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Influence of nitrovasodilators and cyclooxygenase inhibitors on cerebral vasoreactivity in conscious rabbits

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Abstract

Since the nitric oxide (NO) and cyclooxygenase pathways have been suggested to have important roles in most vasodilations, our aim was to study the influence of cyclooxygenase inhibitors and nitrovasodilators on cerebrovascular reserve capacity. Corticocerebral blood flow was measured by hydrogen polarography during hypercapnia and acetazolamide stimuli in conscious rabbits. The measurements were repeated in the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME) and indomethacin as nitric oxide synthase (NOS) and cyclooxygenase inhibitors. The effects of nitroglycerin and isosorbide-5-nitrate were also tested. L-NAME completely, while indomethacin markedly inhibited the hypercapnic corticocerebral blood flow response. Nitroglycerin and isosorbide-5-nitrate significantly attenuated hypercapnia elicited corticocerebral blood flow increase. The different treatments reduced only moderately the acetazolamide-induced corticocerebral blood flow response. These results lend support to the hypothesis that antithrombotic and antiinflammatory medication (cyclooxygenase inhibitors) and nitrovasodilator treatments could interfere with the measurement of cerebrovascular reactivity resulting in underestimation of the cerebrovascular reserve capacity in patients taking these drugs. © 2001 Published by Elsevier Science B.V.

Keywords: Acetazolamide; Carbon dioxide; Nitrovasodilator; Cyclooxygenase inhibitor; Cerebral vasoreactivity

1. Introduction

In patients with occlusive carotid disease, the effectiveness of the collateral circulation to the brain can be measured by determining the cerebrovascular reserve capacity using carbon dioxide or acetazolamide stimuli. Since patients with an impaired cerebral vasoreactivity are at high risk of stroke, cerebrovascular reserve capacity furnishes important information as to the need for revascularization surgery such as carotid endarterectomy or external to internal cranial bypass.

NO produced by the action of NOS has been shown to play a role in the cerebral vasodilatory responses (Iadecola and Zhang, 1994; McPherson et al., 1995; Wang et al., 1995). Although studies on NO in the hypercapnic response have yielded conflicting results, dependent on the species and anaesthetics used in the experiments, they usually agree in that the NO pathway plays a critical role in capnic vasodilation (McPherson et al., 1993, 1994, 1995; Pelligrino et al., 1993; Saito et al., 1993; Ichord et al., 1994). It has been demonstrated that nitrovasodilators, including nitroglycerin, elicit relaxations mediated by guanosine 3′,5′-cyclic monophosphate (cGMP), which is activated by NO liberated from these molecules. This idea is supported by the fact that the responses are suppressed by NO scavengers or inhibitors of soluble guanylate cyclase, while NO compounds increase the production of cGMP in the vascular tissue (Ignarro and Kadowitz, 1985).

Evaluation of the cyclooxygenase pathway in cerebral vasodilation is difficult because of the controversy relating to the studies. Some studies indicated that indomethacin reduces hypercapnia-induced cerebral vasodilation (Eriksson et al., 1983; Wang et al., 1993; Marcus et al., 1994), while others demonstrated that the drug does not influence it (Wei et al., 1980; Busija, 1983; Busija and Heistad, 1983; Toda et al., 1993).

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Although many reports have considered the roles of NO and prostacyclin in cerebral vasodilatory responses such as hypercapnia and blood pressure changes, it appeared worthwhile to compare the acetazolamide-induced vasodilation with the hypercapnic response in the presence of NOS and/or cyclooxygenase inhibitors in conscious animals in order to further elucidate the underlying vasorelaxant mechanisms. Antithrombotic as well as anti-inflammation medication (cyclooxygenase inhibitors) and nitrovasodilators are effective and important in the management of many patients with threatened stroke, myocardial ischaemia or hypertension. As large number of patients are treated with cyclooxygenase inhibitors or nitrovasodilators before and during the estimation of cerebrovascular reserve capacity, it may be speculated that these and other drugs might possibly interfere with the measurement of cerebrovascular reactivity.

This study therefore had two objectives: to analyze the roles of the cyclooxygenase and NO pathways in acetazolamide-induced vasodilation, and to compare the effects of cyclooxygenase inhibitor or acute and long-term nitrovasodilator treatments on hypercapnia and acetazolamide-induced vasoreactivity.

2. Materials and methods

2.1. Surgical procedures

Altogether, 40 New Zealand White rabbits weighing 2.5 to 3.0 kg were used for cerebrovascular reserve capacity measurements.

This work was approved by the Ethical Committee for the Protection of Animals in Research of Albert Szent-Györgyi Medical University, Szeged, Hungary. All experiments involved in this study were conducted in strict compliance with established professional and National Institutes of Health guidelines.

Rabbits were anaesthetized with a solution of intravenously administered diazepam (20 mg/kg) and ketamine (20 mg/kg). Hydrogen-sensitive electrodes consisting of glass-insulated (o.d. 0.5 mm) platinum wire (diameter 0.1 mm) with a bare tip length of 1 mm were placed stereotaxically into the parietal cortex (to the area of the right and left "gyrus suprasylvius anterior and media") through adequately placed bore holes in the skull, and fixed with dental cement under aseptic conditions. The stereotaxic placements were calculated from the atlas of Monnier and Gangloff (1961). Before the wound was closed, antibiotic (Carbenicillin as powder) was applied locally.

In each animal, the left or right central ear artery was cannulated for the monitoring of blood pressure and the withdrawal of arterial blood samples. Mean arterial blood pressure was measured in conscious rabbits through a pressure transducer (Statham) to a Hellige electromanometer. At the same time, electrocardiograms were continuously recorded by means of a radiotelemetry system. Arterial Pa_{CO_2}, Pa_{O_2}, pH and saturation were measured at various times with a blood gas analyzer (model OP-216, Radelkis, Hungary). The ear marginal vein was also cannulated and used for the administration of drugs.

2.2. Corticocerebral blood flow measurements

Rabbits were allowed 3 days to recover after surgery and corticocerebral blood flow was determined at six sites in the brain by means of the hydrogen clearance technique (Aukland et al., 1964; Pasztor et al., 1973). During measurements, the rabbits rested quietly in a comfortable wooden stock and 2–5% hydrogen gas was administered via a funnel. Clearance curves were registered and corticocerebral blood flow was evaluated with the aid of an 80 MHz IBM 486 SX computer. After determination of the basic level of corticocerebral blood flow, Pa_{CO_2} was adjusted to ~50–60 mm Hg by inhalation of a 5% carbon dioxide in air mixture. The level of hypercapnia was maintained until a stable increase in corticocerebral blood flow was observed (usually 3–5 min) and thereafter during the corticocerebral blood flow measurement. When a 20-min normocapnic period following hypercapnia had been achieved, 20 mg/kg acetazolamide was administered intravenously and the corticocerebral blood flow response was tested during the next 8–30 min (time of maximal effects) (Kuwabara et al., 1995). After total cessation of the effect of acetazolamide, 60 min were left as stabilization period, then L-NAME, indomethacin, nitroglycerin were given and the corticocerebral blood flow responses to hypercapnia and to acetazolamide in the presence of drugs were determined. The flow changes to chronic nitroglycerin and isosorbide-5-nitrate administration and the hypercapnic and acetazolamide-stimulated corticocerebral blood flow responses after the treatments were also examined.

2.3. Experimental groups

Rabbits were divided into five groups.

Acetazolamide responses in all of groups were determined using a distilled water solution of substance which was injected in a volume of 1 ml.

2.3.1. Group I (n = 6)

Basal, hypercapnic and acetazolamide-induced corticocerebral blood flow were measured before and after L-NAME administration (40 mg/kg i.v. in 1 ml of aqueous solution). Stabilization of flow occurred 45–60 min following L-NAME injection.

2.3.2. Group II (n = 16)

Corticocerebral blood flow measurements were carried out in normocapnic, hypercapnic and acetazolamide treated

Table 1
Effect of hypercapnia and drug treatments on mean arterial blood pressure (MABP) and heart rate (HR)

Groups	n	MABP (mm Hg)		HR (beats/min)	
		Baseline	Treated	Baseline	Treated
Hypercapnia	6	103±1	107±3	226±5	234±6
Acetazolamide	6	100±2	96±1[a]	230±4	231±8
L-NAME	6	90±2	100±2[a]	220±6	235±5
Indomethacin	6	104±2	103±1	232±5	228±4
Nitroglycerin	6	104±1	93±3[a]	222±4	242±7[a]
Nitroderm	6	103±2	92±2[a]	229±3	253±5[a]
Isosorbide-5-nitrate	6	101±3	89±3[a]	234±5	264±6[a]

Values are means±SEM, n = number of animals.
[a] $P < 0.05$ vs. baseline value for the untreated group.

animals and the measurements were repeated in the presence of water-soluble indomethacin (1, 3, 10 mg/kg).

After administration of indomethacin, constant corticocerebral blood flow responses were registered during 20–30 min, then the different interventions were carried out. Indomethacin was dissolved in distilled water and intravenously administered in a volume of 2 ml in 5 min.

2.3.3. Group III (n = 6)

After registration of the basal, hypercapnia and acetazolamide-induced corticocerebral blood flow responses, nitroglycerin solution in a concentration of 0.025 mg/kg was applied by slow intravenous injection (3–5 min) and the measurements were repeated.

2.3.4. Group IV (n = 6)

We measured the effects of chronically administered nitroglycerin as Nitroderm patches on the normal corticocerebral blood flow and on the hypercapnia and acetazolamide-elicited corticocerebral blood flow responses. Rabbits received a single transdermal nitroglycerin patch, which delivered 10 mg nitroglycerin/24 h, applied to the inner surface of the rabbit's ear and replaced every 24 h, for a period of 3 days, as described by Münzel et al. (1995) and Laursen et al. (1996). On day 4, a new nitroglycerin patch was placed on the ear for the duration of the experiment.

2.3.5. Group V (n = 6)

The effects of chronically administered isosorbide-5-nitrate on the normal and on the hypercapnia and acetazolamide-induced corticocerebral blood flow responses were determined. Animals received a 60 mg capsule of sustained-release isosorbide-5-nitrate once daily for 7 days and the measurements were performed on day 8.

The effects of drugs were investigated in the same animal separately, i.e. each animal was treated with only one dose of drug per day. Degree of hypercapnia and dose of acetazolamide used in the study correspond to the human practice (Sullivan et al., 1987; Oku et al., 1994; Dahl et al., 1995; White et al., 1998). The concentrations of nitroglycerin applied in the study correspond to the human dose (Joshi et al., 1997). To determine the effective dose of indomethacin in rabbits, we investigated a different dose of drugs beginning with the human dose (Wennmalm et al., 1983; Jensen et al., 1993). According to our study, we had to use higher doses than in human to get significant effects on the hypercapnic and acetazolamide-induced corticocerebral blood flow response.

The experiments were supplemented in all cases with the measurements of mean arterial blood pressure, heart rate and blood gas parameters. In the case of the indomethacin, these types of measurements were carried out only with the highest dose, which had significant effects on the hypercapnic and acetazolamide-induced corticocerebral blood flow response.

2.4. Drugs

Acetazolamide (Diamox, Lederle, Germany), L-NAME hydrochloride (Sigma, St Louis, MO), indomethacin (Indosol, Hungary), nitroglycerin (Nitrolingual, G. Pohl-Boskamp, Hohenlockstedt, Germany), Nitroderm TTS 10 (Ciba-Geigy), isosorbide-5-nitrate (Olicard 60 Retard, Solvay Pharma, Hannover, Germany).

2.5. Statistical analysis

The results were expressed as mean ± SEM. Data were collected in pairs from the same measuring sites (electrodes) before and after the experimental intervention or drug administration. The statistical significance of the observed differences was calculated by Student's paired t-test or repeated measures Analysis of Variance (ANOVA). Multiple comparison of different groups and interventions were carried out by means of one-way

Table 2
Arterial blood parameters in conscious rabbits

Groups	n	pH	pO_2 (mm Hg)	pCO_2 (mm Hg)	O_2 saturation
Baseline	6	7.47±0.04	95±2	34±0.6	98±0.4
Hypercapnia	6	7.30±0.03[a]	87±2	52±2[a]	94±2
Acetazolamide	6	7.45±0.01	98±1	36±0.8	98±0.2
L-NAME	6	7.42±0.02	96±2	31±2	98±1
Indomethacin	6	7.44±0.01	92±1	30±2	97±1
Nitroglycerin	6	7.36±0.03	93±2	37±1	98±0.2
Nitroderm	6	7.38±0.02	92±3	38±2	95±1
Isosorbide-5-nitrate	6	7.46±0.02	94±2	35±1	97±0.4

Data are the means±SEM, n = number of animals.
[a] Values significantly differ from untreated baseline values at level: $P < 0.05$.

Table 3
Effect of indomethacin on hypercapnia and acetazolamide (AZ)-induced corticocerebral blood flow response

Groups	*n*	Baseline values	Hypercapnia	Percentage of hypercapnic inhibition	AZ	Percentage of AZ-induced inhibition
Control	5	58 ± 4	85 ± 8[a]		98 ± 6[a]	
1 mg/kg indomethacin	5		75 ± 6[a]	−17	94 ± 8[a]	−7
Control	5	60 ± 4	96 ± 4[a]		107 ± 8[a]	
3 mg/kg indomethacin	5		80 ± 3[a]	−27	96 ± 6[a]	−18
Control	6	56 ± 7	84 ± 7[a]		102 ± 8[a]	
10 mg/kg indomethacin	6		60 ± 8[b]	−43	86 ± 8[ac]	−29

Corticocerebral blood flow is expressed as ml/min/100 g tissue.
Values are means ± SEM, *n* = number of animals.
[a] $P < 0.05$ vs. respective baseline.
[b] $P < 0.05$ control.
[c] $P < 0.05$ hypercapnic values.

ANOVA. The *P* values less than 0.05 when obtained with statistical tests were regarded as significant.

3. Results

As illustrated in Table 1, L-NAME significantly increased mean arterial blood pressure as compared with the baseline values. Significant ($P < 0.05$) decreases in mean arterial blood pressure were observed in the groups treated with acetazolamide, acute or chronic nitroglycerin and isosorbide-5-nitrate. Acute and transdermal nitroglycerin and sustained-release isosorbide-5-nitrate caused significant increases in heart rate as compared with the baseline values of the untreated groups.

No significant changes in arterial blood gas parameters were noted during the experiments, except for moderate hypercapnia (Table 2). During hypercapnia, Pa_{CO_2} increased to ~ 50–60 mm Hg, with an associated significant decrease in pH as compared with the baseline normocapnic values.

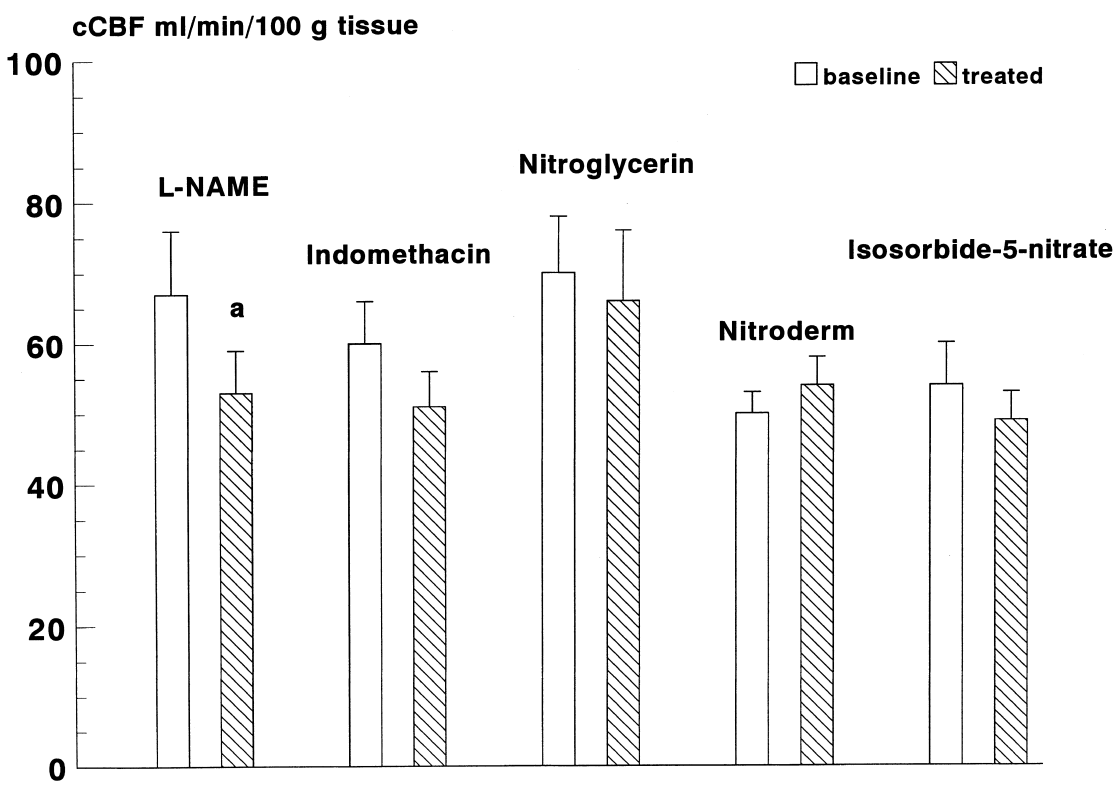

Fig. 1. Effect of different drug treatments on the basal corticocerebral blood flow (cCBF). Each bar represents the mean ± SEM of 30 measurements in six animals. [a] $P < 0.05$ compared to the untreated baseline value.

Moderate hypercapnia and acetazolamide administration caused significant increases in corticocerebral blood flow ($P < 0.05$) (Table 3; Fig. 2). Comparison of the two vasodilatory responses revealed that the acetazolamide response was higher than the hypercapnic response ($55 \pm 5\%$ vs. $101 \pm 12\%$ in hypercapnic response, $P < 0.05$, $n = 40$ (number of animals)).

Administration of L-NAME resulted in a slight but significant decrease in corticocerebral blood flow (from 67 ± 9 to 53 ± 6 ml/min/100 g tissue, $P < 0.05$; Fig. 1). There were no significant differences in corticocerebral blood flow in any other when the drug-treated values were compared with the baseline values (Fig. 1).

Injection of 40 mg/kg L-NAME considerably attenuated the hypercapnic response. In the presence of L-NAME, the corticocerebral blood flow responses to hypercapnia were identical with the normocapnic baseline corticocerebral blood flow values (52 ± 6 and 52 ± 7 ml/min/100 g tissue). The corticocerebral blood flow increasing effect induced by acetazolamide treatment was preserved, but somewhat lower after L-NAME administration (95 ± 12 and 82 ± 9 ml/min/100 g tissue; Fig. 2A).

Indomethacin (1, 3 and 10 mg/kg intravenously) dose-dependently inhibited the hypercapnia and acetazolamide-induced corticocerebral blood flow response (Table 3). Indomethacin, 10 mg/kg, produced significant inhibitory effect on the hypercapnic corticocerebral blood flow values compared to the untreated hypercapnic control values approaching the baseline values. There was a less pronounced and non-significant decrease in the corticocerebral blood flow response to acetazolamide administration when the values with and without indomethacin treatment were compared. Values of hypercapnia and acetazolamide-elicited corticocerebral blood flow response also significantly differed from each other at the dose of 10 mg/kg indomethacin.

Both acutely and chronically administered nitroglycerin were very effective in inhibiting the increase of corticocerebral blood flow due to hypercapnia, but did not significantly affect the vasodilation produced by acetazolamide administration (Fig. 2B,C). The normocapnic baseline values (61 ± 7 and 61 ± 10 ml/min/100 g tissue) and the hypercapnic values in the nitroglycerin treated groups (64 ± 6 and 68 ± 7 ml/min/100 g tissue) did not differ

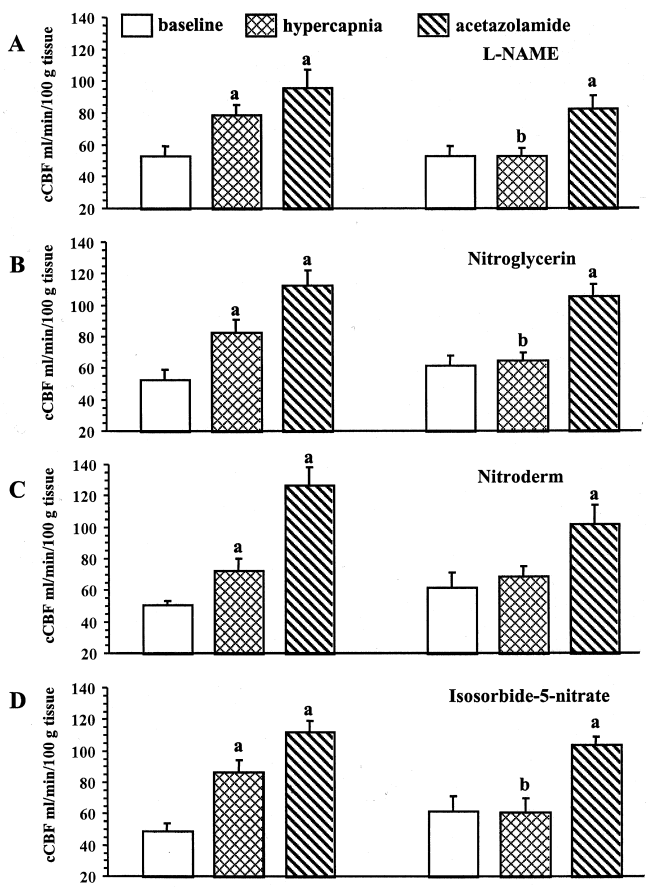

Fig. 2. Effect of L-NAME, acute nitroglycerin, Nitroderm patches (long-term nitroglycerin) as well as long-term isosorbide-5-nitrate treatments on hypercapnia and acetazolamide-induced corticocerebral blood flow (cCBF) responses. Values are the mean ± SEM of 30 measurements in six animals. Statistical significances were calculated in comparison with baseline ($^{a}P < 0.05$) and untreated hypercapnic ($^{b}P < 0.05$) values.

statistically. The effect of acutely administered nitroglycerin on cerebrovascular reserve capacity was short-termed. The maximal changes occurred within 20 min, then gradually returned to the level of the untreated control values and reached it at 30 min.

The long-term administration of isosorbide-5-nitrate similarly to nitroglycerin attenuated the corticocerebral blood flow increase (from 86 ± 8 to 60 ± 10 ml/min/100 g tissue, $P < 0.05$, Fig. 2D) induced by moderate hypercapnia. The values were not significantly different between animals with normocapnia and with hypercapnia after isosorbide-5-nitrate treatment (61 ± 10 and 60 ± 10 ml/min/100 g tissue; Fig. 2D). Treatment with isosorbide-5-nitrate did not significantly affect the acetazolamide-induced corticocerebral blood flow increase (111 ± 8 and 103 ± 6 ml/min/100 g tissue).

4. Discussion

Measurement of cerebrovascular reserve capacity by inhalation of carbon dioxide or acetazolamide administration is used to determine the need for surgical intervention in patients with occlusive cerebrovascular disease. Theoretically, a vasodilator causing maximum vasodilation, and hence expressing maximum cerebrovascular reserve capacity would be more suitable. In our study, moderate hypercapnia (~ 50–60 mm Hg) and acetazolamide treatment (20 mg/kg i.v.) caused significant increases in corticocerebral blood flow. Comparison of the vasodilatory responses of the two methods demonstrated that the corticocerebral blood flow increase following acetazolamide administration was higher than that during moderate hypercapnia (101% vs. 55%; Table 3; Fig. 2). Consistent with our results is the finding of Gambhir et al. (1997), i.e. acetazolamide seems to be more potent and thus more helpful in establishing effective therapy. This is surprising since both methods, i.e. a hypercapnic and an acetazolamide stimulus, are equally used for cerebrovascular reserve capacity measurement.

We have demonstrated that the inhibition of NOS activity by L-NAME administration or by long-term treatment with nitroglycerin or isosorbide-5-nitrate almost completely inhibited the hypercapnic corticocerebral blood flow increase (> 90%), but did not appreciably influence the acetazolamide-induced response in conscious rabbits.

Indomethacin treatment considerably reduced the corticocerebral blood flow response elicited by hypercapnia, and had only a moderate effect on the corticocerebral blood flow response induced by acetazolamide administration. The results of the present study indicate that the underlying vasorelaxant mechanisms of hypercapnic and acetazolamide-elicited response are distinct in conscious rabbits.

Our results confirm those of Kiss et al.'s (1999), who have found that acetazolamide-induced cerebral and ocular vasodilation in humans is independent of NO. The mechanisms of hypercapnia-induced cerebrovasodilation are complex and involve interactions of different mediators and modulators (Iadecola et al., 1993a,b, 1994a; Iadecola and Zhang, 1994; Murphy et al., 1993), which may differ in several species. Iadecola et al. (1994b) demonstrated that the NO donor 3-morpholinosydnonimine or the cGMP analogue 8-bromo-cGMP reversed the attenuation of the hypercapnic cerebrovasodilation elicited by NOS inhibitors, indicating a mechanism related to a decrease in basal levels of NO rather than to inhibition of hypercapnia-induced NOS activation.

Summarizing the conflicting results, it seems unlikely that hypercapnia-induced activation of NOS is the major mechanism of hypercapnic smooth muscle relaxation and the endothelium is the sole source of basal NO production.

It has also been suggested that the extracellular acidosis associated with hypercapnia might activate NOS and increase NO production (Wang et al., 1992; Niwa et al., 1993; Pelligrino et al., 1993).

It remains to be established, however, whether elimination of all potential sources of NO attenuates the cerebral blood flow response to carbon dioxide.

Acetazolamide, a selective inhibitor of carbonic anhydrase, crosses the blood–brain barrier (Rosenthal et al., 1976) and inhibits carbonic anhydrase in the glial cells and the capillary endothelium. In a dose sufficient to block brain carbonic anhydrase, acetazolamide acidifies cerebral extracellular fluids by elevating the brain extracellular fluid Pa_{CO_2} (Ehrenreich et al., 1961; Bickler et al., 1988). In contrast with the NO and cyclooxygenase-dependent mechanism of hypercapnic vasodilation, the working mechanism of acetazolamide, i.e. the link between extracellular acidification and vasodilation, is still unclear. It has been suggested that part of the increase in cerebral blood flow must come from a direct effect of acetazolamide on the smooth muscle in the cerebral arteries (Hauge et al., 1983). Differences between the mechanism of the two stimuli remain to be established.

4.1. Cerebrovascular reserve capacity measurement after L-NAME treatment

NO produced by the action of NOS activates guanylate cyclase in the vascular smooth muscle, increasing the intracellular concentration of cGMP and causing vasorelaxation (Faraci and Brian, 1994). NOS inhibitors attenuate the cerebral blood flow response to hypercapnia (Iadecola and Zhang, 1994; Wang et al., 1995), but only if $Pa_{CO_2} <$ 100 mm Hg. The wide range in the reduction of cerebral vasodilation reported by other authors may reflect differences in NOS inhibitors, in the doses of inhibitors, in the timing of doses, in the degrees of hypercapnia and anaesthesia, and in species (Thomson et al., 1992; Faraci and Brian, 1994; Iadecola and Zhang, 1994; Sandor et al., 1994; McPherson et al., 1995; Wang et al., 1995). Wang et al. (1992) investigated the effect of N^G-nitro-L-arginine at

three dose-levels on cerebral blood flow response in hypercapnia and reported that this inhibitor of NOS dose-dependently attenuated the carbon dioxide reactivity and did not change the cerebral blood flow response to acetazolamide.

In previous studies, different doses of L-NAME were applied depending on the species used. Iadecola and Zhang (1994) ruled out that 40 mg/kg of L-NAME significantly attenuated the hypercapnic corticocerebral blood flow response (~50%), reaching a maximum at 45 min. We found that inhibition of NOS activity by L-NAME administration in the same dose almost completely inhibited the hypercapnic corticocerebral blood flow (>90%), but did not appreciably influence the acetazolamide-induced response in conscious rabbits (Group I; Fig. 2). The present findings are in agreement with previous studies reporting that the intraarterial administration of L-NAME markedly attenuated the corticocerebral blood flow response to hypercapnia in primates. Additionally, the cerebral cortex was the only region where hypercapnic vasodilation was completely blocked after L-NAME administration (McPherson et al., 1995; Thompson et al., 1992). These findings were also supported by other authors, who measured a greater sensitivity of the cerebral cortex as regards the effect of NOS inhibition on the corticocerebral blood flow response to hypercapnia (Sandor et al., 1994).

4.2. Cerebrovascular reserve capacity measurement after indomethacin treatment

Our results demonstrate that indomethacin treatment considerably reduced the corticocerebral blood flow response elicited by moderate hypercapnia, but did not significantly affect the acetazolamide-induced corticocerebral blood flow response. There is evidence that prostacyclin is important in the regulation of the resting cerebral blood flow and the vasodilatory response to hypercapnia. The inhibition of prostaglandin synthesis by the cyclooxygenase inhibitor indomethacin reduced the basal cerebral blood flow (Marcus et al., 1994), both in a variety of animal models and in the few studies performed in humans. In addition, in some animal models indomethacin reduces the cerebral vasodilatory response to hypercapnia (Dahlgren et al., 1981; Wang et al., 1993), although studies in humans have furnished conflicting results (Busija and Heistad, 1983; Wennmalm et al., 1983; Jensen et al., 1993). It has been demonstrated that considerable species differences exist with respect to the predominant prostaglandins produced by the cerebral tissues. Furthermore, the prostaglandins exert different vascular effects in various species. Differences in experimental procedures may also be responsible for the divergent findings.

4.3. Cerebrovascular reserve capacity measurement after acute and long-term nitroglycerin and isosorbide-5-nitrate treatment

Despite the widespread use of nitroglycerin, little is known about its cerebral haemodynamic effects. Our results show that both acute treatment with nitroglycerin and long-term treatment with nitroglycerin or isosorbide-5-nitrate virtually completely inhibited the hypercapnic corticocerebral blood flow increase (>90%), but did not markedly influence the acetazolamide-induced response.

NO released exogenously from clinically administered NO donors (nitroglycerin, sodium nitroprusside, 3-morpholinosydnonimine, isosorbide mono- and dinitrates) activates cyclooxygenase, leading to the release of prostacyclin-causing vasorelaxation (Salvemini et al., 1993). Although nitroglycerin releases NO only upon metabolic bioconversion, the mechanisms by which NO is formed from the NO donors vary (Feelisch and Kelm, 1991; Salvemini et al., 1996).

Evidence has accumulated to support an endothelium-dependent mechanism of nitroglycerin-induced vasodilation in addition to its action as NO donor (Malta, 1989a,b; Abou-Mohamed et al., 1995). It was shown using in vitro vascular models that effects of nitroglycerin involve activation of the endothelial NOS pathway acting as NOS agonist and partially dependent on L-arginine (Abou-Mohamed et al., 2000). Nitroglycerin had a biphasic effect on uptake of L-arginine, namely acute exposure to nitroglycerin stimulated while longer treatment suppressed the uptake.

Our suggestion is that the attenuation of hypercapnia induced corticocerebral blood flow increase following acute nitroglycerin administration developed on a haemodynamic basis. Nitrovasodilator treatment in our experiments slightly, but not significantly, increased the basal corticocerebral blood flow (by 20–30%). This is supported in part by the results of Joshi et al. (1997), who reported that an infusion of nitroglycerin, presumed to work through an NO-mediated pathway, did not increase corticocerebral blood flow. Oliver and Dormehl (1999) observed a slightly insignificant cerebral blood flow increasing effect of nitroglycerin in a non-human primate model and Niehaus et al. (1998) registered dilation of basal cerebral arteries without affecting cerebral blood flow measured by transcranial Doppler sonography in humans. We suppose that attenuation of the carbon dioxide response by acutely given nitroglycerin may be attributed to the vasodilatory effect of nitroglycerin as dilated cerebral blood vessels are not able to have additional dilation in hypercapnia.

At low concentrations of nitroglycerin, the relaxant effect may also be due to a cGMP-independent mechanism, such as a direct action on ion channels. This is supported by Bolotina et al. (1994), who demonstrated that NO itself may have a direct effect on calcium-dependent potassium channels in the rabbit thoracic aorta. The dual formation of NO and prostacyclin, and their subsequent interaction at the levels of the vasculature and circulating cells, such as the platelets, may represent a powerful mechanism followed by therapeutically administered nitrovasodilators to restore an abnormal vascular tone in pathological states associated with an altered endothelium.

Münzel et al. (1995) have found that chronically treated rabbits (nitroglycerin patches for 3 days like in our experiments) demonstrate greater degrees of tolerance to nitroglycerin when the endothelium is present than if it is removed. The development of nitrate tolerance is associated with significant impairments of the vascular effects of the endothelium-dependent NO agonist (acetylcholine) and antagonist (L-NAME) treatment. Thus, prolonged administration of an exogenous nitrovasodilator and the development of an in vivo nitrate tolerance significantly interfere with the endogenous nitrovasodilator pathway for vascular relaxation (Laursen et al., 1996).

On the basis of previous results and our findings, we suppose that if nitroglycerin-induced tolerance is due to a reduction of NOS, then responses of vessels to hypercapnia, which have NO-dependent mechanism, should be reduced.

Our results that indomethacin and nitrovasodilator (nitroglycerin, isosorbide-5-nitrate) treatments almost completely inhibited the hypercapnic corticocerebral response ($>90\%$), but did not markedly influence the acetazolamide-induced response support our idea that treatments with these drugs can influence the cerebrovascular reserve capacity measurements, especially in tests involving hypercapnic stimulation. It is speculated that the vasomotor reserve testing in patients undergoing antithrombotic (cyclooxygenase inhibitors) and nitrovasodilator medications (antihypertensive therapy or treatment of heart failure) could lead to an underestimation of cerebrovascular reserve capacity, i.e. a normal vasoreactivity could be measured as decreased or even exhausted especially after long-term treatments. These findings also suggest that such agents can worsen the compensatory state of cerebral autoregulation, and patients taking these drugs may be prone to TIA (transient ischemic attack) or stroke on a haemodynamic basis (a sudden blood pressure decrease). Since acetazolamide-induced vasodilation is less sensitive to nitrovasodilators than hypercapnic stimulation, acetazolamide-elicited vasodilation seems preferable for cerebrovascular reserve capacity measurements in patients with intra- and extracranial vascular occlusive disease. Further studies are required to clarify the mechanisms underlying the differences between the two vasodilations.

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