Cerebral blood flow and cerebrovascular reactivity after inhibition of nitric oxide synthesis in conscious goats

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1 The role of nitric oxide in the cerebral circulation under basal conditions and after vasodilator stimulation was studied in instrumented, conscious goats, by examining the action of inhibiting endogenous nitric oxide production with N^G-nitro-L-arginine methyl ester (L-NAME).

2 In 6 unanaesthetized goats, blood flow to one brain hemisphere (electromagnetically measured), systemic arterial blood pressure and heart rate were continuously recorded. L-NAME (35 mg kg⁻¹ by i.v. bolus) decreased resting cerebral blood flow by 43 ± 3%, increased mean arterial pressure by 21 ± 2%, and decreased heart rate by 41 ± 2%; cerebrovascular resistance increased by 114 ± 13% (*P* < 0.01); the immediate addition of i.v. infusion of L-NAME (0.15–0.20 mg kg⁻¹ during 60–80 min) did not significantly modify these effects. Cerebral blood flow recovered at 72 h, arterial pressure and cerebrovascular resistance at 48 h, and heart rate at 6 days after L-NAME treatment.

3 A second treatment with L-NAME scheduled as above reproduced the immediate haemodynamic effects of the first treatment, which (except bradycardia) reversed with L-arginine (200–300 mg kg⁻¹ by i.v. bolus).

4 Acetylcholine (0.01–0.3 µg), sodium nitroprusside (3–100 µg) and diazoxide (0.3–9 mg), injected into the cerebral circulation of 5 conscious goats, produced dose-dependent increases in cerebral blood flow, and decreases in cerebrovascular resistance; sodium nitroprusside (30 and 100 µg) also caused hypotension and tachycardia.

5 The reduction in cerebrovascular resistance from resting levels (in absolute values) to lower doses, but not to the highest dose, of acetylcholine was diminished, to sodium nitroprusside was increased, and to diazoxide was unaffected after L-NAME, compared to control conditions. The effects on cerebrovascular resistance to acetylcholine normalized within 24 h and to sodium nitroprusside within 48 h after L-NAME treatment.

6 This study provides information about the evolution of the changes in cerebral blood flow and cerebrovascular reactivity after inhibition of endogenous nitric oxide in conscious animals. The results suggest: (a) endogenous nitric oxide is involved in regulation of the cerebral circulation by producing a resting vasodilator tone, (b) the cerebral vasodilatation to acetylcholine is mediated, at least in part, by nitric oxide release, and (c) inhibition of nitric oxide production induces supersensitivity of cerebral vasculature to nitrovasodilators.

Keywords: Endothelium; N^G-nitro-L-arginine methyl ester (L-NAME); cerebral blood vessels; vasodilator tone; acetylcholine; nitrovasodilators; supersensitivity

Introduction

Nitric oxide, or a closely related compound, seems to be at least one type of endothelium-derived relaxing factor that is synthesized from L-arginine and relaxes vascular smooth musculature via the stimulation of guanylate cyclase (Ignarro, 1990; Moncada *et al.*, 1991a). The synthesis of endothelial nitric oxide can be inhibited by several L-arginine analogues and this inhibition induces vascular contraction *in vitro* and *in vivo* (Moncada & Higgs, 1990), and increases resistance in several vascular beds (Gardiner *et al.*, 1990; 1991b). Thus, the basal release of nitric oxide (Martin *et al.*, 1986; Rees *et al.*, 1989a) appears to be responsible for maintaining a vasodilator tone in the cardiovascular system (Rees *et al.*, 1989b; Gardiner *et al.*, 1990).

In the cerebral circulation, experimental observations suggest that nitric oxide mediates the dilatation of cerebral blood vessels to acetylcholine (Fujiwara *et al.*, 1986; Faraci, 1990; Fischer-Nakielski *et al.*, 1990; Rosenblum *et al.*, 1990) and could play a role in maintaining cerebrovascular tone *in vivo* (Faraci, 1990; Fischer-Nakielski *et al.*, 1990; Rosenblum *et al.*, 1990). Studies measuring cerebral blood flow have been performed in anaesthetized animals and have produced controversial results as inhibition of nitric oxide synthesis

decreases (Kováč *et al.*, 1992; Kozniowska *et al.*, 1992; Pellegrino *et al.*, 1992) or does not change (Faraci & Heistad, 1992; Iadecola, 1992) resting cerebral blood flow. Thus, more studies are necessary to clarify the role of nitric oxide in the regulation of basal cerebral blood flow, as well as in cerebrovascular reactivity. Since anaesthetics can modify cerebrovascular response, the use of conscious animals could contribute to the elucidation of this issue.

The present study was carried out to analyse the role of endogenous nitric oxide in basal cerebral blood flow and in dilatation of the cerebral circulation. The experiments were performed by using an experimental model in goats that permits the blood supply to one brain hemisphere to be continuously measured on a beat-to-beat basis in the unanaesthetized animal, a situation that is near to normal conditions (Reimann *et al.*, 1972; Gómez *et al.*, 1977; García *et al.*, 1991). Inhibition of endogenous nitric oxide production was induced by i.v. administration of N^G-nitro-L-arginine methyl ester (Moore *et al.*, 1990; Rees *et al.*, 1990) and acetylcholine, sodium nitroprusside and diazoxide were used as vasodilators. This study allowed us to evaluate throughout several days the time course of the effects of inhibition of nitric oxide production on basal cerebral blood flow, systemic arterial blood pressure and heart rate, as well as on cerebral vasodilatation.

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Methods

The present experiments were performed in six female goats (32 to 49 kg weight). In this species, each internal maxillary artery, a branch of the external carotid artery, provides the total blood flow to each cerebral hemisphere via the rete mirabile; the vertebral arteries do not contribute to brain blood flow, and the extracranial internal carotid artery is absent (Daniel *et al.*, 1953; Reimann *et al.*, 1972). The circle of Willis in the goat is similar to that in man except that the blood flows in a caudal direction in the basilar artery (Daniel *et al.*, 1953; Reimann *et al.*, 1972). Analysis of the distribution of radioactively labelled microspheres in the cerebral circulation of the goat after the surgical procedure described by Reimann *et al.* (1972) indicates that nearly all of the blood carried by the internal maxillary artery passes directly to cerebral tissue (Miletich *et al.*, 1975). Extracerebral blood flow is minimal, <5% of total flow.

The operative procedure has been described elsewhere (Reimann *et al.*, 1972). Briefly, the extracerebral vessels from one of the internal maxillary arteries were ligated and thrombosed with 1 000 units of thrombin (Thrombostat, Parke Davis, Morris Plains, NJ, U.S.A.) dissolved in 1 ml of 0.9% NaCl solution. This manoeuvre produces an almost immediate obliteration of the ethmoidal, ophthalmic, and buccinator arteries and thus eliminates blood flow to the eye and other facial structures. This is confirmed on recovery from surgery by the presence of ipsilateral blindness. However, obliteration of the extracerebral vessels from the internal maxillary artery does not cut off vascular supply to half of the face. The areas supplied by the ethmoidal, buccinator, dental, and temporal arteries are nourished by anastomotic channels that are normally in a state of dynamic balance but in which the direction of blood flow can be quickly changed, depending on the pressure differential from one side of the union to the other (Daniel *et al.*, 1953; Reimann *et al.*, 1972). There is no necrosis, and the functions related to these areas such as eating, drinking, and rumen are intact. Obliteration and thrombosis of the ophthalmic artery permanently cuts off vascular supply to the ipsilateral eye. This procedure becomes necessary for the successful isolation of the cerebral circulation (Reimann *et al.*, 1972; Miletich *et al.*, 1975). The ipsilateral blindness that ensues does not seem to alter normal behaviour and the physical condition of the animals.

An electromagnetic flow transducer (Biotronex, Silver Spring, MD, U.S.A.) was placed on the internal maxillary artery to measure blood flow to the ipsilateral cerebral hemisphere. A polyethylene catheter (PE-90) inserted in the temporal artery permitted the injection of drugs directly into the internal maxillary artery in the conscious goat; the same catheter was used to measure arterial blood pressure with a Statham P 23 ID transducer. A snare-type occluder was placed on the external carotid, close to the temporal artery, to obtain zero-flow base lines. The external connecting leads from the flow transducer and occluder, and the temporal artery catheter were led out subcutaneously and secured to the horn of the goat.

Heart rate was measured from the arterial pressure pulse with a ratemeter. Flow measurements were made with a Biotronex electromagnetic flowmeter (model BL-610). Cerebral blood flow systemic arterial blood pressure, and heart rate were recorded on a Dynograph Recorder (model R611, Sensor Medics, Bilthoven, The Netherlands).

The experiments on the conscious goats were started 2–3 days after the operative procedure, at which time the goats had fully recovered and were in good condition. The various measurements were made with the goat unrestrained in a large cage, except for a Lucite stock fitting loosely around the neck that limited forward and backward motion. Once placed in the cage the animal stood quietly during the experiments and showed no signs of disturbance. However, the experiments were stopped whenever the animals showed signs of excitation or uneasiness as also evidenced by altera-

tions in the recordings of blood pressure and heart rate. In this event, the goat was brought back to the animal quarters, and a period of ≥ 24 h was allowed before attempting a new experiment. The administration of small amounts of drugs into the internal maxillary artery can be carried out with reproducible results on different days in the conscious state without causing any discomfort to the animal (Lluch *et al.*, 1975; García *et al.*, 1991).

In this work the following experiments were performed: (1) after resting control measurements were recorded, all the animals received an i.v. bolus of N^G -nitro-L-arginine methyl ester (L-NAME, 35 mg kg^{-1} during 15–18 min), and 5 min after the haemodynamic variables had reached a new steady state, animals received an i.v. infusion of L-NAME (0.15–0.20 mg kg^{-1} during 60–80 min); the animals received in total 47 mg kg^{-1} of this substance. The effects of L-NAME were continuously recorded during about 2 h after stopping the infusion, and periodically during the subsequent 6–8 days; (2) four of these animals received a second treatment with L-NAME 8–10 days after the first treatment in the same way and doses as indicated above; these four goats, immediately after stopping the infusion of L-NAME, were also treated with L-arginine by i.v. route (200–300 mg kg^{-1} during 15–20 min), the haemodynamic variables being recorded during about 2 h. L-NAME and L-arginine were dissolved in isotonic saline at 5 mg ml^{-1} and 40 mg ml^{-1} , respectively; and (3) the effects of acetylcholine (0.01, 0.03, 0.1 and 0.3 μg), sodium nitroprusside (3, 10, 30 and 100 μg) and diazoxide (0.3, 1, 3 and 9 mg) were recorded in 5 goats. These substances, dissolved in isotonic saline, were given in volumes of 0.3 ml through the catheter placed into the temporal artery, and their effects were evaluated under control conditions, during the infusion and periodically after administration of the first treatment of L-NAME.

Arterial blood PO_2 , PCO_2 and pH were measured before and after injection of L-NAME by standard electrometric methods (Radiometer, ABL 300, Copenhagen, Denmark). Cerebrovascular resistance was calculated as the mean systemic arterial blood pressure (mmHg) divided by blood flow to one brain hemisphere (ml min^{-1}). In previous experiments (unpublished observations) and in 2 animals of the present study, we found that the goat intracranial pressure (intracranial venous pressure) in the cisterna magna is 0–3 mmHg under control conditions. Also, as in the 2 animals of the present study intracranial pressure did not appreciably change during the experimental conditions, we considered cerebral venous pressure to be of minor relevance for cerebrovascular resistance calculations.

Drugs used were: N^G -nitro-L-arginine methyl ester (Sigma), acetylcholine chloride (Sigma), sodium nitroprusside (Nitroprussiat Fides, Barcelona, Spain), and diazoxide (Hyperstat, Schering Corporation, NJ, U.S.A.). Administration of isotonic saline alone intravenously or into the internal maxillary artery at the volumes employed in the present study had no systemic or cerebrovascular effects.

Statistics

All haemodynamic measurements before and after L-NAME treatment were compared using the same animal as its own control. An analysis of variance for repeated measures, followed by Dunnett's test was applied to the results with L-NAME, acetylcholine, sodium nitroprusside, diazoxide and L-arginine. Percentage changes in cerebral blood flow and cerebrovascular resistance after L-NAME or L-arginine, and decreases in cerebrovascular resistance from resting levels taken in absolute values for the effects of acetylcholine, sodium nitroprusside and diazoxide before and after L-NAME were used. $P < 0.05$ was considered statistically significant.

Figure 1 Actual recordings of the mean cerebral blood flow (CBF), systemic arterial blood pressure (AP) and heart rate (HR) obtained before and during administration of N^G -nitro-L-arginine methyl ester (L-NAME) in one conscious goat. Deflections of CBF and AP records correspond to occlusions of external carotid artery to obtain zero flow.

Results

Effects of L-NAME

With the first treatment, the i.v. bolus of L-NAME induced arterial hypertension, bradycardia and decreases in cerebral blood flow in all 6 goats. These effects began to be evident at 2–3 min after beginning the injection, the systemic and cerebral blood flow changes being practically simultaneous (Figure 1). The maximal effects of the i.v. bolus were reached at about 3–5 min after ending the injection, and cerebral blood flow decreased by $43 \pm 3\%$, mean systemic arterial blood pressure increased by $21 \pm 2\%$, heart rate decreased by $41 \pm 2\%$, and calculated cerebrovascular resistance increased by $114 \pm 13\%$ ($P < 0.01$). These effects were not significantly modified by adding the i.v. infusion of L-NAME (cerebral blood flow decreased by $48 \pm 4\%$, mean arterial pressure increased by $28 \pm 3\%$, heart rate decreased by $37 \pm 4\%$ and cerebrovascular resistance increased by $156 \pm 27\%$) and remained for at least 2 h after stopping the infusion. The haemodynamic variables returned to control values after treatment with L-NAME, arterial pressure was the first variable to do so at about 48 h, cerebral blood flow at about 72 h, and heart rate at about 6 days (Figure 2).

The second treatment with L-NAME (i.v. bolus and infusion) in 4 of the 6 goats produced immediate systemic and cerebral blood flow effects comparable to those induced by the first treatment. In these 4 animals, the administration of L-arginine following infusion of L-NAME reversed the effects of L-NAME on systemic arterial pressure and cerebral blood flow, but not on heart rate (Table 1). The systemic arterial pressure and cerebral blood flow normalized at 10–20 min after stopping the administration of L-arginine and they remained within the control values during at least 4–6 days. Bradycardia persisted for this period in spite of L-arginine treatment.

We also observed that the first or second treatment with L-NAME produced clinical impairment in all the animals, which were moderately obtunded and less responsive to laboratory stimuli during 6–8 days after drug administration, an effect that was not apparently affected by L-arginine. Administration of L-NAME or L-arginine did not modify significantly arterial blood gases and pH.

Reactivity of the cerebral circulation

Figure 3 summarizes the absolute values for cerebrovascular resistance obtained before and after administration of acetylcholine, sodium nitroprusside and diazoxide in the animals

Figure 2 Summary of changes in cerebral blood flow (CBF), cerebrovascular resistance (CVR), mean systemic arterial blood pressure (MAP) and heart rate (HR) induced by the first treatment (i.v. bolus and infusion) with N^G -nitro-L-arginine methyl ester (L-NAME) in 6 conscious goats. * $P < 0.01$, † $P < 0.05$ compared with the control (C).

Table 1 Haemodynamic values (means $\pm$ s.e.mean) obtained before (control), immediately after the second N^G-nitro-L-arginine methyl ester (L-NAME) treatment (i.v. bolus + i.v. infusion) and after i.v. administration of L-arginine following L-NAME in 4 unanaesthetized goats

	Control	L-NAME (47 mg kg ⁻¹)	L-Arginine (200–300 mg kg ⁻¹)
CBF (ml min ⁻¹)	66 $\pm$ 3.2	39 $\pm$ 3.6 *	69 $\pm$ 3.4
CVR (mmHg ml ⁻¹ min ⁻¹)	1.51 $\pm$ 0.12	3.21 $\pm$ 0.20*	1.61 $\pm$ 0.13
MAP (mmHg)	101 $\pm$ 5.1	126 $\pm$ 7.3 †	110 $\pm$ 3.6
HR (beats min ⁻¹)	65 $\pm$ 3.8	45 $\pm$ 2.7 *	51 $\pm$ 3.4†

CBF = cerebral blood flow; CVR = cerebrovascular resistance; MAP = mean systemic arterial blood pressure; HR = heart rate.

* $P < 0.01$ and † $P < 0.05$ compared to control.

Figure 3 Values (mean $\pm$ s.e.mean) for cerebrovascular resistance under resting conditions in control (C) and after effects of acetylcholine, sodium nitroprusside and diazoxide obtained before (●), during treatment with N^G-nitro-L-arginine methyl ester (L-NAME) (○), at 24 h (□), and at 48 h (Δ) after L-NAME treatment. * $P < 0.05$ and ** $P < 0.01$ for resting values before and after L-NAME. † $P < 0.05$ and †† $P < 0.01$ for decreases in resistance from resting values before and after L-NAME.

with and without L-NAME. Resting cerebrovascular resistance under control (without any treatment) was about 1.55 ± 0.11 mmHg ml⁻¹ min⁻¹, and after L-NAME treatment were 3.45 ± 0.35 (immediate, $P < 0.01$), 2.51 ± 0.30 (at 24 h, $P < 0.5$) and 2.15 ± 0.25 (at 48 h, $P > 0.05$) mmHg ml⁻¹ min⁻¹.

Acetylcholine (0.01–0.3 µg), injected into the internal maxillary artery, produced dose-dependent increases in cerebral blood flow and decreases in cerebrovascular resistance, without affecting systemic variables. However, the decreases in cerebrovascular resistance (in absolute values) by the three lower doses of acetylcholine were significantly lower during the i.v. infusion of L-NAME; the reduction in cerebrovascular resistance by the highest dose (0.3 µg) of acetylcholine was comparable before and after L-NAME. At 24 h after L-NAME, the effects of acetylcholine were not significantly different from the control situation.

Sodium nitroprusside (3–100 µg), injected into the internal maxillary artery, produced marked, dose-dependent increases in cerebral blood flow and decreases in cerebrovascular resistance; and the doses of 30 and 100 µg also caused a small hypotension and tachycardia. These effects of sodium nitroprusside, however, were significantly higher after than before L-NAME. The decreases in cerebrovascular resistance (in absolute values) induced by sodium nitroprusside were significantly higher during and at 24 h after L-NAME than under control conditions; at 48 h after L-NAME these decreases were comparable to those obtained in controls.

Diazoxide (0.3–9 mg), injected into the internal maxillary artery, caused dose-dependent increases in cerebral blood flow and decreases in cerebrovascular resistance, without affecting systemic arterial pressure or heart rate. The decreases in cerebrovascular resistance (in absolute values) produced by diazoxide were not significantly different before and after treatment with L-NAME.

Discussion

The present study shows that i.v. administration of L-NAME reduced considerably resting cerebral blood flow by increasing cerebrovascular resistance in unanaesthetized animals, and these effects were accompanied by systemic arterial hypertension and bradycardia. The effects of L-NAME on cerebral blood flow and systemic variables reversed spontaneously and were reproduced when a second treatment was administered to the animals after recovery from the first treatment. Also, the cerebrovascular effects and systemic hypertension, but not bradycardia, induced by L-NAME were reversed by L-arginine. As L-NAME has proved to be a potent inhibitor of nitric oxide production (Moore *et al.*, 1990; Rees *et al.*, 1990), and the observed effects of this

substance were reversed by L-arginine, precursor of endothelial nitric oxide synthesis (Ignarro, 1990; Moncada *et al.*, 1991a), our results suggest that the reduction in cerebral blood flow by L-NAME is due to decreases in basal nitric oxide production and, consequently, to inhibition of nitric oxide-mediated basal vasodilator tone in the cerebral vasculature under normal conditions. This is in line with observations by others in the cerebral blood flow of anaesthetized animals (Gardiner *et al.*, 1991b; Kovách *et al.*, 1992; Kozniowska *et al.*, 1992; Pellegrino *et al.*, 1992) and supports the idea that an important basal vasodilator tone mediated by nitric oxide is present in the cerebral circulation as in other vascular beds (Moncada *et al.*, 1991a). Systemic hypertension and bradycardia have been previously observed with nitric oxide inhibitors (Moncada & Higgs, 1990). Results found in anaesthetized animals suggest that bradycardia after administration of inhibitors of nitric oxide synthesis is mainly caused by an increased vagal efferent activity (Aisaka *et al.*, 1989; Rees *et al.*, 1989b; Widdop *et al.*, 1992), whereas data from conscious rats suggest that it is related to inhibition of sympathetic activity, rather than potentiation of parasympathetic nerve activity (Wang & Pang, 1991). Widdop *et al.* (1992) observed that in the presence of supramaximal doses of atropine and atenolol, a small bradycardia persisted following L-NAME administration in anaesthetized rats, and they suggest that this persistent bradycardia could be due to coronary vasoconstriction induced by L-NAME. Other studies in conscious animals show that L-NAME (Gardiner *et al.*, 1991a) or N^G-nitro-L-arginine (L-NOARG) (Du *et al.*, 1992) did not affect the cardiac baroreflex sensitivities to pressor or depressor stimuli. Interestingly, we found that bradycardia remained for relatively longer periods than the reduction in cerebral blood flow and hypertension after treatment with L-NAME, and that it did not reverse with L-arginine. Du *et al.* (1992) also observed in conscious rabbits that bradycardia persisted for a longer period than hypertension after L-NOARG treatment. Although we have no confident data for the observed clinical effects after L-NAME, we can speculate that this substance may induce a sedative effect by depressing the central nervous system, including the basal nervous activity on the heart. This depression might account for the prolonged bradycardia found in our experiments. Data from mice suggest that L-NAME produces a long-lasting antinociception most probably by a direct effect within the central nervous system (Moore *et al.*, 1991).

The reduction in resting cerebral blood flow by L-NAME remained during 48 h and these effects were of longer duration than those on systemic arterial blood pressure, thus suggesting that cerebral blood vessels are particularly sensitive to the blockade of nitric oxide production, and that this substance plays a major role in regulating cerebral blood flow.

Therefore, the present experimental model permitted us to evaluate the time course of the changes in cerebral blood flow after inhibition of endogenous nitric oxide formation in the animals without anaesthesia, in conditions near to the normal situation. However, this model has potential limitations. First, with respect to cerebrovascular resistance, we cannot determine the vascular segments (arteries, microvessels, veins) of the cerebral circulation where changes in cerebrovascular resistance take place, as the changes in cerebral blood flow induced by L-NAME reflect the effects on the cerebral circulation as a whole. Also, because we do not consider outflow (venous) pressure, an error probably exists in our calculations of cerebrovascular resistance. However, this error should be small since in two animals we did not find appreciable changes in intracranial pressure during the experiments. In addition, it is accepted that changes in cerebrovascular resistance occur mainly in the arterial side when vasoactive stimuli are applied and that changes in cerebral venous caliber affect cerebral blood volume rather than cerebral blood flow (Auer & MacKenzie, 1984). Second, in

the goat an arterial rete (carotid rete mirabile) is interposed between the internal maxillary artery and the circle of Willis. Thus, the changes in cerebral blood flow after L-NAME could reflect the overall effects of this substance on nitric oxide synthesis in retial and cerebral vessels. In our laboratory we have observed (unpublished observations) that the i.v. administration of L-NAME also reduces resting blood flow in the middle cerebral artery, thus suggesting that nitric oxide produces a vasodilator tone in cerebral vessels under basal conditions. Therefore, the reduction in cerebral blood flow observed in the present study after L-NAME could be due, at least in part, to the reduction of nitric oxide production in the cerebral vasculature. Retial vessels, however, could also contribute to these effects and this should be taken into account. The last point is related to the interpretation of the increase in cerebrovascular resistance after L-NAME. One could argue that the increase in cerebrovascular resistance represents an intrinsic vascular response to the simultaneous hypertension. However, if this were the case, cerebral blood flow probably would remain at the same level as before the raise in arterial pressure. On the other hand, if the intrinsic vascular response were disturbed, then one would expect an increase and not a decrease in cerebral blood flow. Therefore, although we cannot exclude the possibility that part of the increase in cerebrovascular resistance may be due to an intrinsic cerebrovascular response to hypertension after L-NAME, the decrease in cerebral blood flow found in the present study is not.

With regard to the site(s) of the cerebral circulation where synthesis of nitric oxide takes place, experimental data show the cerebral microvessels do not release (Kontos *et al.*, 1988) or release a minimal amount (Rosenblum, 1986) of nitric oxide, and that basal production of this substance seems to be dependent on cerebral vessels size, being greater in large than in small arteries (Faraci, 1991). Also, although the endothelium appears to be a main source of nitric oxide, other sources of this vasodilator such as cerebral arterial nerves (Bredt *et al.*, 1990) and cerebrovascular musculature (Katusic, 1991) seem also to exist. Thus, the reduction in cerebral blood flow by L-NAME in our study could be due to inhibition of nitric oxide production in the different sources of formation, the importance of which we cannot determine. The reduction in brain metabolism during L-NAME treatment might also be involved in the observed changes in cerebral blood flow.

In evaluating the effects of the vasodilators acetylcholine, sodium nitroprusside and diazoxide on cerebral circulation before and after L-NAME administration, the fact that basal haemodynamic conditions before and after L-NAME are different, should be taken into account as this might influence the subsequent responses to vasodilators. In Figure 3 we present the values for cerebrovascular resistance obtained before and after the vasodilators used, and the cerebrovascular responses to these vasodilators can be evaluated as follows: (1) by expressing the changes in cerebrovascular resistance as a percentage of the immediately preceding basal resistance; this approach could be questionable when changes in vascular resistance are analysed, as it could lead to underestimation of the effects of the vasodilators during L-NAME administration, when vascular resistance is higher than under control conditions; (2) by expressing the changes as the level of resistance reached after each dose of the vasodilators, and (3) by expressing the response as the resistance changes in absolute values induced by vasodilators from the preceding basal resistance level before and after L-NAME. When the second approach (levels of resistance reached) is considered, the comparison before and after L-NAME could present a problem as cerebrovascular resistance is considerably higher after L-NAME. In this case, it may be reasonable to expect that the cerebrovascular resistance reached after vasodilators frequently will remain more elevated after than before L-NAME, and it would not mean necessarily that vasodilator responses were lower after L-

NAME. The third approach considered could be the least dependent on the preceding basal resistance level of the three approaches. In addition, it seems that increases in arterial pressure would not, necessarily, enhance the response to vasodilator agents (Gardiner *et al.*, 1991b). Thus, evaluation of reductions in cerebrovascular resistance from resting levels in absolute values as in Figure 3 may be a more appropriate approach to analyse specific interactions between the effects of L-NAME and of vasodilators. Using this approach we found that absolute reductions in cerebrovascular resistance induced by diazoxide were comparable before and after L-NAME, indicating that effects of this vasodilator are not affected by the basal cerebrovascular resistance, and suggesting that cerebral vasodilatation by diazoxide is not related to nitric oxide. Diazoxide, a benzothiadiazine derivative, seems to produce relaxation of the vascular smooth muscle by hyperpolarizing arterial smooth muscle cells through activation of ATP-sensitive K^+ channels (Standen *et al.*, 1989). The results with acetylcholine and sodium nitroprusside, however, differed from those with diazoxide. The reduction of resistance caused by lower doses, but not by the highest dose, of acetylcholine were attenuated after L-NAME, suggesting that at least a component of the cerebral vasodilatation to acetylcholine is mediated by nitric oxide. This suggestion agrees with that previously proposed by others (Fujiwara *et al.*, 1986; Faraci, 1990; Fischer-Nakielski *et al.*, 1990; Rosenblum *et al.*, 1990). Although basal release of nitric oxide in the cerebral circulation could be greater in large arteries than in small vessels, the role of nitric oxide in mediating responses to acetylcholine in the cerebral circulation seems to be similar in large arteries and the microcirculation (Faraci, 1991). However, other factors distinct to the L-arginine-nitric oxide pathway could be involved because in the present study, L-NAME did not affect the cerebral vasodilatation to the highest dose of acetylcholine used. This feature has also been observed by recording internal carotid blood flow of rats, where L-NAME reduced the effects of lower, but not of higher dose, of acetylcholine (Gardiner *et al.*, 1991b). Our data also indicate that the duration of the changes in reactivity of the cerebral circulation to acetylcholine was shorter than that of the changes in cerebral blood flow after L-NAME. Thus after inhibition of nitric oxide synthesis, the ability of cerebral blood vessels to release

nitric oxide upon stimulation appears to normalize more quickly than the capacity for nitric oxide release under basal conditions.

The reductions in cerebrovascular resistance induced by sodium nitroprusside were increased during and at 24 h, and normalized at 48 h, after L-NAME treatment suggesting that cerebral vasodilatation to sodium nitroprusside is increased after inhibition of nitric oxide synthesis. Sodium nitroprusside, a nitrous compound used as a donor of exogenous nitric oxide, produces vasodilatation in a similar way to nitric oxide (Ignarro, 1990), and enhanced vasorelaxant responses to sodium nitroprusside and other nitrous compounds have been found in the absence of a functional endothelium or in the presence of L-arginine analogues (Shirasaki & Su, 1985; Lüscher *et al.*, 1989; Moncada *et al.*, 1991b; García *et al.*, 1992). This enhanced response has been related to an increased sensitivity of guanylate cyclase in vascular smooth muscle to exogenous nitric oxide when endogenous nitric oxide production is reduced (Gardiner *et al.*, 1991b; Moncada *et al.*, 1991b). Thus, it appears that the removal of endogenous nitric oxide in the vasculature could lead to supersensitivity to vasodilators that act by stimulating soluble guanylate cyclase (Gardiner *et al.*, 1991b; Moncada *et al.*, 1991b) and this phenomenon could also occur in the cerebral circulation of the unanaesthetized animals as suggested from our study. We also observed that supersensitivity of cerebral vasculature to sodium nitroprusside remained for a shorter period than that required for the cerebral blood flow to recover.

In conclusion, the present study performed in the conscious goat suggests that endogenous nitric oxide plays an important role in regulating cerebral blood flow by producing a basal vasodilator tone in the cerebral circulation under normal conditions. It also suggests that the cerebral vasodilatation to acetylcholine is mediated, at least in part, by nitric oxide release, and that inhibition of endogenous nitric oxide production induces supersensitivity of cerebral vasculature to nitrovasodilators.

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