Effect of inhibition of nitric oxide synthase on dynamic cerebral autoregulation in humans

R. P. WHITE*, P. VALLANCE† and H. S. MARKUS*‡
*Clinical Neurosciences, Guy’s, King’s and St Thomas’ School of Medicine and Institute of Psychiatry, De Crespigny Park, London SE5 8AF, U.K., †Centre for Clinical Pharmacology Unit, Wolfson Institute for Biochemical Research, University College, Gower Street, London WC1E 6BY, U.K., and ‡Clinical Neuroscience, St George’s Hospital Medical School, Cranmer Terrace, London SW17 ORE, U.K.

ABSTRACT

Cerebral blood flow is maintained constant over a range of cerebral perfusion pressures by cerebral autoregulation. Impaired cerebral autoregulation may be important in the pathogenesis of cerebral ischaemia. The mechanisms mediating normal cerebral autoregulation in humans are poorly understood. We used a recently described transcranial Doppler technique, which allows non-invasive measurement of dynamic cerebral autoregulation, to test the hypothesis that nitric oxide mediates cerebral autoregulation. The rate of rise of middle cerebral artery blood flow velocity, compared with that of arterial blood pressure, was determined following a stepwise fall in arterial blood pressure, in order to calculate an autoregulatory index. The effect of the nitric oxide synthase inhibitor \(N^G\)-monomethyl-L-arginine (l-NMMA) on dynamic autoregulation was compared with that of noradrenaline titrated to result in a similar rise in blood pressure. Six healthy subjects were studied in each group. The mean (S.D.) change in autoregulatory index following noradrenaline at a similar pressor dose was significantly greater than the change following the l-NMMA bolus: 1.1 (1.2) compared with −0.8 (0.8) for the left middle cerebral artery \((P = 0.002)\), and 1.1 (0.8) compared with −0.8 (0.8) for the right middle cerebral artery \((P = 0.002)\). There was no difference in the mean (S.D.) blood pressure increase resulting from the two agents: l-NMMA, 19.7 (7.4) mmHg; noradrenaline, 15.5 (4.8) mmHg \((P = 0.281)\). These results suggest that nitric oxide mediates at least part of the dynamic phase of cerebral autoregulation in humans. Reduced nitric oxide release may play a role in the impaired cerebral autoregulation seen in patients with, or at risk of, cerebral ischaemia.

INTRODUCTION

Cerebral autoregulation provides an important protective mechanism by ensuring that the cerebral circulation maintains near-constant cerebral blood flow over a wide range of perfusion pressures. It occurs rapidly following a change in perfusion pressure [1]. Several mechanisms have been proposed to explain cerebral autoregulation, including components dependent on chemical mediators, perivascular neural innervation and myogenic tone, but the underlying mechanisms remain incompletely understood. Impaired cerebral autoregulation is found in patients at risk of stroke, such as those with hypertension [2], and may also occur during acute cerebral ischaemia. Understanding the mechanisms underlying cerebral autoregulation may offer new therapeutic possibilities.

Nitric oxide (NO) has been shown to regulate basal cerebral blood flow in both animals [3] and humans [4],...
and in some animal models it mediates at least part of the vasodilatory response to hypercapnia and regional vasoneuronal coupling [3]. However, few studies to date have addressed the role of NO in cerebral autoregulation, and results are conflicting; some authors report impaired autoregulation following NO inhibition [5,6], but others report no effect [7,8]. No studies have examined the role of NO in humans. These animal studies have assessed steady-state flow after a mechanical or pharmacological manipulation of cerebral perfusion pressure. However, the autoregulatory response to perfusion pressure change is rapid and occurs within seconds [9]. Recently a technique for estimating dynamic autoregulation in humans has been developed, which takes advantage of the high temporal resolution of transcranial Doppler ultrasound [10]. This allows the beat-to-beat measurement of cerebral blood flow velocity, changes in which correlate well with changes in cerebral blood flow. A sudden fall in blood pressure is induced by inflation, and then rapid deflation, of bilateral leg cuffs. In the presence of normal autoregulation, cerebral blood flow will return to baseline more rapidly than the systemic blood pressure. Therefore, by comparing the rate of rise of middle cerebral artery (MCA) blood flow velocity with the rate of rise of continuously monitored arterial blood pressure, an index of cerebral autoregulatory capacity can be derived [10].

We have studied the effects of the NO synthase inhibitor, Nω-monomethyl-L-arginine (L-NMMA), on dynamic autoregulatory responses in normal volunteers. L-NMMA increases blood pressure, and this is likely to increase the rate of dynamic autoregulation. Therefore we examined the hypothesis that inhibition of NO synthase with L-NMMA would decrease the speed of dynamic autoregulation compared with noradrenaline (NA), titrated to result in a similar increase in blood pressure to that seen with L-NMMA.

**METHODS**

**Subjects**

Normal healthy volunteers were recruited. Two studies were performed, the first with L-NMMA and the second with NA. Each was carried out in a separate group of six subjects. Four subjects were common to both studies. There was no difference in mean (S.D.) age between the two groups [27.0 (3.0) versus 27.7 (2.7) years; P = 0.71] or in mean (S.D.) weight [60.0 (9.9) versus 56.7 (5.4) kg; P = 0.49]. Six females were studied in the L-NMMA group, and five females and one male in the NA group. Local Hospital Ethics Committee approval was obtained, and all subjects gave written informed consent.

**Dynamic autoregulation testing**

All subjects were studied in a supine position. MCA velocity was recorded bilaterally simultaneously via the transtemporal window using a commercially available transcranial Doppler ultrasound system with 2 MHz transducers (DWL, Langerach, Germany). Continuous arterial blood pressure recording was performed using a servo-controlled finger plethysmograph (Finapres 2300; Ohmeda, Louisville, KY, U.S.A.), with the hand maintained level with the head. A typical tracing is shown in Figure 1. Baseline measurement of resting blood pressure was carried out using an automated arm cuff (Omega 1400 series; In Vivo Laboratories Inc., Orlando, FL, U.S.A.). To induce a stepwise drop in blood pressure, bilateral thigh cuffs were inflated suprasystolically for 3 min, and then deflated suddenly. Only falls in blood pressure of more than 10 mmHg were considered to be a sufficient stimulus. Autoregulatory responses were analysed off-line using the time-averaged mean velocities of the maximum velocity outlines of the Doppler spectrum and mean arterial blood pressure. This software program compares the rate of return of blood pressure and MCA blood flow velocity to baseline following the drop in blood pressure. Starting at the moment of cuff release and based on the actual blood pressure curve over the following 30 s, a series of ten hypothetical autoregulatory curves are calculated. The theoretical curves are calculated on the basis that, if the rate of rise in flow velocity is identical with that of blood pressure, an autoregulatory index (ARI) of 0 is obtained, while greater rates of rise in MCA velocity result in an increased ARI (maximum = 9). Each model curve is compared with the actual flow velocity recording for the best fit. Full details of the equations used have been published previously [10]. Three cycles of inflation/deflation were performed per subject at each study measurement point, with a 3 min rest interval between cycles, and at least a 10 min rest interval between study points.

In the L-NMMA group, clinical-grade L-NMMA with a purity of 99.8% (Glaxo-Wellcome) was used. This was administered at a dose of 10 mg/kg, diluted in 20 ml of normal saline, and given as a 30 s intravenous bolus dose into the antecubital fossa; this was followed by an intravenous infusion of L-NMMA at 10 mg h⁻¹·kg⁻¹. Two sets of baseline autoregulatory measurements were made before administration of L-NMMA. After a 5 min pause to allow for the peak effect of the L-NMMA bolus on cerebral blood flow, a first set of autoregulatory readings was taken. This was followed by a second set recorded 25 min after infusion.

NA (Sanofi Winthrop), diluted in 5% (w/v) dextrose to a concentration of 10 μg/ml, was infused intravenously via an antecubital vein. For the NA group, a similar protocol was followed as for the L-NMMA group, with two baseline sets of readings. NA was then given at an infusion rate titrated to effect a 10 mmHg increase in blood pressure over baseline, after which the next data set was collected. The infusion was then increased to effect a 15–20 mmHg increase in blood pressure, a pressor effect.
NO and autoregulation

Figure 1 Typical tracing showing the measurement of dynamic autoregulation
MCA velocity is displayed on the upper panel, and continuous blood pressure monitoring is displayed on the lower panel. At the time point arrowed, the leg cuffs are suddenly deflated, resulting in a sudden drop in blood pressure. There is a secondary drop in MCA flow velocity. Following the sudden drop, blood pressure rises gradually, but MCA flow velocity rises more rapidly, representing cerebral autoregulation. A series of ten hypothetical curves are calculated (see Methods section) and are shown in shades of grey around the MCA tracing; the best fit is selected, in this case giving an ARI of 6.

equivalent to that seen after the 10 mg/kg L-NMMA bolus, and the next reading set was taken. Blood pressure was observed to be stable for at least 10 min before each set of readings was taken.

A mean ARI was calculated for each subject only from runs where a mean fall in blood pressure of sufficient magnitude (> 10 mmHg) was obtained. Only one autoregulatory measurement in one set of three measurements in three of the NA group, and in one of the t-NMMA group, gave an insufficient fall in blood pressure to be used in ARI estimation. In these cases, mean ARI was calculated from the mean of the remaining two measurements at that time point. Within-study comparisons of ARI and haemodynamic variables were carried out using paired-sample t tests.

RESULTS

Dynamic ARI
The effect of t-NMMA on dynamic autoregulation is shown in Figure 2. There was no difference in mean (S.D.) resting ARI between the two baseline runs: 4.6 (1.0) and 4.7 (1.1) for the right MCA (P = 0.736); and 4.4 (1.0) and 4.7 (1.0) for the left MCA (P = 0.290). Following the t-NMMA bolus, ARI fell to 3.9 (0.7) for the right MCA and to 3.9 (0.6) for the left MCA (P = 0.06 and P = 0.05 respectively, compared with second baseline). During the t-NMMA infusion, ARI returned towards baseline, but remained relatively suppressed at 4.3 (0.9) for the right MCA and 4.3 (0.8) for the left MCA (P = 0.02 and P = 0.08 respectively, compared with the second baseline).
The effect of NA on dynamic autoregulation is shown in Figure 3. There were no significant differences in Resting mean (S.D.) ARI between the two baseline runs: 5.3 (0.7) and 5.4 (0.8) for the right MCA (P = 0.744); and 5.3 (0.7) and 5.4 (0.6) for the left MCA (P = 0.73). There was no significant difference between the NA and L-NMMA groups in baseline ARI for either MCA. Following a dose of NA resulting in an equivalent elevation of blood pressure to that caused by the L-NMMA bolus, mean (S.D.) ARI was 6.4 (0.9) for the right MCA, and 6.3 (0.7) for the left MCA (P = 0.08 and P = 0.12 respectively, compared with second baseline).

The mean (S.D.) change in ARI following NA administration was significantly greater than the change following the L-NMMA bolus, and an effect of similar magnitude was seen for both MCAs: 1.1 (1.2) compared with −0.8 (0.8) for the left MCA (P = 0.002), and 1.1 (0.8) compared with −0.8 (0.8) for the right MCA (P = 0.002).

**Systemic haemodynamic response**

**L-NMMA group**

Following a bolus of 10 mg/kg L-NMMA, the mean (S.D.) arterial blood pressure increased from 75.8 (7.2) mmHg to 93.8 (9.1) mmHg (P = 0.002), and the mean (S.D.) pulse rate fell from 65.3 (10.4) min⁻¹ to 48.2 (6.8) min⁻¹ (P = 0.003). Blood pressure remained elevated during the L-NMMA infusion, compared with baseline values, at 93.8 (8.4) mmHg (P = 0.001); pulse rate was suppressed at 50.8 (6.8) min⁻¹ (P = 0.02).

**NA group**

NA was titrated to effect an increase in mean (S.D.) blood pressure from 78.7 (6.7) mmHg to 88.5 (6.2) mmHg and then 94.2 (6.9) mmHg (P = 0.001 in both cases with respect to baseline). The maximal increase of 15.5 (4.8) mmHg was not significantly different from the 19.7 (7.4) mmHg increase observed following the L-NMMA bolus (P = 0.28). A fall in mean (S.D.) pulse rate was observed following the higher NA pressor dose equivalent to L-NMMA, from 65.8 (8.1) min⁻¹ at baseline to 59.5 (7.9) min⁻¹ (P = 0.06).

**DISCUSSION**

The results of the present study show that inhibition of NO with L-NMMA reduces the speed of dynamic autoregulation compared with the effect of an equivalent pressor dose of NA. This provides the first evidence that NO mediates dynamic autoregulation in humans. This effect occurred despite an increase in vascular tone, secondary to systemic inhibition of endothelial NO synthase, sufficient to increase systemic blood pressure by 20 mmHg. Empirically, one would expect an increase in vascular tone to result in more rapid autoregulatory responses, and this has been demonstrated following hyperventilation to induce hypocapnia [11]. This was confirmed by our pressor control study, in which NA increased the speed of dynamic autoregulation. Therefore the appropriate control for the effect of L-NMMA is an agent that results in a similar pressor effect, and we achieved this by the use of NA. Following L-NMMA there was highly significant reduction in the speed of autoregulation, compared with that after an equivalent pressor dose of NA.

Despite the compelling evidence for a role for NO in maintaining cerebral blood flow, its role in this autoregulation has been little studied to date, and the results from animal models have been conflicting. Autoregulation to hypotension in the rat has been reported to be unaffected [7] or impaired [6] following systemic NO inhibition. In a feline model, intravenous L-NMMA has been reported to inhibit pressure-dependent autoregulation [5], but a primate study has suggested that intra-carotid arterial NO inhibition has no effect on autoregulation [8].

However, the conventional techniques used for measuring autoregulation in such models are based on static flow measurements during stable blood pressure excursions, and do not have sufficient temporal resolution to measure the dynamics of the initial vascular response. We have used a technique which is non-invasive and utilizes the excellent temporal resolution of transcranial Doppler to compare the rate of recovery of MCA flow velocity with that of systemic blood pressure in response to a sudden transient hypotensive event [12]. The technique depends upon changes in MCA velocity correlating with changes in MCA blood flow, and thus assumes constancy of the MCA luminal diameter. Previous validation studies support this assumption. Firstly, a good correlation was found between dynamic auto-
regulation measured using this technique and static autoregulation measured using conventional techniques [10]. Secondly, there was a close correlation between both the degree and time course of the fall and subsequent rate of rise in MCA flow velocity and absolute internal carotid artery flow measured using a magnetic flowmeter during the procedure; furthermore, dynamic autoregulation was similar whether it was determined from the carotid flow data or MCA flow velocity data [13]. Therefore there is strong evidence for the validity of the technique that we used. A potential criticism is that l-NMMA itself may result in MCA constriction [4] to a greater extent than NA, despite their similar pressor effect. However, this would be expected to increase the tone in the MCA wall, and therefore to increase the speed of dynamic autoregulation. In contrast, l-NMMA reduced the speed. Therefore our results suggest that NO mediates at least part of dynamic cerebral autoregulation. Even following a large bolus dose of l-NMMA, the dynamic autoregulatory response was not abolished, but only reduced. This may represent a failure to fully inhibit NO synthase, but is perhaps more likely to be consistent with other mechanisms mediating part of the autoregulatory response.

Due to the time taken to perform each autoregulatory run, repeating the protocol with different doses of l-NMMA in the same subject, as has been performed when studying the effect of l-NMMA on resting cerebral blood flow [4], was not practical. Therefore we used a dose of l-NMMA which has been shown to have a clear effect on resting cerebral blood flow, namely 10 mg/kg [4]. This was followed by an l-NMMA infusion at 10 mg·h\(^{-1}\)·kg\(^{-1}\). This was designed to determine whether there was any delayed effect of l-NMMA on autoregulation. However, at 25 min, the decrease in the speed of autoregulation was less than that seen after the bolus dose, and the blood pressure remained elevated to a similar level as that seen post-bolus, suggesting a similar degree of systemic NO inhibition. It is possible that other mechanisms come into play to preserve dynamic autoregulation following sustained NO inhibition.

l-NMMA is a non-specific inhibitor of NO synthase, and therefore we cannot determine whether the effects of l-NMMA on autoregulation are mediated via the endothelial or neuronal isoform of NO synthase. The systemic haemodynamic effects are consistent with significant inhibition of endothelial NO synthase; because of the slow transport of l-NMMA across the blood–brain barrier [14], we can be less certain about the degree of inhibition of neuronal NO synthase achieved. Our results are consistent with those from endothelial NO synthase knockout mice, in which there was a greater fall in cerebral blood flow during haemorrhagic hypotension in the mutant animals; however, that study investigated static rather than dynamic autoregulation [15].

Our findings may have important clinical implications. Impaired cerebral autoregulation plays a role in the pathogenesis of both acute stroke and more chronic white-matter ischaemic damage [2]. Impaired production and sensitivity to NO in the peripheral arteries has been described in hypertension [16] and diabetes [17]. A similar situation in the cerebral circulation may contribute to this increased risk of stroke.

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REFERENCES


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