration

The animals could be divided into two distinct groups according to different responses of CBF to CO_2 before and after administration of L-NAME. In 11 rats (group 1), as expected, the CBF response to CO_2 after L-NAME was decreased (Fig. 1). However, in the remaining 5 rats (group 2) CBF response to a CO_2 stimulus was significantly augmented (Fig. 2). This dual pattern of response was identical in each group after both 15 mg/kg and 70 mg/kg of L-NAME and lasted over the whole period of hypercapnia. No significant differences in MAP, CBF and CVR responses to NOS inhibition alone were observed between the two groups of animals.
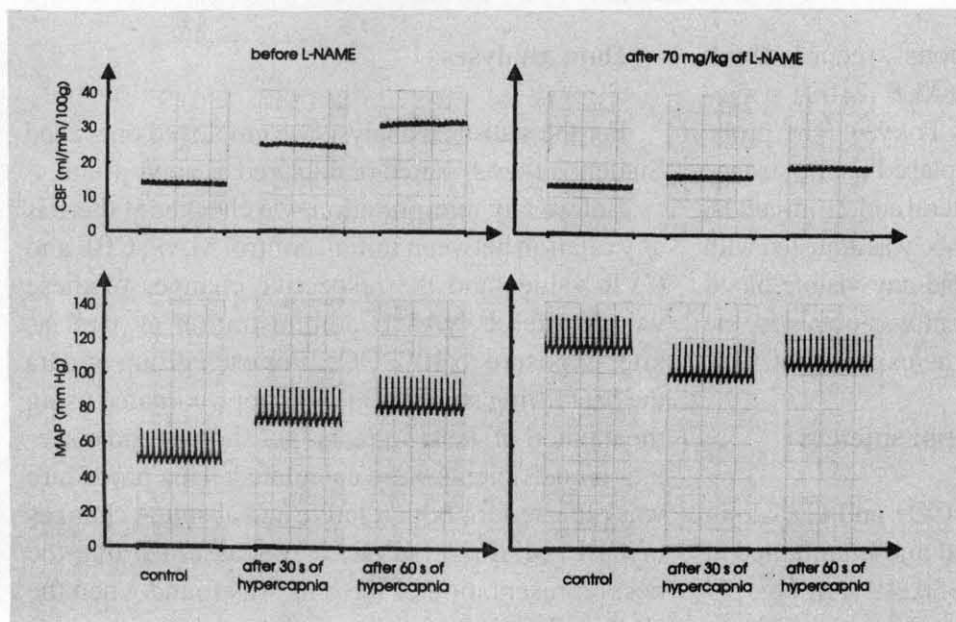
Fig. 1. A representative recording of CO_2-induced changes of CBF and MAP in the first 60 s of hypercapnia before and after 70 mg/kg L-NAME administration in group 1 of the rats. As can be seen in the upper panel L-NAME diminished the response of CBF to CO_2. This was accompanied by a lack of the pressor response to CO_2 (lower panel). Administration of L-NAME alone increased MAP, but did not change CBF significantly.

Characteristics of the groups

We analysed these two groups of animals separately. All values are presented as mean ±SEM. In group 1 before L-NAME administration MAP was 84.6±6.7 mm Hg, CVR 5.5±0.6, and in group 2 respectively: MAP 99.2±6.7 mm Hg, CVR 7.9±1.0. There was no significant difference between basal CBF values in the two groups. After L-NAME administration (15 mg/kg) MAP increased by 25.3±4.9 mm Hg ($P<0.005$) in group 1 and by 27.0±6.4 mm Hg ($P<0.025$) in group 2. CBF did not change significantly either in group 1 or in group 2. CVR increased

by 34±10% ($P<0.005$) in group 1 and by 26±10% ($P<0.05$) in group 2.

After administration of a high dose of L-NAME (70 mg/kg), the values of all the parameters characterizing each group increased slightly.

Responses of the cortical blood flow to CO_2 after L-NAME administration

Figure 3 illustrates two different patterns of responsiveness to CO_2 after L-NAME administration. A CO_2-induced increase in CBF during hypercapnia was decreased after L-NAME in group 1,
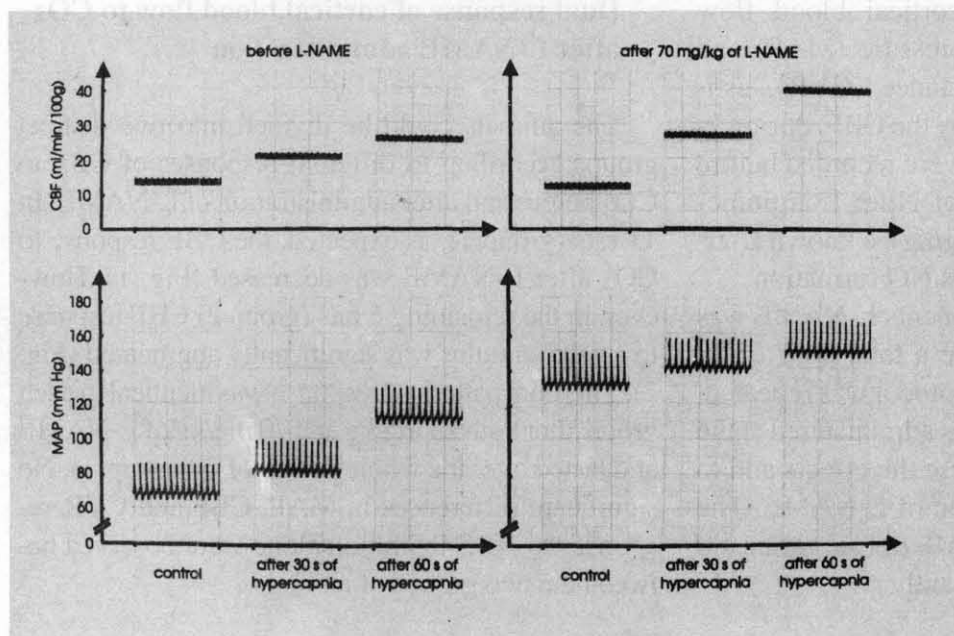

Fig. 2. A representative recording of CO_2-induced changes of CBF and MAP in the first 60 s of hypercapnia before and after 70 mg/kg L-NAME in group 2 of the rats. L-NAME augmented a CO_2-induced increase in CBF (upper panel). Arterial blood pressure response to CO_2 was preserved (lower panel). Administration of L-NAME alone increased MAP, but did not change CBF significantly.

whereas it was augmented in group 2. In group 1 the difference between pre- and post-L-NAME CBF values was the most significant at 30 s of the duration of hypercapnia (35.1±6.3% before L-Name vs. 8.8±5.6% after 15 mg/kg L-NAME, $P<0.01$ and 1.8±8.3% after 70 mg/kg, $P<0.01$). In group 2 there was no significant difference between pre- and post-L-NAME CBF values at 30 s of hypercapnia but there was a strong positive tendency for this difference to become greater after longer hypercapnia, reaching 43.4±24.0% before L-NAME vs. 137.8±38.8% after 70 mg/kg L-NAME at 120 s of the duration of hypercapnia. There was no significant difference between pre-L-NAME values of CBF in group 1 and group 2 at any time point of hypercapnia, but after L-NAME, at any time point, the CBF values in group 1 and in group 2 were significantly different from each other during hypercapnia. The magnitude of L-NAME effect was about the same regardless of whether a low or a high dose was administered.

Responses of the arterial blood pressure to CO_2 after L-NAME administration

Both groups were analysed in respect to absolute changes in MAP during hypercapnia before and

after L-NAME administration. In both groups a significant increase in MAP during hypercapnia appeared in the pre-L-NAME conditions. After L-NAME administration (70 mg/kg) in group 1 MAP decreased at 30 s of hypercapnia (-15.2±5.7 mm Hg, $P<0.025$). After this "dip", MAP rose gradually, but in most cases did not reach the respective control value (Fig. 4). This effect seems to be L-NAME dose-dependent. In group 2, after L-NAME administration a continuous pressor response to hypercapnia was preserved, and the response was not significantly depressed even by a high dose of L-NAME (Fig. 4).

Changes of the cerebrocortical vascular resistance during hypercapnia after L-NAME administration

Figure 5 shows relative changes of the cerebrovascular resistance (CVR) during hypercapnia before and after L-NAME.

In both groups a similar decrease in CVR occurred during hypercapnia before administration of L-NAME (in group 1: by -13.7±2.3% at 30 s, $P<0.005$; -15.8±6.5% at 120 s, $P<0.025$, and in group 2: -11.2±5.3% at 30 s, NS; -11±8% at 120 s,
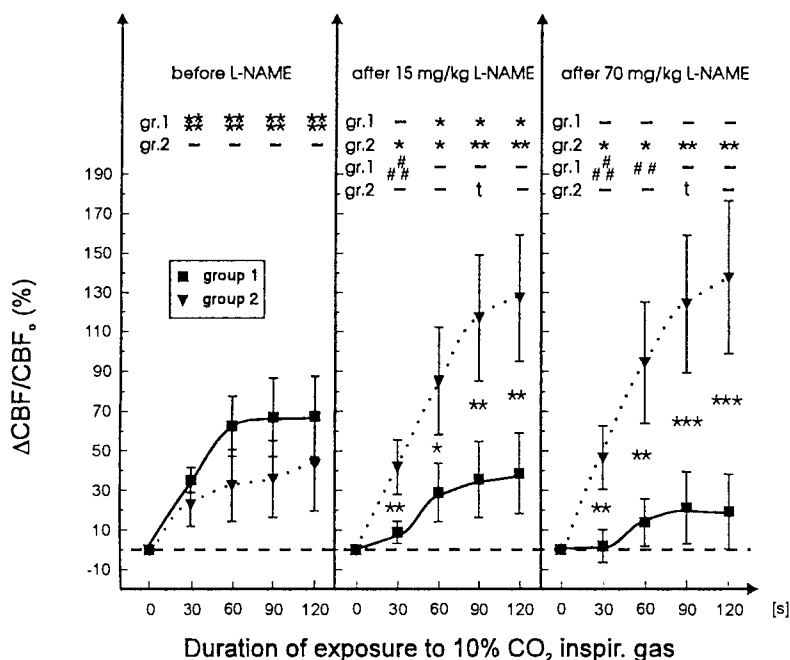

Fig. 3. Relative increases of CBF in response to a CO_2-stimulus of 120 s duration are shown before L-NAME and after two different doses of L-NAME . In group 1 the response of CBF was decreased by L-NAME, whereas it was facilitated by L-NAME in group 2. Symbols in the rows at the top of the figure indicate the statistical significance of the difference between the points on the graph below a given symbol and their respective control values before the beginning of hypercapnia (*) and before administration of L-NAME at the same time point of the duration of hypercapnia (#). Symbols between the points on the graph indicate the statistical significance of the difference between these points. * (or #) - $P<0.05$; ** (or ##) - $P<0.025$; *** (###) - $P<0.01$; **** (or ####) - $P<0.005$; "t" tendency; "-" "not significant". Each point is the mean ±SD.
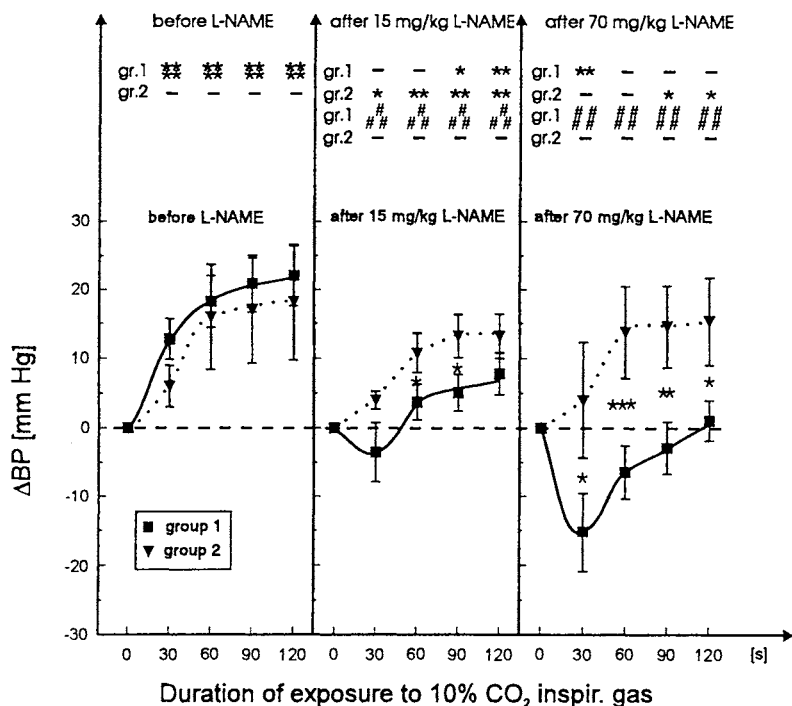
Fig. 4. Absolute changes of mean arterial blood pressure (BP) during hypercapnia before and after administration of L-NAME. In group 2 the pressor response was preserved after L-NAME. In group 1, L-NAME eliminated the pressor response to hypercapnia, the effect being dose-dependent. Markings as in Fig. 3.

ns). In group 2 the changes were not significant due to a small sample size.

After L-NAME administration, the decrease in CVR during hypercapnia was significantly greater in group 2 (after 15 mg/kg L-NAME: -24.8±6.5% at 30 s, $P<0.025$; -47.1±7.6% at 120 s, $P<0.005$), whereas in group 1 the CO_2-induced decrease in CVR was not significantly affected by L-NAME (after 15 mg/kg L-NAME: -10.1±5.1% at 30 s, $P<0.05$; -10.5±8.5% at 120 s, NS). The magnitude of L-NAME effects did not depend on the dose applied.
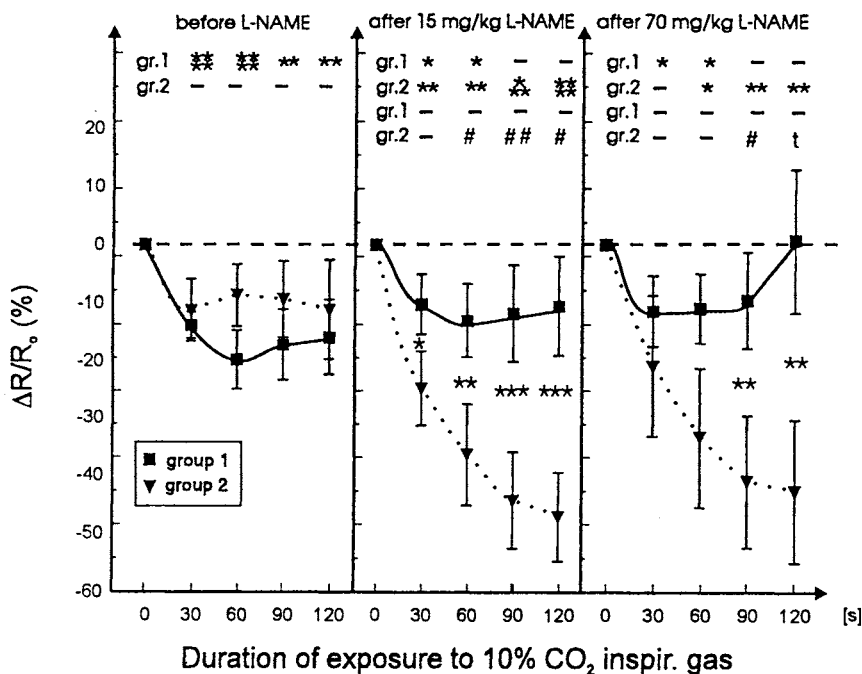

Fig. 5. Relative changes of local cerebrocortical vascular resistance (R) during hypercapnia in groups 1 and 2 before and after L-NAME administration. Hypercapnia induced a decrease of R in both groups before L-NAME. This effect was significantly larger after L-NAME in group 2. In group 1 the response of R to CO_2 was about the same before and after L-NAME administration. However, after a high dose of L-NAME (70 mg/kg), longer hypercapnia (120 s) did not elicit any significant decrease in cerebrocortical vascular resistance. Markings as in Fig. 3.
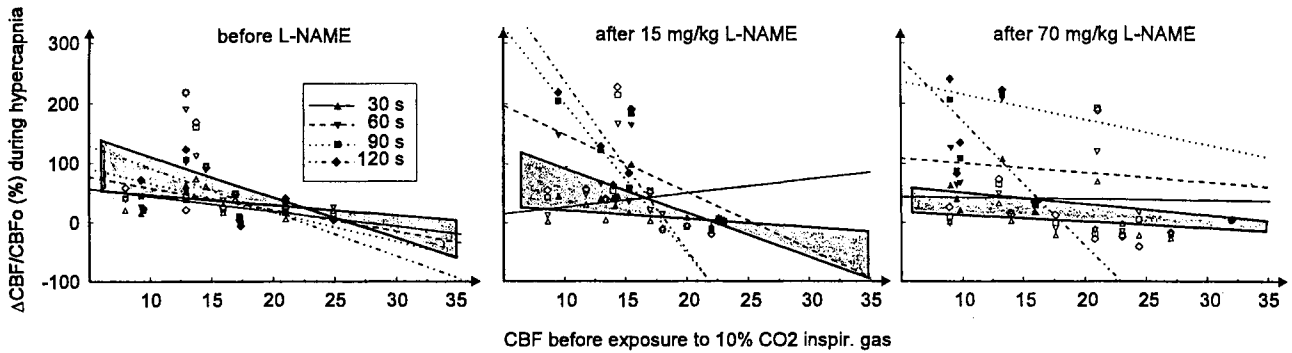
Fig. 6. CBF responses to CO_2 (ordinate) at a given time point of hypercapnia (30 s, 60 s, 90 s, 120 s) plotted against respective control CBF values. A magnitude of this response is related to an initial control CBF value. For each particular time point a regression line can be drawn for either of the two groups. The shadowed area contains all the regression lines for group 1, whereas the regression lines for group 2 and the points, on which these lines are based, are drawn separately. Before L-NAME, all the regression lines for group 2 were contained within the shadowed area. After L-NAME these lines became more slanted and different from those contained within the shadowed area. The slant was greater for longer periods of hypercapnia during the same 2-min exposure to CO_2. Duration of hypercapnia of 30 s, 60 s, 90 s and 120 s is represented by time points respectively at 30 s, 60 s, 90 s and 120 s from the onset of hypercapnia.

Reversibility of L-NAME effects by L-arginine

In both groups i.v. administration of L-arginine gradually reduced MAP and CVR to values sligthly higher, but not significantly different from the pre-NAME values and it did not influence CBF values consistently.

The effects of L-NAME (70 mg/kg) on the responses to CO_2 were only partially reversed. In group 1, the pattern of MAP responses to CO_2 after L-arginine administration was similar to that after L-NAME, and not to that prior to L-NAME administration. At 30 s of the duration of hypercapnia MAP decreased by -5.9±3.8 mm Hg (vs. -15.2±5.7 mm Hg after 70 mg/kg L-NAME) and CBF increased by 10.1±4.2% (vs. 1.8±8.3%). The response of CVR to CO_2 was not markedly affected by L-arginine. In group 2 the pressor response to CO_2 was similar both before and after L-arginine administration. At 120 s of hypercapnia CBF increased by 111±18% (vs. 137.8±38.8% after 70 mg/kg L-NAME) and CVR decreased by -42±6.6% (vs. -47.1±7.6%).

Other differences between the groups

The two groups could not be distinguished from each other on the basis of the control values of MAP, CBF and CVR, or on the basis of their respective responses to L-NAME alone or to CO_2 before L-NAME administration. The differences became apparent by different responses of CBF and MAP to a CO_2-stimulus in each group only after L-NAME administration.

Figure 6 illustrates another feature which discriminates between the two groups. The shadowed area contains all the regression lines (not drawn) obtained at different time points of hypercapnia in group 1. The regression lines for group 2 are drawn for the following periods of hypercapnia: 30 s, 60 s, 90 s, 120 s. Before L-NAME administration the regression lines for group 2 were contained within the shadowed area, whereas after NOS inhibition they became negatively slanted, the effect being the most pronounced at later time points of hypercapnia. In group 2, the magnitude of CBF response to hypercapnia after L-NAME administration depended in an inverse relation on the control, steady state values of CBF before hypercapnia.

DISCUSSION

L-NAME administration produced a well-known increase in the mean arterial blood pressure (MAP) and local vascular resistance (CVR) in the

cortex. No significant reduction of the cortical blood flow (CBF) was observed, a result different from those reported by Kozniewska et al. (1992), Pellegrino et al. (1993) and Sandor et al. (1994), but consistent with those of Buchanan and Phillis (1993) and Reid et al. (1994), all of whom applied an NOS blocker to systemic circulation.

Perhaps discrepancies are due to differences in NOS activity in different segments of the cerebral vessels. It appears that NO-dependent vasodilatatory tone is less evident in microcirculation, which is analysed in our study by Laser-Doppler flowmetry, than in large cerebral arteries (Katusic and Cosentino 1994). Whatever the reason, a significant increase in CVR after L-NAME administration, confirmed also in our experiments, indicates that endogenous NO accounts for a significant vasodilatatory tone in the cortical microvessels.

Inhibition of NO synthesis modulated CO_2 responsiveness of CBF and of MAP differently in two groups of animals of the same Wistar strain studied in similar experimental conditions (Figs. 1 and 2). In 11 rats L-NAME administration attenuated the increase in CBF elicited by a CO_2 stimulus (Fig. 3, group 1), yet did not change CVR response (Fig. 5). Reduction of CBF response was apparently secondary to the reversal of the arterial blood pressure response, as CO_2 induced a fall instead of a rise of MAP after inhibition of NO synthesis in this group of animals (Fig. 4). Our result does not support the concept that NO is a mediator of CO_2-induced vasodilatation in the cortex. Immediate, short-lasting vasodilatatory effects of CO_2 may not require NO derived from the enthothelium or other sources. Increased membrane K^+ conductance and hyperpolarization of vascular smooth muscle cells by endothelium derived hyperpolarising factor (EDHF) (Brayden et al. 1991, Chen et al. 1991) or *via* activation of the electrogenic Na^+ pump by extracellular acidification (Toda et al. 1989) should be considered.

A number of studies using topical application of NO inhibitors, in which blood pressure remained constant, found reduced CO_2 reactivity after prolonged treatment (Iadecola 1992, Dirnagl et al.

1993, Niwa et al. 1993, Fabricius and Lauritzen 1994, Irikura et al. 1994). However, these studies differ from our study in two important respects. Firstly, they tested the reactivity of the cerebral circulation to 5% CO_2, whereas in our study 10% CO_2 was applied. It is possible that at higher partial pressures CO_2 may produce vasodilatation by a direct effect on smooth muscle cells (this problem is discussed in more detail by Iadecola, 1992). Secondly, the above-mentioned authors studied steady state conditions, because the attenuation of vasodilatation in animals treated with NOS inhibitors was noted after a long exposure to CO_2, eg.: 7 min (Iadecola 1992), 5-10 min (Fabricius and Lauritzen 1994), 5 min (Niwa et al. 1993, Irikura et al. 1994). In our study dynamic conditions are studied, as we apply a short-lasting, transient CO_2-stimulus for 120 s only. Interestingly, at the end of 2 min-exposure to hypercapnia a decrease in CVR appears to be slightly attenuated by L-NAME (Fig. 5), a finding which suggests that NO may play some role in CO_2-induced vasodilatation only at some later stage of hypercapnia. Also, it must not be forgotten, that with topical application higher local concentration of an NOS blocker may be achieved.

A novel finding was a reversal of the pressor response to CO_2 in 11 out of 16 animals after L-NAME administration. A pressor response to CO_2 is due to central sympathoexcitatory mechanisms (Trzebski et al. 1974, Hanna et al. 1981, Trzebski and Kubin 1981) involving activation of the rostral ventrolateral medullary neurones (RVLM) (for review see Ciriello et al. 1986). Recently it has been reported that NOS is present in RVLM neurones (Iadecola et al. 1993). Blockers of NO synthesis produce central sympathoexcitatory effects (Togashi et al. 1990, 1992, Sakuma et al. 1992, Harada et al. 1993, Tagawa et al. 1994). Inhibitory role of the centrally generated NO in the modulation of sympathetic activity and arterial blood pressure appears inconsistent with attenuation or reversal of the central pressor effect of CO_2 after inhibition of NO synthesis. Perhaps inhibition of NOS could depress central CO_2-sensitive neurones which project to sympathoexcitatory neuronal population of

RVLM. This problem requires, however, further research for elucidation. A transient fall of the arterial blood pressure induced by hypercapnia in this group of animals is probably due to the peripheral vasodilatatory and cardioinhibitory action of CO_2 (Suutarinen 1966, Beręsewicz and Trzebski 1983).

Microinjections of NO-providing drugs into RVLM sympathoexcitatory neuronal population attenuate renal nerve sympathetic activity and decrease arterial blood pressure, while injections of the same drugs into the sympatho-inhibitory neuronal population within the caudal ventrolateral medulla (CVLM) enhance renal nerve sympathetic activity and raise systemic blood pressure (Shapoval et al. 1991). Therefore, it may be that the CO_2-induced sympathoexcitatory action depends on NO-dependent inhibition of the CVLM inhibitory neurons with following disinhibitory sympathetic activation. If so, L-NAME would be expected to abolish this central disinhibition of sympathetic activity and to reduce or to eliminate the sympathoexcitatory and pressor effect of CO_2.

A major finding of this study was a significant facilitatory effect of L-NAME on the vasodilatatory response to CO_2 in the cortical microcirculation in 5 out of the 16 rats (Figs. 3 and 5). As the facilitatory effect was not diminished by a very high dose of L-NAME (70 mg/kg i.v.), its mediation by NO-dependent vasodilatation can be excluded. In this group of rats the pressor response to CO_2 was preserved. A significant potentiation of the CO_2-induced cortical vasodilatation restricted only to those rats in which the central pressor effect of CO_2 was present, is difficult to explain. The magnitude of the potentiation was related to the control, steady state values of CBF measured after administration of L-NAME: the lower the control CBF, the more pronounced vasodilatatory response to CO_2 in the group of animals responding with vasodilatatory facilitation (Fig. 6). This inverse relationship may suggest that blocking of NOS removed some CO_2-dependent central vasoconstrictive mechanism - an effect opposite to the peripheral vascular response to NOS inhibition which is characterized by removal of vasodilatatory tone and by predominance of

vasoconstrictive influences. Therefore we propose a central rather than a peripheral mechanism to account for the increase in CO_2-induced vasodilatation after NOS inhibition.

Neurones producing cortical vasodilatation *via* an intracerebral vasodilatatory pathway have been demonstrated within RVLM by local microinjections of glutamate in rats (Saeki et al. 1989). Central sympathoexcitatory action of CO_2 may activate this vasodilatatory RVLM neuronal population. Under control conditions vasodilatatory neurogenic effect of CO_2 could be overriden by simultaneous excitation of vasoconstrictive neurones within RVLM and CVLM via cervical sympathetic nerves supplying blood vessels in the cortex (Maeda et al. 1991, 1994). This neurogenic and sympathetically mediated vasoconstrictive effect possibly prevents excessive vasodilatation in cortical microcirculation during hypercapnia. After blocking NO-synthesis mutual antagonistic central vasoconstrictive and vasodilatatory effects of CO_2 *via* RVLM neurones could be diminished or abolished. If, however, in some animals central vasoconstrictive neurogenic mechanisms were more effectively depressed by NOS blockade than the central vasodilatatory mechanism mediated by RVLM neurones, the net result could be a facilitation of the vasodilatation elicited by a brief CO_2 stimulus.

At the present stage any interpretation of the mechanisms by which inhibition of NOS facilitates CO_2 responsiveness of the cortical microvessels in some rats would be speculative. For resolving this problem further research is needed.

L-arginine prevented only part of the effect of L-NAME. This is not a novel finding, as it has been reported that inhibitory effects of certain NOS blockers may be irreversible (Mülsch and Busse 1990, Dwyer et al. 1991) Also, as mentioned in the Results, the difference between the effects of both doses of L-NAME on control CVR, CBF and MAP was slight, so it is conceivable that after 70 mg/kg L-NAME the system is saturated and any competitive action of L-arginine is not apparent.

In summary, the present results do not support the concept that endogenous NO plays a significant

role as a mediator in the transient vasodilatatory response to brief hypercapnia in the cortical microvessels of the rat. These results are consistent with those of Adachi et al. (1992).

In two groups of Wistar rats we found opposite effects of NOS inhibition upon arterial blood pressure and cortical blood flow responses to CO_2. This result indicates that extreme caution is needed in interpretation of experimental data processed with routine statistical tests as they may provide conflicting results. Controversies as to the role of the endogenous NO in the vasodilatatory action of hypercapnia in the cerebral cortex may be due to complex central and vascular mechanisms modulated in variable ways by NO and by NOS blockers.

ACKNOWLEDGEMENTS

REFERENCES

Adachi T., Inanami O., Sato A. (1992) Nitric Oxide (NO) is involved in increased cerebral cortical blood flow following stimulation of the nucleus basalis of Meynert in anesthetized rats. Neurosci. Lett. 139: 201-204.

Amano T., Fukuuchi Y., Yamawaki T., Obara K., Muramatsu K., Shirai T., Takahashi K., Gotoh J., Gotoh F. (1993) Does NO participate in CO_2 reactivity of cerebral vessels? J. Cereb. Blood Flow Metab. 13 (Suppl. 1): XVI-6.

Beręsewicz A., Trzebski A. (1983) Chronotropic response of isolated guinea pig atria to alterations in pCO_2, bicarbonate and pH. Pol. J. Pharmacol. 35: 481-487.

Brayden J.E., Quayle J.M., Standen N.B., Nelson M.T. (1991) Role of potassium channels in the vascular response to endogenous and pharmacological vasodilators. Blood Vessels 28: 147-153.

Bredt D.S., Snyder S.H. (1992) Nitric oxide, a novel neuronal messenger. Neuron 8: 3-11.

Buchanan J.E., Phillis J.W. (1993) The role of nitric oxide in the regulation of cerebral blood flow. Brain Res. 610: 248-255.

Busija D.W., Heistad D.D. (1984) Factors involved in the physiological regulation of the cerebral circulation. Rev. Physiol. Biochem. Pharmacol. 101: 162-211.

Chen G., Yamamoto Y., Miwa K., Suzuki H. (1991) Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am. J. Physiol. 260: H1888-H1892.

Ciriello J., Caverson M.M., Polosa C. (1986) Function of the ventrolateral medulla in the control of the circulation. Brain Res. Rev. 11: 359-391.

Cosentino F., Still J.C., Katusic Z.S. (1993) Endothelial L-arginine pathway and relaxation to vasopressin in canine basilar artery. Am. J. Physiol. 264: H413-H418.

Dirnagl U., Lindauer U., Villringer A. (1993) Role of nitric oxide in the coupling of cerebral blood flow to neuronal activation in rats. Neurosci. Lett. 149:43-46.

Dwyer M.A., Bredt D.S., Snyder S.H. (1991) Nitric oxide synthase: irreversible inhibition by L-N-nitroarginine in brain in vivo and in vitro. Biochem. Biophys. Res. Commun. 176:1136-1141.

Fabricius M., Lauritzen M. (1994) Examination of the role of NO for the hypercapnic rise of CBF in rats. Am. J. Physiol. 266: H1457-H1464.

Faraci F.M. (1991) Role of endothelium-derived relaxing factor in cerebral circulation: Large arteries vs microcirculation. Am. J. Physiol. 261: H1038-H1042.

Hanna B.D., Lioy F., Polosa C. (1979) The effect of cold blockade of the medullary chemoreceptors on CO_2 modulation of vascular tone and heart rate. Can. J. Physiol. Pharmacol. 57: 461-468.

Hanna B.D., Lioy F., Polosa C. (1981) Role of carotid and central chemoreceptors in the CO_2 response of sympathetic preganglionic neurons. J. Aut. Nerv. Syst. 3: 421-435.

Harada S., Tokunaga S., Momohara M., Masaki H., Tagawa T., Imaizumi T., Takeshita A. (1993) Inhibition of nitric oxide formation in the nucleus tractus solitarius increases renal sympathetic nerve activity in rabbits. Circ. Res. 72: 511-516.

Iadecola C. (1992) Does NO mediate the increases in CBF elicited by hypercapnia. Proc. Natl. Acad. Sci. USA 89: 3913-3916.

Iadecola C., Faris P.L., Hartman B.K., Xiaohong Xu (1993) Localization of NADPH diaphorase in neurons of the rostral ventral medulla: possible role of nitric oxide in central autonomic regulation and oxygen chemoreception. Brain Res. 603: 173-179.

Iadecola C., Zhang F. (1994) NO-dependent and independent components of cerebrovasodilation elicited by hypercapnia. Am. J. Physiol. 266: R546-R552.

Irikura K., Maynard K.J., Lee W.S., Moskowitz A. (1994) L-NNA decrease cortical hyperemia and brain cGMP levels following CO_2 inhalation in Sprague-Dawley rats. Am. J. Physiol. 267: H837-H843.

Katusic Z.S., Cosentino F. (1994) Nitric oxide synthase: from molecular biology to cerebrovascular physiology. News Physiol. Sci. 9: 64-67.

Kozniewska E., Oseka M., Stys T. (1992) Effects of endothelium-derived nitric oxide on cerebral circulation during

normoxia and hypoxia in the rat. J. Cereb. Blood Flow Metab. 12: 311-317.

Lioy F., Trzebski A. (1984) Pressor effect of CO_2 in the rat: different thresholds of the central cardiovascular and respiratory responses to CO_2. J. Auton. Nerv. Syst. 10: 43-54.

Maeda M., Inoue M., Takao S., Hayashida Y., Nakai M., Krieger A.J., Sapru H.N. (1994) Caudal ventrolateral medullary depressor area controls cerebral circulation via rostral ventrolateral medullary pressor area. Pflüger's Arch. (Eur. J. Physiol.) 427: 556-558.

Maeda M., Krieger A.J., Sapru H.N. (1991) Chemical stimulation of the ventrolateral medullary depressor area decreases ipsilateral cerebral blood flow in anesthetized rats. Brain Res. 543: 61-68.

Moncada S., Palmer R.M.J., Higgs E.A. (1991) Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev. 43: 109-142.

Mülsch A., Busse R. (1990) N^G-nitro-L-arginine (N^5-[imino(nitroamino)methyl]-L-ornithine) impairs endothelium-dependent dilatations by inhibiting cytosolic nitric oxide synthesis from L-arginine. Naunyn-Schmiedeberg's Arch. Pharmacol. 341: 143-147.

Niwa K., Lindauer U., Villringer A., Dirnagl U. (1993) Blockade of Nitric Oxide synthesis in rats strongly attenuates the CBF response to extracellular acidosis. J. Cereb. Blood Flow Metab. 13:535-539.

Norman Y., Kitamura Y. (1993) Inducible nitric oxide synthase in glial cells. Neurosci. Rev. 18: 103-107.

Nunokawa Y., Ishida N., Tanaka S. (1993) Cloning of inducible nitric oxide synthase in rat vascular smooth muscle cells. Biophys. Res. Commun. 191: 89-94.

Pellegrino D.A., Koenig H.M., Albrecht R.F. (1993) NO synthesis and regional CBF response to hypercapnia and hypoxia in the rat. J. Cereb. Blood Flow Metab. 13: 80-87.

Persson M.G., Wiklund N.P., Hedgvist P., Gustafsson L.E. (1991) Microvascular effects of local or systemic inhibition of endogenous endothelium-derived relaxing factor (nitric oxide) production. J. Cardiovasc. Pharmacol. 17 (Suppl. 3): S169-S172.

Reid J.M., Davies A.G., Paterson D.J. (1994) Effect of N-monomethyl-L-arginine on CBF during hypercapnia, eucapnia and hypoxia in the anaesthetized rat. Proc. Physiol. Soc. 80P-81P.

Saeki Y., Sato A., Sato Y., Trzebski A. (1989) Stimulation of RVLM neurons increases cortical CBF via activation of the intracerebral neural pathway. Neurosci. Lett. 107: 26-32.

Sakuma I., Togashi H., Yoshioka M., Saito H., Yanagid M., Tamura M., Kobayashi T., Yasuda H., Gross S.S., Levi R. (1992) N^G-methyl--L-arginine, an inhibitor of L-arginine-derived NO synthesis, stimulates renal sympathetic nerve activity in vivo. Circ. Res. 70: 607-611.

Sandor P., Komiati K., Reivich M., Nyary J. (1994) Major role of NO in the mediation of regional CO_2 responsiveness of the cerebral and spinal cord vessels of the cat. J. Cereb. Blood Flow Metab. 14: 49-58.

Shapoval L.N., Sagach V.F., Pobegailo L.S. (1991) Nitric oxide influences ventrolateral medullary mechanisms of vasomotor control in the cat. Neurosci. Lett. 132: 47-50.

Suutarinen T. (1966) Cardiovascular responses to changes in arterial carbon dioxide tension. Acta Physiol. Scand. (Suppl.) 266: 1-75.

Tagawa T., Imaizumi T., Harada S., Endo T., Masanasi S., Hirooka Y., Takeshita A. (1994) Nitric oxide influences neuronal activity in the nucleus tractus solitarii of rat brain stem slices. Circ. Res. 75: 70-76.

Tanaka K., Gotoh F., Gomi S., Takashima S., Miharo B., Shirai T., Nogawa S., Nagata E. (1991) Inhibition of nitric oxide synthesis induces a significant reduction in local cerebral blood flow in the rat. Neurosci. Lett. 127: 129-132.

Toda N., Hatano Y., Mori K. (1989) Mechanisms underlying response to hypercapnia and bicarbonate of isolated dog cerebral arteries. Am. J. Physiol. 257: H141-H146.

Toda N., Okamura T. (1990a) Mechanism underlying the response to vasodilator nerve stimulation in isolated dog and monkey cerebral arteries. Am. J. Physiol. 259: H1511-H1517.

Toda N., Okamura T. (1990b) Modification by L-N^G-monomethylarginine (L-NMMA) of the response to nerve stimulation in isolated dog mesenteric and cerebral arteries. Jap. J. Pharmacol. 52: 170-173.

Toda N., Okamura T. (1991) Role of NO in neurally induced cerebroarterial relaxation. J. Pharmacol. Exp. Ther. 258: 1027-1032.

Togashi H., Sakuma I., Yoshioka M., Kobayashi T., Yasuda H., Kitabatake A., Saito H., Gross S.S., Levi R. (1992) A central nervous system action of nitric oxide in blood pressure regulation. J. Pharmacol. Exp. Ther. 262: 343-347.

Togashi H., Yoshioka M., Saito H., Sakuma I., Kobayashi T., Yasuda H., Gross S.S., Levi R. (1990) Central sympatho-excitatory effects of L-N^G-methylarginine, a selective inhibitor of NO synthesis from L-arginine. Circulation 82 (Suppl. 3): III-457.

Trzebski A., Kubin L. (1981) Is the central inspiratory activity responsible for pCO_2-dependent drive of the sympathetic discharge? J. Auton. Nerv. Syst. 3: 401-420.

Trzebski A., Zieliński A., Majcherczyk S., Lipski J., Szulczyk P. (1974) Effect of chemical   stimulation and depression of the medullary superficial areas on the respiratory motoneurones discharges, sympathetic activity and efferent control of carotid area receptors. In: Central rhythmic and regulation (Eds. W.Umbach and H.P.Koepchen). Hippokrates, Stuttgart, p. 170-177.

Wang Q., Paulson O.B., Lassen N.A. (1992) Effect of NO blockade by N^G-Nitro-L-arginine on cerebral blood flow responses to changes in CO_2 tension. J. Cereb. Blood Flow Metab. 12: 947-953.

Wang Q., You J.P., Jansen I., Edvinsson L., Paulson O.B., Lassen N.A. (1993) Mechanisms underlying effects of NO inhibition on hypercapnic vasodilation in isolated basilar arteries from rats. J. Cereb. Blood Flow Metab. 13 (Suppl. 1): XVIII-22.