British Journal of Pharmacology (1997) 121, 49–56

Cerebrovascular effects of nitric oxide manipulation in spontaneously hypertensive rats

Ioannis P. Fouyas, [1]Paul A.T. Kelly, Isobel M. Ritchie & Ian R. Whittle

Department of Clinical Neurosciences, University of Edinburgh, Western General Hospital, Crewe Road South, Edinburgh EH4 2XU

1 Evidence that nitric oxide (NO) bioactivity is altered in chronic hypertension is conflicting, possibly as a result of heterogeneity in both the nature of the dysfunction and in the disease process itself. The brain is particularly vulnerable to the vascular complications of chronic hypertension, and the aim of this study was to assess whether differences in the cerebrovascular responsiveness to the NO synthase (NOS) inhibitors, N^G-nitro-L-arginine methyl ester (L-NAME) and 7-nitroindazole (7-NI), and to the NO donor 3-morpholinosydnonimine (SIN-1) might indicate one possible source of these complications.

2 Conscious spontaneously hypertensive (SHR) and WKY rats, were treated with L-NAME (30 mg kg^{-1}, i.v.), 7-NI (25 mg kg^{-1}, i.p.), SIN-1 (0.54 or 1.8 mg kg^{-1} h^{-1}, continuous i.v. infusion) or saline (i.v.), 20 min before the measurement of local cerebral blood flow (LCBF) by the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique.

3 With the exception of mean arterial blood pressure (MABP), there were no significant differences in physiological parameters between SHR and WKY rats within any of the treatment groups, or between treatment groups. L-NAME treatment increased MABP by 27% in WKY and 18% in SHR groups, whilst 7-NI had no significant effect in either group. Following the lower dose of SIN-1 infusion, MABP was decreased to a similar extent in both groups (around −20%). There was no significant difference in MABP between groups following the higher dose of SIN-1, but this represented a decrease of −41% in SHR and −21% in WKY rats.

4 With the exception of one brain region (nucleus accumbens), there were no significant differences in basal LCBF between WKY and SHR. L-NAME produced similar decreases in LCBF in both groups, ranging between −10 and −40%. The effect of 7-NI upon LCBF was more pronounced in the SHR (ranging from −34 to −57%) compared with the WKY (ranging from −14 to −43%), and in seven out of the thirteen brain areas examined there were significant differences in LCBF.

5 Following the lower dose of SIN-1, in the WKY 8 out of the 13 brain areas examined showed significant increases in blood flow compared to the saline treated animals. In contrast, only 2 brain areas showed significant increases in flow in the SHR. In the rest of the brain areas examined the effects of SIN-1 upon LCBF were less marked than in the WKY.

6 Infusion of the higher dose of SIN-1 resulted in further significant increases in LCBF in the WKY group (ranging between +30% and +74% compared to saline-treated animals), but no significant effects upon LCBF were found in the SHR. As a result, there were significant differences in LCBF between SIN-1-treated WKY and SHR in six brain areas. In most brain areas examined, cerebral blood flow in SHR following the higher dose of SIN-1 was less than that measured with the lower dose of SIN-1.

7 Despite comparable reductions in MABP (~20%) in both groups, calculated cerebrovascular resistance (CVR) confirmed that the vasodilator effects of the lower dose of SIN-1 were significantly more pronounced throughout the brain in the WKY (ranging between −3% and −50%; median = −38%) when compared to the SHR (ranging between −10% and −36%; median = −26%). In the animals treated with the higher dose of SIN-1, CVR changes were broadly similar in both groups (median = −45% in WKY and −42% in SHR), but with the reduction in MABP in SHR being twice that found in WKY, this is in keeping with an attenuated blood flow response to SIN-1 in the SHR.

8 The results of this study indicate that NO-dependent vasodilator capacity is reduced in the cerebrovasculature of SHR. In addition, the equal responsiveness to a non-specific NOS inhibitor but an enhanced effectiveness of a specific neuronal NO inhibitor upon LCBF in the SHR could be consistent with an upregulation of the neuronal NO system.

Keywords: Cerebral blood flow; hypertension; SHR; nitric oxide; L-NAME; 7-nitroindazole; 3-morpholinosydnonimine (SIN-1); quantitative autoradiography

Introduction

Although it has been proposed that an impaired release of endothelial vascular relaxing factors might underlie the pathogenesis of hypertension (Lüscher & Vanhoutte, 1986), the involvement of nitric oxide in the aetiology of the disease process remains controversial. Studies in hypertensive human subjects have shown that there is abnormal nitric oxide (NO) activity associated with hypertension (Calver *et al*., 1992), but divergent results have also emerged (Cockroft *et al*., 1994). In experimental animal models of chronic hypertension, there is increasing evidence that endothelium-dependent relaxation is heterogeneously affected (Lüscher, 1992), with normal function maintained in the renal and coronary arteries (Tschudi *et al*., 1991), but impaired function in aorta, mesenteric, carotid and cerebral circulation (Lüscher & Vanhoutte, 1986; Dohi *et*

[1] Author for correspondence.

al., 1990; Cuevas *et al*., 1996). These observations could explain not only contradictory experimental observations, but also the selective vulnerability of certain tissues, such as the brain, to hypertensive complications.

The brain is particularly vulnerable to complications associated with hypertension and both the incidence (Whisnant, 1996) and severity of cerebrovascular ischaemia (Coyle, 1984) are increased in hypertension. Studied performed *in vitro* have raised the possibility that cerebrovascular NO systems are altered in hypertensive rats (Miyata *et al*., 1990; Malinski *et al*., 1993), and perturbations in endothelium-dependent relaxation have been identified in pial arteries examined *in situ* (Yang *et al*., 1991a; Mayhan, 1992). In contrast, cerebrovascular responsiveness to NO inhibition is preserved *in vivo* (Izuta *et al*., 1995), and basal local cerebral blood flow (LCBF) appears to be unaffected by hypertension (Wei *et al*., 1992).

Whilst it might appear from these observations that there may be no functional basis to the increased susceptibility to ischaemia in chronic hypertensives, this cannot be discounted in favour of structural dysfunction without first examining the integrity of NO-dependent cerebrovascular dilator reserve and the role of perivascular neuronal NO systems in cerebrovascular control (Kelly *et al*,. 1995). The fact that NO donors reduce infarct size in spontaneously hypertensive rats (SHR) subjected to middle cerebral artery (MCA) occlusion (Zhang *et al*., 1994), is consistent with a reduced NO-dependent dilator capacity of the hypertensive brain. In the present studies, we have measured local cerebral blood flow (LCBF) in SHR and normotensive WKY controls following treatment with an exogenous NO donor 3-morpholinosydnonimine (SIN-1) and 7-nitro indazole, which shows *in vivo* selectivity for neuronal nitric oxide synthase (NOS) inhibition (Moore *et al*., 1993a,b). We have also re-examined the effects of the non-selective NOS inhibition N^G-nitro-L-arginine methyl ester (L-NAME) to allow comparisons with 7-NI.

Methods

These studies were performed with a total of 25 SHR and 27 WKY adult male rats between 14 and 16 weeks of age (Charles River, U.K.). Animals were held under normal animal house conditions with free access to food and water.

Measurement of local cerebral blood flow

On the day of the experiment the animals were anaesthetized with halothane (1.5% in a gas mixture of 70% nitrous oxide and 30% oxygen) and prepared for the measurement of LCBF as described previously (Kelly *et al*., 1994). Following surgery, general anaesthesia was withdrawn and 2 h allowed to elapse before any further experimental manipulation. Approximately equal numbers of SHR and WKY rats were treated with either L-NAME (30 mg kg^{-1} in saline, i.v. over 60 s; $n=4$ from each group), 7-NI (25 mg kg^{-1} in sesame oil, i.p. over 5 s; $n=4$ from each group), SIN-1 (0.54 or 1.8 mg kg^{-1} h^{-1} in saline, continuous i.v. infusion; $n=4$ from each group). In previous studies from this laboratory we have found no difference in LCBF between control animals injected with oil (i.p.) or saline (i.v.), or between saline infusion or bolus injection. In this study, control animals were injected with saline. (1.0 ml i.v.; $n=5$ SHR and $n=6$ WKY). The doses of L-NAME and 7-NI were chosen on the basis of previously published work from this and other laboratories (Macrae *et al*., 1993; Moore *et al*., 1993a,b; Kelly *et al*., 1994; 1995). Doses of 7-NI higher than that used in this study, have been shown to produce further reductions in cerebral blood flow in normal rats (Kelly *et al*., 1995; Wang *et al*., 1995) but they may also produce anomalous focal hyperaemia (Kelly *et al*., 1995).

In a series of preliminary studies, two doses of SIN-1 were identified which produced either similar magnitude of reduction in MABP in WKY and SHR (lower dose, 0.54 mg kg^{-1} h^{-1}), or which reduced MABP to comparable absolute values in both groups (higher dose, 1.8 mg kg^{-1} h^{-1}). In these studies, we also found that an i.v. infusion of SIN-1 at 1.8 mg kg^{-1} h^{-1} for 20 to 30 min completely blocked the expected cardiovascular and cerebrovascular effects of subsequent i.v. injection of L-NAME (30 mg kg^{-1}).

The measurement of LCBF was initiated 20 min after the start of L-NAME, 7-NI, SIN-1 or saline treatments by use of the fully quantitative [^{14}C]-iodoantipyrine autoradiographic technique. As far as possible, control (saline) experiments were performed contemporaneously to the various drug treatments. The protocols were in complete accordance with the methodology as originally published (Sakurada *et al*., 1978) and as described previously from this laboratory (Kelly *et al*., 1994). Autoradiographic images were analysed by quantitative densitometry relative to ^{14}C-containing standards and LCBF was calculated by use of the appropriate operational equation for the technique (Sakurada *et al*., 1978). Areas of interest were chosen to represent brain areas in the vascular territories of the anterior, middle and posterior cerebral arteries. Arterial blood pressure and rectal temperature were monitored continuously in each animal throughout the experiments and heart rate was measured intermittently. Samples of arterial blood were withdrawn before and after treatments, for the measurement of pH, P_{CO_2}, and P_{O_2}.

Calculation of cerebrovascular resistance

Cerebrovascular resistances (CVR) were calculated in animals treated with SIN-1 and the relevant saline-treated rats by dividing mean arterial blood pressure (mmHg) by LCBF values (ml 100 g^{-1} min^{-1}) for each brain area in each individual animal.

Drugs

With the exception of SIN-1 which was a gift from Cassella AG, Frankfurt, Germany, all drugs were purchased from the Sigma Chemical Co.

Statistical analysis

Physiological and LCBF data (presented as mean ± s.e.mean) were analysed by Student's *t* test with Bonferroni correction applied to allow multiple pair-wise comparisons between appropriate groups (maximum number of comparisons = 3). Differences in the CVR response to SIN-1 treatment between the two rat strains were analysed by Mann-Whitney U-test. Acceptable levels of significance were set at $P<0.05$ for all statistical tests.

Results

Physiological parameters

With the exception of mean arterial blood pressure, there were no significant differences in physiological parameters between SHR and WKY rats within any of the treatment groups, or between treatment groups (Table 1). As expected, mean arterial blood pressure (MABP) was 46% greater in SHR compared to WKY rats before any treatment, and saline injection had no effect. Not withstanding the initial difference in blood pressure, L-NAME treatment increased MABP to a similar extent in both groups of rats, whilst 7-NI had no significant effect in either group. Following treatment with the lower SIN-1 dose MABP decreased by −21% in the SHR and by −20% in the WKY (Table 2). However, following the higher dose of SIN-1, MABP was decreased by −41% in SHR but by only −21% in WKY rats, so that after treatment there was no

Table 1 Physiological variables in WKY and SHR groups following saline, L-NAME or 7-NI treatment

	WKY			*SHR*		
	Saline	*L-NAME*	*7-NI*	*Saline*	*L-NAME*	*7-NI*
pH	7.49±0.01	7.47±0.01	7.48±0.01	7.48±0.01	7.46±0.01	7.46±0.01
P_{CO_2} (mmHg)	41.6±2.0	40.3±1.0	40.0±0.7	39.3±2.0	37.7±1.0	36.7±0.4
P_{O_2} (mmHg)	88.1±4.0	90.6±4.0	97.8±4.0	91.4±2.0	86.8±1.0	89.2±0.7
MABP (mmHg)	120±3	152±3*	123±3	175±6#	207±1*#	180±6#
Temperature (°C)	36.3±0.2	36.6±0.4	37.1±0.2	36.7±0.2	36.7±0.1	36.5±0.1
Group *n*	6	4	4	5	4	4

Data are presented as mean±s.e.mean. #Significant difference between WKY and SHR given the same treatment ($P<0.05$). *Significantly different from saline treated rats of the same sub-strain ($P<0.05$).

Table 2 Physiological variables in WKY and SHR groups following saline or SIN-1 treatment

	WKY			*SHR*		
	Saline	*Low SIN-1*	*High SIN-1*	*Saline*	*Low SIN-1*	*High SIN-1*
pH	7.49±0.01	7.48±0.01	7.48±0.01	7.48±0.01	7.46±0.01	7.44±0.01
P_{CO_2} (mmHg)	41.6±2.0	38.4±2.0	37.9±1.0	39.3±2.0	7.9±1.0	40.4±1.0
P_{O_2} (mmHg)	88.1±4.0	85.5±1.0	94.6±3.0	91.4±2.0	90.2±3.0	97.4±1.0
MABP (mmHg)	120±4	96±8*	95±6*	178±6#	147±2#*	104±5*
Temperature (°C)	36.3±0.2	35.8±0.2	37.0±0.2	36.7±0.2	37.4±0.1	37.3±0.3
Group *n*	5	4	4	4	4	4

Data are presented as mean±s.e.mean. #Significant difference between WKY and SHR given the same treatment ($P<0.05$). *Significantly different from saline-treated rats of the same sub-strain ($P<0.05$).

Table 3 Local cerebral blood flow in normotensive (WKY) and spontaneously hypertensive (SHR) rats treated with saline, L-NAME or 7-NI

	Saline		*L-NAME*		*7-NI*	
	WKY	*SHR*	*WKY*	*SHR*	*WKY*	*SHR*
Neocortex						
Parietal	141±6	132±8	124±7	105±6	121±5	78±4*#
Cingulate	98±4	117±6	99±5	91±6	82±1	64±6*
Corpus callosum	37±2	32±2	20±1*	29±2#	21±1*	21±1*
Basal ganglia						
Striatum	99±4	94±5	87±4	83±5	78±3*	59±5*#
Globus pallidus	64±4	68±8	53±3	54±3	49±2	38±1#
Accumbens	98±4	116±3#	78±5	82±5*	77±5	56±1*#
Thalamus						
Hypothalamus	75±4	83±5	49±3*	45±3*	50±3*	36±3*#
Lateral geniculate	106±5	116±9	89±6	93±5	87±3	69±2*#
Hippocampus						
CA3	76±4	85±3	60±1	63±4*	57±2*	49±3*
CA2	71±3	83±4	56±2*	59±5*	51±1*	45±2*
CA1	68±5	74±3	56±1	61±3	49±2	44±2*
Molecular layer	73±4	85±2	57±1	61±5*	55±3	45±2*
Dentate hilus	76±4	93±6	61±1	62±5*	58±3	46±1*#
Group *n*	6	5	4	4	4	4

Data are presented as mean local cerebral blood flow (ml $100\,g^{-1}\,min^{-1}$)±s.e.mean. #Significant difference between WKY and SHR animals given the same treatment ($P<0.05$). *Significantly different from saline-treated rats of the same sub-strain ($P<0.05$).

significant difference in MABP between these two groups (Table 2).

Local cerebral blood flow following L-NAME

Significant differences in basal LCBF between WKY and SHR were found in only of one of the thirteen brain regions examined (nucleus accumbens) (Table 3). Following injection of L-NAME, LCBF in the WKY group was significantly reduced (compared to saline treated animals) in the corpus callosum (−46%), the hypothalamus (−35%) and the CA2 layer of the hippocampus (−21%). Elsewhere in the brain there was a tendency towards decreases in LCBF (with the exception of cingulate cortex) ranging between −12 and −22%, but these differences were not significant. Similarly, in the L-NAME-treated SHR group there was a global trend towards reductions in LCBF ranging between −12 and −22%, which reached acceptable levels of significance in the nucleus accumbens (−29%), hypothalamus (−46%), hippocampal fields CA2 and CA3 (−29% and −26%), and in the molecular layer (−28%) and hilus of the dentate gyrus (−33%). There were no significant differences in the LCBF between L-NAME treated WKY and SHR, apart from in the corpus callosum (Table 3).

Local cerebral blood flow following 7-NI

Following the intraperitoneal injection of SHR with 7-NI, significant reductions in LCBF (ranging between −34 and −57%) were found in 12 of the 13 areas examined when compared to the appropriate saline treated group (Table 3). Although the response to 7-NI in the WKY group was qualitatively similar, significant reductions in LCBF were limited to the corpus callosum (−43%), striatum (−21%), hypothalamus (−33%), and CA2 (−28%) and CA3 (−25%) fields of the hippocampus. However, throughout the brain, the re-

Table 4 Local cerebral blood flow in normotensive (WKY) and spontaneously hypertensive (SHR) rats treated with saline or SIN-1

	Saline		*Low SIN-1*		*High SIN-1*	
	WKY	*SHR*	*WKY*	*SHR*	*WKY*	*SHR*
Neocortex						
Parietal	138±6	127±9	139±12	142±10	206±9*	148±11#
Cingulate	95±3	112±5	152±7*	143±5*	171±6*	138±5#
Corpus callosum	35±2	32±3	29±3	29±1	36±3	41±2
Basal ganglia						
Striatum	96±3	90±4	117±5*	109±8	129±6*	107±9
Globus pallidus	62±4	61±4	90±2*	82±4*	80±4	66±4
Accumbens	96±4	114±4	136±7*	138±11	153±4*	130±8
Thalamus						
Hypothalamus	71±3	80±4	79±5	75±7	89±4	70±5
Lateral geniculate	103±5	111±10	138±6*	139±14	164±7*	118±8#
Hippocampus						
CA3	74±4	83±3	98±4*	100±8	104±3*	81±9
CA2	68±3	80±3	82±7	81±9	92±5*	82±5
CA1	65±4	73±3	80±6	81±6	100±5*	76±5#
Molecular layer	71±4	84±2	95±4*	88±8	103±3*	82±4#
Dentate hilus	73±5	90±5	94±5*	89±9	112±6*	87±4#
Group *n*	5	4	4	4	4	4

Data are presented as mean local cerebral blood flow (ml $100\,g^{-1}\,min^{-1}$)±s.e.mean. #Significant difference between WKY and SHR animals given the same treatment ($P<0.05$). *Significantly different from saline-treated rats of the same sub-strain ($P<0.05$).

Table 5 Calculated cerebrovascular resistance in normotensive (WKY) and hypertensive (SHR) animals, treated with saline or SIN-1

	WKY					*SHR*				
	Saline	*Low SIN-1*	*% change*	*High SIN-1*	*% change*	*Saline*	*Low SIN-1*	*% change*	*High SIN-1*	*% change*
Neocortex										
Parietal	0.87±0.02	0.69±0.05	−21	0.46±0.03	−46	1.43±0.11	1.06±0.08	−26	0.71±0.06	−48
Cingulate	1.27±0.05	0.63±0.05	−50	0.56±0.02	−55	1.58±0.08	1.04±0.05	−34	0.76±0.06	−50
Corpus callosum	3.37±0.21	3.28±0.10	−3	2.66±0.23	−19	5.85±0.79	5.17±0.31	−12	2.55±0.22	−54
Basal ganglia										
Striatum	1.24±0.05	0.82±0.05	−34	0.74±0.04	−39	1.99±0.09	1.38±0.11	−31	0.99±0.11	−48
Globus pallidus	1.95±0.12	1.06±0.08	−46	1.19±0.40	−37	2.85±0.24	1.82±0.12	−36	1.59±0.09	−40
Accumbens	1.26±0.03	0.72±0.03	−43	0.62±0.02	−50	1.54±0.11	1.10±0.10	−29	0.81±0.06	−47
Thalamus										
Hypothalamus	1.68±0.10	1.21±0.04	−28	1.08±0.09	−34	2.26±0.16	2.02±0.21	−11	1.52±0.14	−29
Lateral geniculate	1.17±0.08	0.69±0.05	−41	0.58±0.02	−49	1.66±0.20	1.10±0.13	−34	0.89±0.08	−43
Hippocampus										
CA3	1.64±0.08	0.97±0.07	−41	0.92±0.06	−43	2.17±0.13	1.51±0.15	−30	1.33±0.14	−36
CA2	1.76±0.08	1.18±0.09	−33	1.04±0.03	−39	2.13±0.12	1.91±0.27	−10	1.25±0.09	−41
CA1	1.86±0.13	1.19±0.04	−36	0.95±0.02	−47	2.36±0.16	1.87±0.19	−21	1.39±0.12	−42
Molecular layer	1.71±0.08	1.02±0.09	−40	0.92±0.04	−45	2.03±0.09	1.74±0.21	−14	1.27±0.08	−38
Dentate gyrus	1.65±0.09	1.03±0.10	−38	0.85±0.01	−47	1.92±0.10	1.73±0.23	−10	1.21±0.10	−36
Median effect	–	–	−38	–	−45	–	–	−26§	–	−42
Group *n*	5	4		4		4	4		4	

Cerebrovascular resistances were calculated by dividing mean arterial blood pressure (mmHg) by LCBF values (ml $100\,g^{-1}\,min^{-1}$) for each individual animal and are presented as mean±s.e.mean. The effects of SIN-1 treatments (percentage change) were compared between rat strains by Mann-Whitney U-test. §Significantly different from similarly-treated WKY group ($P<0.01$).

sponse to 7-NI was less marked in the WKY group (ranging between −14 and −43%) when compared to SHR, and in seven brain areas (parietal cortex, striatum, globus pallidus, nucleus accumbens, hypothalamus, lateral geniculate and hilus of the hippocampal dentate gyrus) the decreases in LCBF were significantly greater in the SHR group (Table 3).

Local cerebral blood flow following SIN-1

Following treatment with the lower dose of SIN-1 there were significant increases in LCBF in all but five brain areas (parietal cortex, corpus callosum, hypothalamus, CA1 and CA2 layers of the hippocampus) in the WKY group (Table 4). Significant effects ranged from +22% in striatum, to +60% in cingulate cortex. In contrast, in the SHR group, there were significant effects upon LCBF in only two brain regions (cingulate cortex and globus pallidus) (Table 4). In the rest of the brain areas examined, the effects of this lower dose of SIN-1 upon LCBF were less marked than in the WKY.

Following intravenous infusion of the higher dose of SIN-1 there were significant increases in LCBF in all but three brain areas (corpus callosum, globus pallidus and hypothalamus) in the WKY group (Table 4). Significant effects ranged from +30% in hippocampal field CA2 and striatum, to +74% in cingulate cortex. The CBF values in most of the brain areas examined were higher compared to those obtained following treatment with the lower dose of SIN-1. In contrast, in the SHR group, there were no significant effects of this higher dose of SIN-1 upon LCBF in any region of the brain. In this group, LCBF in grey matter areas ranged from −16% in hypothalamus to +18% in cingulate cortex (Table 4). A comparison of LCBF between WKY and SHR groups treated with the higher dose of SIN-1 revealed significant differences in six of the thirteen brain areas examined (parietal cortex, cingulate cortex, lateral geniculate, hippocampal field CA1, and molecular layer and hilus of the dentate gyrus) (Table 4). Although there were no significant differences between the effects upon LCBF of the two doses of SIN-1 in

SHR, the values obtained following treatment with the higher dose of SIN-1 were generally lower in most of the areas examined (Table 4).

Cerebrovascular resistance (CVR)

Despite comparable reductions in MABP (~20%) in both groups, calculated CVR confirmed that the vasodilator effects of the lower dose of SIN-1 were significantly more pronounced ($P<0.01$, Mann-Whitney U-test) throughout the brain in the WKY (ranging between −3% and −50%; median = −38%) when compared to the SHR (ranging between −10% and −36%; median = −26%) (Table 5). In the groups treated with the higher dose of SIN-1, CVR changes were broadly similar in both groups (median = −45% in WKY and −42% in SHR), but with the reduction in MABP in SHR being twice that found in WKY, this is in keeping with an attenuated blood flow response to SIN-1 in the SHR (Table 5).

Discussion

In the present study we found little evidence of any fundamental difference in basal LCBF between WKY and SHR groups, although in one area of the brain, the nucleus accumbens, blood flow in SHR was significantly higher. These observations are largely in keeping with previous data, where either similar quantitative autoradiographic techniques were used to measure LCBF in conscious rats of these two strains (Wei *et al*., 1992), or where rats of a similar age to those used in this study were examined with different measurement protocols (Grabowski & Johansson, 1985). Moreover, our physiological studies are in keeping with morphological studies of cerebral capillary bed structure (Lin *et al*., 1990) and precapillary arterioles on the pial surface (Harper & Bohlen, 1984) which show no differences between SHR and WKY. Although structural changes have been described in larger blood vessels of the cerebrovascular bed in SHR (Folkow, 1990), it is not these vessels which regulate LCBF.

Early *in vitro* investigations identified a decrease in NO-mediated activity in cerebral blood vessels taken from SHR (Miyata *et al*., 1990; Malinski *et al*., 1993). Subsequent examination of the basilar artery *in situ* showed that L-NAME induced greater constriction in hypertensive rats (Kitazono *et al*., 1995). These authors suggested that basal release of NO might be somewhat enhanced in SHR over that in WKY, and *in vivo* studies confirmed that the cerebrovascular response to NO inhibition with N^G-monomethyl-L-arginine (L-NMMA) was greater in SHR (Izuta *et al*., 1995), although no significant difference in LCBF was found in SHR and WKY treated with N^G-nitro-L-arginine (L-NOARG). In our studies, acute treatment with L-NAME had broadly similar effects upon LCBF in the WKY and SHR groups and were in keeping with previously published results (Izuta *et al*., 1995; Yang, 1996). The dose of L-NAME used in the present study has previously been found in Sprague-Dawley (SD) rats to produce significant reductions in LCBF at 15 min post-injection (Kelly *et al*., 1994) which are maintained for at least 3 h (Macrae *et al*., 1993), but in general it appears from our study that L-NAME is not as efficacious in reducing LCBF in WKY and SHR as it is in Sprague-Dawley (SD) rats. Although it was outwith the present experimental design to make such inter-strain comparisons, it is also noteworthy that differences in vascular structure have been found between normotensive WKY and SD in the cerebral capillary bed (Lin *et al*., 1990).

Whilst there was no evidence of any difference between WKY and SHR in the response to the non-selective NOS inhibitor L-NAME, the response to the intraperitoneal injection of 7-NI, which *in vivo* is a selective inhibitor of the neuronal isoform of NOS (nNOS; Moore *et al*., 1993a,b), was significantly greater in the majority of brain areas of hypertensive animals. Although previous studies have suggested that there may be an upregulation of cerebrovascular NO systems in hypertension (Kitazono *et al*. 1995), our observations point to there being a more specific upregulation of neuronal NOS. Studies specifically addressing the role of neuronal NOS in the brains of SHRs are lacking, although it does seem that nNOS expression is normal in the cerebellum and brain stem of 4-, 16- and 24-week-old SHR, compared to age-matched WKY (Iwai *et al*., 1995). It is interesting to note that the activity of nNOS in cerebral ischaemia is potentially detrimental (Huang *et al*., 1994) and although perhaps speculative at this stage our findings, consistent with an upregulated nNOS system in hypertension, could offer one explanation for the predisposition of hypertensives to ischaemia following stroke (Coyle, 1984). Our findings of comparable cerebrovascular responses to L-NAME but enhanced responses to 7-NI in the SHR could also be compatible with a down-regulation of endothelial NOS in these animals together with an up-regulation of the neuronal NOS. Testing of this hypothesis awaits the development of specific endothelial NOS inhibitors.

There is growing evidence that endothelium-dependent vascular dilatation is heterogeneously affected in hypertension (Nava *et al*., 1995) with both regional and species differences (Deng *et al*., 1995). Studies in hypertensive humans and animals have demonstrated preserved dilatator responses to sodium nitroprusside in peripheral vascular beds (Taddei *et al*., 1993; Küng & Lüscher, 1995). In contrast to the qualitatively similar effects of SIN-1 upon blood pressure in both WKY and SHR, the effects of SIN-1 upon LCBF were attenuated in our SHR group when compared with WKY. These apparent differences in the response to SIN-1 between vascular beds in the SHR group suggest that they may be regional perturbations of NO-specific vasodilator reserve, and would be consistent with an up-regulation of endogenous cerebral NOS activity. In support of our observations, L-arginine was found to have no effect upon LCBF in the contralateral hemisphere of SHR subjected to middle cerebral artery (MCA) occlusion (Prado *et al*., 1996), and SIN-1 had no significant effect upon cortical blood flow in SHR subjected to sham-occlusion of the MCA (Zhang *et al*., 1994). Contradictory results have also been obtained with *in situ* methodology to measure responsiveness of pial arteries, or the basilar artery, to superfusion of NO donors in the stroke-prone substrain of the SHR (Mayhan *et al*., 1988; Yang *et al*., 1991a,b; 1993; Kitazono *et al*., 1993) and also in stroke-resistant SHR (Mayhan *et al*., 1987; Mayhan, 1991). There are several potential explanations for the apparent differences between these results and the data presented in this paper, including the fact that the previous experiments were performed under the influence of anaesthetics, with their potential influence on cerebrovascular responsiveness (Edvinsson & McCulloch, 1981); the possibility that NO bioactivity might differ markedly between the stroke-prone substrain of SHR and the SHR used in our study (Dominiczak & Bohr, 1995); the fact that our animals were considerably younger than those used previously, and NO responsiveness has been shown to change – at least in renovascular hypertension – as the duration of hypertension progresses (Dubey *et al*., 1996); most importantly, neither the basilar artery nor the pial vessels constitute the principal source of resistance to flow in the cerebrovascular bed, and are therefore not responsible for the control of LCBF. Recent investigations in conscious patients with arterial hypertension have also confirmed impaired responsiveness to NO-donors (Preik *et al*., 1996), although once again other, earlier studies have reached contradictory conclusions (Creager & Roddy, 1994; Panza *et al*., 1995).

Cerebrovascular resistance (CVR), calculated from LCBF and MABP values measured from each individual rat, was found to decrease to a greater extent in WKY (median effect = −38%) treated with the lower dose of SIN-1 when compared to SHR (median effect = −26%). Since the reduction in MABP following this treatment was similar in both WKY and SHR, these differences in CVR directly reflect blood flow changes between the two groups. In the rats treated with a higher dose of SIN-1, CVR changes were broadly similar in both groups, but with the reduction in MABP in SHR

(−41%) being twice that found in WKY (−20%), this is in keeping with an attenuated blood flow response to SIN-1 in the SHR. However, the vessels of the cerebrovascular bed are normally endowed with the ability to alter their calibre in response to fluctuations in perfusion pressure. The resulting changes in vascular resistance ensure the maintenance of constant cerebral blood flow over a wide range of arterial blood pressure, a phenomenon known as autoregulation (Paulson *et al*., 1990). The lower limit of autoregulation below which the relationship between cerebral blood flow and perfusion pressure becomes linear is not a fixed point, and chronic hypertension is known to raise the arterial pressure threshold at the lower limit of the autoregulatory range (Strandgaard, 1978; Paulson *et al*., 1990). Although the levels of MABP measured in our SHR in response to the higher dose of SIN-1 (104 mmHg) were above the lower limit of autoregulation reported for this strain (90 mmHg) (Barry *et al*., 1982; Harper & Bohlen, 1984), the observation that LCBF was higher – though not significantly – in most of the areas examined in the SHR treated with the lower SIN-1 dose compared to those treated with the higher dose, could be interpreted as indicating pressure-dependency in flow to some extent at least. It is known that NO inhibition shifts the upper limit of cerebrovascular autoregulation to higher pressure levels in normal animals (Kelly *et al*., 1994), so it is conceivable that NO could have a role in determining the lower limit of autoregulation. A definitive conclusion regarding this possibility cannot be derived simply by calculating CVR.

SIN-1 generates NO and superoxide, which can potentially react with NO to form peroxynitrite (Moncada & Higgs, 1995; Plane *et al*., 1997). The dilator actions of SIN-1 in extracranial tissues are also modulated by the basal production of endothelium-derived NO (Plane *et al*., 1997). Studies performed *in vitro* with endothelial cells from peripheral tissues of 5 week old SHR showed that superoxide may be responsible for the decreased activity of NO (Grunfeld *et al*., 1995). There is also growing speculation of an enhanced oxidative stress in the pathogenesis of hypertensive complications (Alexander, 1995). It is clearly possible that the effects of SIN-1 upon LCBF could be related to generation of superoxide, complicated further by the potential involvement of free radicals in the evolution of hypertensive complications. The exact mechanism by which SIN-1 effects changes in cerebral blood flow may therefore be quite complex, but whatever the underlying mechanism there are differences in the cerebrovascular response to SIN-1 in WKY and SHR.

Morphological analysis of those cerebral blood vessels which are largely responsible for the control of LCBF found no structural differences between WKY and SHR (Lin *et al*., 1990). Whilst it is possible that subtle changes might go undetected in these rather small vessels, the method of analysis did prove sufficiently sensitive to detect differences between vessels from both WKY and SHR, when compared to those from SD rats (Lin *et al*., 1990). It has been argued that structural changes in the vessel wall associated with chronic hypertension could explain altered responses to vasoactive agents (constrictor and dilator) (Calver *et al*., 1992), but it might be expected that the response to all vasoactive agents would be affected in a non-specific manner (Harper & Bohlen, 1984; Folkow, 1990). It is possible that structural changes might have been present in our relatively young (14 weeks old) animals, particularly as structural changes appear even before frank hypertension has been established (Folkow, 1990), but structural differences cannot readily provide an explanation for the differential response to the two NOS inhibitors (L-NAME and 7-NI) and nor could it explain the attenuated vasodilator response to SIN-1. Thus our results would appear to support the concept of hypertension-induced functional changes (Winquist & Bohr, 1983) in the cerebrovasculature.

Cerebrovascular dysfunction associated with hypertension is most probably multifactorial. Studies with pial arteries from hypertensive rats have identified altered dilator responses which appear to involve vasoconstrictor prostanoids (Yang *et al*., 1991a; Mayhan, 1992), and *in situ* studies have shown that the prostaglandin-induced pial arterial vasodilatation is related to NO production (Armstead, 1995). Interestingly, enhanced responses of the basilar artery to activation of endothelin$_B$ (ET_B) receptors in hypertensive rats, independent of NO or prostanoid pathways, have also been observed (Kitazono *et al*., 1995). The same group also found that the mechanisms responsible for the impaired responses of the basilar artery in SHRs (Mayhan, 1990) are not the same as those responsible for the attenuated responses of the pial vessels.

This present study provides further evidence for heterogeneous perturbation of NO systems in the cerebrovasculature of the SHR strain, with a reduced vasodilator capacity and possibly an up-regulation of neuronally derived NO systems. The importance of these findings may become pronounced in situations of cerebral ischaemia, which is one of the commonest complications of hypertension.

These studies were funded with contributions from the James A.M. Kennedy Bequest to the University of Edinburgh and the Royal College of Surgeons of Edinburgh. I.P.F. is a British Brain and Spine Foundation Research Fellow. We gratefully acknowledge the gift of SIN-1 from Dr Henning, Cassella AG, Frankfurt.

References

ALEXANDER, R.W. (1995). Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. *Hypertension*, **25,** 155 – 161.

ARMSTEAD, W.M. (1995). Role of nitric oxide and cAMP in prostaglandin-induced pial arterial vasodilation. *Am. J. Physiol.*, **268,** H1436 – H1440.

BARRY, D.I., STRANDGAARD, S., GRAHAM, D.I., BRAENDSTRUP, O., SVENDSEN, U.G., VORSTRUP, S., HEMMINGSEN, R. & BOLWIG, T.G. (1982). Cerebral blood flow in rats with renal and spontaneous hypertension: resetting of the lower limit of autoregulation. *J. Cereb. Blood Flow Metab.*, **2,** 347 – 353.

CALVER, A., COLLIER, J., MONCADA, S. & VALLANCE, P. (1992). Effect of local intra-arterial N^G-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *J. Hypertension*, **10,** 1025 – 1031.

COCKROFT, J.R., CHOWIENCZYK, P.J., BENJAMIN, N. & RITTER, J.M. (1994). Preserved endothelium-dependent vasodilatation in patients with essential hypertension. *New Engl. J. Med.*, **330,** 1036 – 1040.

COYLE, P. (1984). Outcomes to middle cerebral artery occlusion in hypertensive and normotensive rats. *Hypertension*, **6** (suppl I), I-69 – I-74.

CREAGER, M.A. & RODDY, M.-A. (1994). Effect of captopril and enalapril on endothelial function in hypertensive patients. *Hypertension*, **24,** 499 – 505.

CUEVAS, P., GARCIA-CALVO, M., CARCELLER, F., REIMERS, D., ZAZO, M., CUEVAS, B., MUNOZ-WILLERY, I., MARTINEZ-COSO, V., LAMAS, S. & GIMENEZ-GALLEGO, G. (1996). Correction of hypertension by normalization of endothelial cells of fibroblast growth factor and nitric oxide synthase in spontaneously hypertensive rats. *Proc. Natl. Acad. Sci. U.S.A.*, **93,** 11996 – 12001.

DENG, L.-Y., LI, J.-S. & SCHIFFRIN, E.L. (1995). Endothelium-dependent relaxation of small arteries from essential hypertensive patients: mechanisms and comparison with normotensive subjects and with responses of vessels from spontaneously hypertensive rats. *Clin. Sci.*, **88,** 611 – 622.

DOHI, Y., THIEL, M.A., BÜHLER, F.R. & LÜSCHER, T.F. (1990). Activation of endothelial L-arginine pathway in resistance arteries: Effect of age and hypertension. *Hypertension*, **15,** 170–179.

DOMINICZAK, A.F. & BOHR, D.F. (1995). Nitric oxide and its putative role in hypertension. *Hypertension*, **25,** 1202–1211.

DUBEY, R.K., BOEGEHOLD, M.A., GILLESPIE, D.G. & ROSSELLI, M. (1996). Increased nitric oxide activity in early renovascular hypertension. *Am. J. Physiol.*, **270,** R118–R124.

EDVINSSON, L. & MCCULLOCH, J. (1981). Effects of pentobarbital on contractile responses of feline cerebral arteries. *J. Cereb. Blood Flow Metab.*, **1,** 437–440.

FOLKOW, B. (1990). ''Structural factor'' in primary and secondary hypertension. *Hypertension*, **16,** 89–101.

GRABOWSKI, M. & JOHANSSON, B.B. (1985). Nifedipine and nimodipine: effect on blood pressure and regional cerebral blood flow in conscious normotensive and hypertensive rats. *J. Cardiovasc. Pharmacol.*, **7,** 1127–1133.

GRUNFELD, S., HAMILTON, C.A., MESAROS, S., MCCLAIN, S.W., DOMINICZAK, A.F., BOHR, D.F. & MALINSKI, T. (1995). Role of superoxide in the depressed nitric oxide production by the endothelium of genetically hypertensive rats. *Hypertension*, **26,** 854–857.

HARPER, S.L. & BOHLEN, H.G. (1984). Microvascular adaptation in the cerebral cortex of adult spontaneously hypertensive rats. *Hypertension*, **6,** 408–419.

HUANG, Z., HUANG, P.L., PANAHIAN, N., DALKARA, T., FISHMAN, M.C. & MOSKOWITZ, M.A. (1994). Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. *Science*, **265,** 1883–1885.

IWAI, N., HANAI, K., TOOYAMA, I., KITAMURA, Y. & KINOSHITA, M. (1995). Regulation of neuronal nitric oxide synthase in rat adrenal medulla. *Hypertension*, **25,** 431–436.

IZUTA, M., CLAVIER, N., KIRSCH, J.R. & TRAYSTMAN, R.J. (1995). Cerebral blood flow during inhibition of brain nitric oxide synthase activity in normal, hypertensive, and stroke-prone rats. *Stroke*, **26,** 1079–1085.

KELLY, P.A.T., RITCHIE, I.M. & ARBUTHNOTT, G.W. (1995). Inhibition of neuronal nitric oxide synthase by 7-nitroindazole: effects upon local cerebral blood flow and glucose use in the rat. *J. Cereb. Blood Flow Metab.*, **15,** 766–773.

KELLY, P.A.T., THOMAS, C.L., RITCHIE, I.M. & ARBUTHNOTT, G.W. (1994). Cerebrovascular autoregulation in response to hypertension induced by N^G-nitro-L-arginine methyl ester. *Neuroscience*, **59,** 13–20.

KITAZONO, T., HEISTAD, D.D. & FARACI, F.M. (1995). ATP-sensitive potassium channels in the basilar artery during chronic hypertension. *Hypertension*, **22,** 677–681.

KITAZONO, T., HEISTAD, D.D. & FARACI, F.M. (1995). Enhanced responses of the basilar artery to activation of endothelin-B receptor in stroke-prone spontaneously hypertensive rats. *Hypertension*, **25,** 490–494.

KÜNG, C.F. & LÜSCHER, T.F. (1995). Different mechanisms of endothelial dysfunction with aging and hypertension in rat aorta. *Hypertension*, **25,** 194–200.

LIN, S.-Z., SPOSITO, N., PETTERSEN, S., RYBACKI, L., MCKENNA, E., PETTIGREW, K. & FENSTERMACHER, J. (1990). Cerebral capillary bed structure or normotensive and chronically hypertensive rats. *Microvasc. Res.*, **40,** 341–357.

LÜSCHER, T.F. (1992). Heterogeneity of endothelial dysfunction in hypertension. *Eur. Heart J.*, **13** [suppl D], 50–55.

LÜSCHER, T.F. & VANHOUTTE, P.M. (1986). Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension*, **8,** 344–348.

MACRAE, I.M., DAWSON, D.A., NORRIE, J.D. & MCCULLOCH, J. (1993). Inhibition of nitric oxide synthesis: Effects on cerebral blood flow and glucose utilisation in the rat. *J. Cereb. Blood Flow Metab.*, **13,** 985–992.

MALINSKI, T., KAPTURCZAK, M., DAYHARSH, J. & BOHR, D. (1993). Nitric oxide synthase activity in genetic hypertension. *Biochem. Biophys. Res. Commun.*, **194,** 654–658.

MAYHAN, W.G. (1990). Impairment of endothelium-dependent dilatation of basilar artery during chronic hypertension. *Am. J. Physiol.*, **259,** H1455–H1462.

MAYHAN, W.G. (1991). Responses of the basilar artery to products released by platelets during chronic hypertension. *Brain Res.*, **545,** 97–102.

MAYHAN, W.G. (1992). Role of prostaglandin H_2-thromboxane A_2 in responses of cerebral arterioles during chronic hypertension. *Am. J. Physiol.*, **262,** H539–H543.

MAYHAN, W.G., FARACI, F.M. & HEISTAD, D.D. (1987). Impairment of endothelium dependent responses of cerebral arterioles in chronic hypertension. *Am. J. Physiol.*, **253,** H1435–H1440.

MAYHAN, W.G., FARACI, F.M. & HEISTAD, D.D. (1988). Responses of cerebral arterioles to adenosine 5′-diphosphate, serotonin, and the thromboxane analogue U-46619 during chronic hypertension. *Hypertension*, **12,** 556–561.

MIYATA, N., TSUCHIDA, K., TANAKA, M. & OTOMO, S. (1990). Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in carotid arteries from stroke-prone spontaneously hypertensive rats. *J. Pharm. Pharmacol.*, **42,** 763–766.

MONCADA, S. & HIGGS, E.A. (1995). Molecular mechanisms and therapeutic strategies related to nitric oxide. *FASEB J.*, **9,** 1319–1330.

MOORE, P.K., BABBEDGE, R.C., WALLACE, P., GAFFEN, Z.A. & HART, S.L. (1993a). 7-Nitro indazole, an inhibitor of nitric oxide synthase, exhibits anti-nociceptive activity in the mouse without increasing blood pressure. *Br. J. Pharmacol.*, **108,** 296–297.

MOORE, P.K., WALLACE, P., GAFFEN, Z.A., HART, S.L. & BABBEDGE, R.C. (1993b). Characterization of the novel nitric oxide synthase inhibitor 7-nitro indazole and related indazoles: antinociceptive and cardiovascular effects. *Br. J. Pharmacol.*, **110,** 219–224.

NAVA, E., NOLL, G. & LÜSCHER, T.F. (1995). Nitric oxide in cardiovascular diseases. *Ann. Med.*, **27,** 343–351.

PANZA, J.A., GARCIA, C.E., KILCOYNE, C.M., QUYYUMI, A.A. & CANNON, R.O. (1995). Impaired endothelium-dependent vasodilatation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation*, **91,** 1732–1738.

PAULSON, O.B., STRANDGAARD, S. & EDVINSSON, L. (1990). Cerebral autoregulation. *Cerebrovasc. Brain Metab. Rev.*, **2,** 161–192.

PLANE, F., HURRELL, A., JEREMY, J.Y. & GARLAND, C.J. (1997). Evidence that potassium channels make a major contribution to SIN-1-evoked relaxation of rat isolated mesenteric artery. *Br. J. Pharmacol.*, **119,** 1557–1562.

PRADO, R., WATSON, B.D., ZHAO, W., YAO, H., BUSTO, R., DIETRICH, W.D. & GINSBERG, M.D. (1996). L-Arginine does not improve cortical perfusion or histopathological outcome in spontaneously hypertensive rats subjected to distal middle cerebral artery photothrombotic occlusion. *J. Cereb. Blood Flow Metab.*, **16,** 612–622.

PREIK, M., KELM, M., FEELISCH, M. & STRAUER, B.E. (1996). Impaired effectiveness of nitric oxide-donors in resistance arteries of patients with arterial hypertension. *J. Hypertens.*, **14,** 903–908.

SAKURADA, O., KENNEDY, C., JEHLE, J., BROWN, J.D., CARBIN, G.L. & SOKOLOFF, L. (1978). Measurement of cerebral blood flow with iodo-[^{14}C]-antipyrine. *Am. J. Physiol.*, **234,** H59–H66.

STRANDGAARD, S. (1978). Autoregulation of cerebral circulation in hypertension. *Acta Neurol. Scand.*, **57** (Suppl. 66), 1–82.

TADDEI, S., VIRDIS, A., MATTEI, P. & SALVETTI, A. (1993). Vasodilatation to acetylcholine in primary and secondary forms of human hypertension. *Hypertension*, **21,** 929–933.

TSCHUDI, M.R., CRISCIONE, L. & LÜSCHER, T.F. (1991). Effect of ageing and hypertension on endothelial function of rat coronary arteries. *J. Hypertens.*, **9** (Suppl 6), 164–165.

WANG, Q., PELLIGRINO, D.A., BAUGHAM, V.L., KOEING, H.M. & ALBRECHT, R.F. (1995). The role of neuronal nitric oxide synthase in regulation of cerebral blood flow in normocapnia and hypercapnia in rats. *J. Cereb. Blood Flow Metab.*, **15,** 774–778.

WEI, L., LIN, S.-Z., TAJIMA, A., NAKATA, H., ACUFF, V., PATLAK, C., PATTIGREW, K. & FENSTERMACHER, J. (1992). Cerebral glucose utilization and blood flow in adult spontaneously hypertensive rats. *Hypertension.*, **20,** 501–510.

WHISNANT, J.P. (1996). Effectiveness versus efficacy of treatment of hypertension for stroke prevention. *Neurology*, **46,** 301–307.

WINQUIST, R.J. & BOHR, D.F. (1983). Structural and functional changes in cerebral arteries from spontaneously hypertensive rats. *Hypertension*, **5,** 292–297.

YANG, S.-T. (1996). Role of nitric oxide in the maintenance of resting cerebral blood flow during chronic hypertension. *Life Sci.*, **58,** 1231–1238.

YANG, S.-T., FARACI, F.M. & HEISTAD, D.D. (1993). Effects of cilazapril on cerebral vasodilatation in hypertensive rats. *Hypertension*, **22,** 150–155.

YANG, S.-T., MAYHAN, W.G., FARACI, F.M. & HEISTAD, D.D. (1991a). Endothelium-dependent responses of cerebral blood vessels during chronic hypertension. *Hypertension*, **17,** 612–618.

YANG, S.-T., MAYHAN, W.G., FARACI, F.M. & HEISTAD, D.D. (1991b). Mechanisms of impaired endothelium-dependent cerebral vasodilatation in response to bradykinin in hypertensive rats. *Stroke*, **22,** 1177–1182.

ZHANG, F., WHITE, J.G. & IADECOLA, C. (1994). Nitric oxide donors increase blood flow and reduce brain damage in focal ischemia: evidence that nitric oxide is beneficial in the early stages of cerebral ischemia. *J. Cereb. Blood Flow Metab.*, **14,** 217–226.

(Received August 27, 1996
Revised January 22, 1997
Accepted January 27, 1997)