Br. J. Pharmacol. (1993), **109**, 1219–1225

Effects of N^G-nitro-L-arginine methyl ester on the cardiovascular system of the anaesthetized rabbit and on the cardiovascular response to thyrotropin-releasing hormone

[1]Eva E. Seligsohn & Anders Bill

Department of Physiology and Medical Biophysics, University of Uppsala, Biomedical Center, Box 572, S-751 23 Uppsala, Sweden

1 The effects of 300 mg kg^{-1} of the nitric oxide (NO) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on the regional blood flow, on the flow response to 1 mg kg^{-1} of thyrotropin-releasing hormone (TRH) and on cerebral blood flow autoregulation were studied in urethane anaesthetized rabbits subjected to unilateral sectioning of the cervical sympathetic chain. The blood flow measurements were performed by the tracer microspheres method.

2 The cerebral arteriovenous difference in oxygen saturation (CAVOD) was measured before and after the administration of L-NAME and TRH in order to ascertain whether the effects on cerebral blood flow that were observed were secondary to changes in cerebral metabolism.

3 L-NAME caused a significant decrease in blood flow in several cerebral regions; CBF_{tot} decreased to $72 \pm 4\%$ of control ($P < 0.001$). An increase in blood pressure and a concurrent decrease in heart rate and cardiac output were noted.

4 In the eye, L-NAME caused a reduction in uveal blood flow which was more pronounced on the sympathetically intact side; in the retina the blood flow decreased to 50% of control on both sides.

5 The administration of TRH in animals pretreated with L-NAME caused a significant increase in blood pressure and cerebral blood flow.

6 In L-NAME-treated animals the CBF was not affected when the mean arterial blood pressure was increased by ligation of the abdominal aorta.

7 The CAVOD increased from 56.0 ± 5.2 to $73.6 \pm 3.5\%$, 20 min after the administration of L-NAME. In animals given 1 mg kg^{-1} TRH after L-NAME the CAVOD decreased to $54.6 \pm 4.6\%$, 5 min after the injection of TRH.

8 The results of the present study indicate that endogenous NO is involved in the regulation of regional blood flow and blood pressure in the anaesthetized rabbit. The reduction in cerebral blood flow that was caused by L-NAME was not due to a reduction in cerebral metabolism. An interaction between the NO synthesis/release/effect and the sympathetic nervous system was found in the uvea. There was no evidence for a major involvement of NO in the cardiovascular responses to TRH and autoregulation of cerebral blood flow was not abolished by L-NAME.

Keywords: Blood flow; blood pressure; brain; eye; cardiovascular control; cerebral autoregulation; N^G-nitro-L-arginine methyl ester (L-NAME); nitric oxide; thyrotropin releasing hormone (TRH); rabbit

Introduction

The neuropeptide thyrotropin-releasing hormone (TRH) and its receptors have been found in various sites in the brain, spinal cord and in some peripheral organs (Hökfelt *et al.*, 1975; Leppäluoto *et al.*, 1978). TRH has been found to influence several important physiological functions including those of the autonomic nervous system (Feuerstein *et al.*, 1983; Koskinen, 1986a; Seligsohn & Koskinen, 1991). Complex effects on the cardiovascular system have been demonstrated (Feuerstein *et al.*, 1983; Sirén *et al.*, 1988). In the rabbit, TRH has been found to elicit cerebral vasodilatation by activation of an intrinsic cerebral vasodilating neural pathway (Koskinen, 1986a) which involves a yohimbine-sensitive mechanism (Seligsohn & Koskinen, 1991). In some peripheral organs TRH causes vasoconstriction and in others vasodilatation (Koskinen & Bill, 1984; Hugoson-Seligsohn & Koskinen, 1989; Seligsohn, 1992a). Recently, evidence has been found for the involvement of endogenous TRH in cardiovascular regulation during bleeding (Okuda *et al.*, 1988) and in the regulation of the blood pressure in the spontaneously hypertensive rat (Nurminen, 1992).

Various effects of TRH on several neurotransmitter systems have been proposed and its effects on neuronal activity have been described (Yarbrough, 1979; Horita *et al.*, 1986). The mechanisms by which TRH produces cerebral vasodilatation are not fully understood. The potent vasodilator substance, nitric oxide (NO), which is synthesized from L-arginine by vascular endothelial cells (Palmer *et al.*, 1988), has been found to influence cerebral blood flow in rats (Tanaka *et al.*, 1991) and induce vasodilatation of bovine cerebral arteries *in vitro* (González & Estrada, 1991). The administration of stable arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine methyl ester (L-NAME) has been shown to cause a dose-dependent inhibition of the formation of NO from L-arginine (Rees *et al.*, 1990).

The aim of the present study was to investigate the involvement of endogenous NO in the cardiovascular system of the anaesthetized rabbit by using a stable L-arginine analogue, N^G-nitro-L-arginine methyl ester (L-NAME), which inhibits NO synthesis (Rees *et al.*, 1990), as well as ascertain whether the cardiovascular effects of TRH are modified by pretreatment with L-NAME. It was also of interest to investigate whether the cerebral and ocular vascular effects of the drugs were influenced by cervical sympathotomy.

[1] Author for correspondence.

The results were reported in part at the Scandinavian Physiological Society Meeting, Copenhagen, Denmark (Seligsohn, 1992b).

Methods

New Zealand white rabbits of either sex, weighing between 1.9–4.2 kg, were used. The general procedure has previously been described (Seligsohn, 1992a). In brief, the animal was anaesthetized with urethane (7 ml mg^{-1}, i.v., of a 25% solution), tracheotomized and artificially ventilated. Both femoral arteries were cannulated and used for measurements of mean arterial blood pressure (MAP), heart rate (HR) and blood sampling. One vein was cannulated and used for drug delivery. The left ventricle was retrogradely cannulated for injections of 15 μm labelled spheres (NEN Chemicals, Boston, Mass., U.S.A.) and the tracer microspheres method was used for determining regional blood flows. Blood flow was calculated according to the free flow method (Alm & Bill, 1972).

Arterial blood gases (P_{O_2}, P_{CO_2}) and pH were determined regularly with an ABL 300 acid-base analyser (Radiometer, Copenhagen, Denmark). Any deviations in the acid-base balance were corrected by administering sodium bicarbonate and/or changing the ventilation rate. Heparin (Kabi Vitrum, Stockholm, Sweden) (500 iu kg^{-1}) was given intravenously (i.v.) to prevent clotting. Tubocurarine (Tubocuran, Nordisk Droge, Copenhagen, Denmark) (0.5–1 mg kg^{-1}) was administered i.v. to induce skeletal muscle relaxation. Body temperature was recorded by a rectal thermistor and maintained at about 38°C with a heating pad.

Unilateral sectioning of the cervical sympathetic chain was performed about 1 cm below the upper cervical ganglion. Blood flow data for the sectioned side were compared with data for the intact side. The following experiments were performed (Figure 1).

(I) In the first series of experiments ($n = 6$) a control blood flow measurement preceded the i.v. administration of 300 mg kg^{-1} of L-NAME, which was followed 12 min later by the second microsphere injection. The third microsphere injection was performed 20 min after the administration of L-NAME. In this study a high dose of L-NAME (300 mg kg^{-1}) was used to ensure a total inhibition of the NO synthesis and maximal effect on regional blood flow. This high dose of L-NAME was chosen after preliminary experiments where the administration of 30 mg kg^{-1} of L-NAME elicited an increase in MAP which was sustained for at least 20 min. However, in these experiments the decrease in CBF_{tot} measured after the administration of L-NAME was maintained only for a relatively short period of time (<20 min). It has recently been shown that in the rabbit, administration of L-NAME caused a dose-dependent reduction of microvascular diameters in the dose-interval 1–100 mg kg^{-1} while a maximal increase in MAP was already achieved at a dose of 30 mg kg^{-1} (Persson *et al.*, 1991).

(II) In the second series of experiments ($n = 5$) the first two blood flow determinations were made in the same manner as in the first series. Then TRH was given, in a dosage of 1 mg kg^{-1}, i.v., 15 min after the administration of L-NAME. A third blood flow measurement was performed 5 min after the TRH injection (20 min after L-NAME).

(III) In order to investigate if the cerebral autoregulatory response was affected by treatment with L-NAME, a third series of experiments was performed. In these experiments ($n = 5$) the procedure differed in some respects from that described above. Blood pressure recording was performed through an ear artery and a brachial artery was cannulated in order to collect blood samples. A brachial vein was cannulated for drug injections. The abdomen was opened and a loose ligature was placed around the abdominal part of the aorta. A blood flow measurement, which was regarded as the control, was performed 12 min after the injection of L-NAME. Then blood pressure was elevated by ligation of the aorta and blood flow was measured 4–5 min later. In all the experiments the animals were killed by intracardial administration of saturated KCl after the last microsphere injection. Organs and tissue samples were autopsied and placed in preweighed plastic tubes. The radioactivity of the samples was analysed by gammaspectrometry. Total cerebral blood flow (CBF_{tot}) was calculated to include all parts of the brain, except medulla oblongata and cerebellum. Cardiac output (CO) was calculated as $CO = Q_r \times CPM_i \times CPM_r^{-1}$, where Q_r = reference blood flow, CPM_i = injected radioactivity and CPM_r = reference radioactivity. CO was further indexed by unit of weight (CO_W) (g min^{-1} kg^{-1}). Peripheral vascular

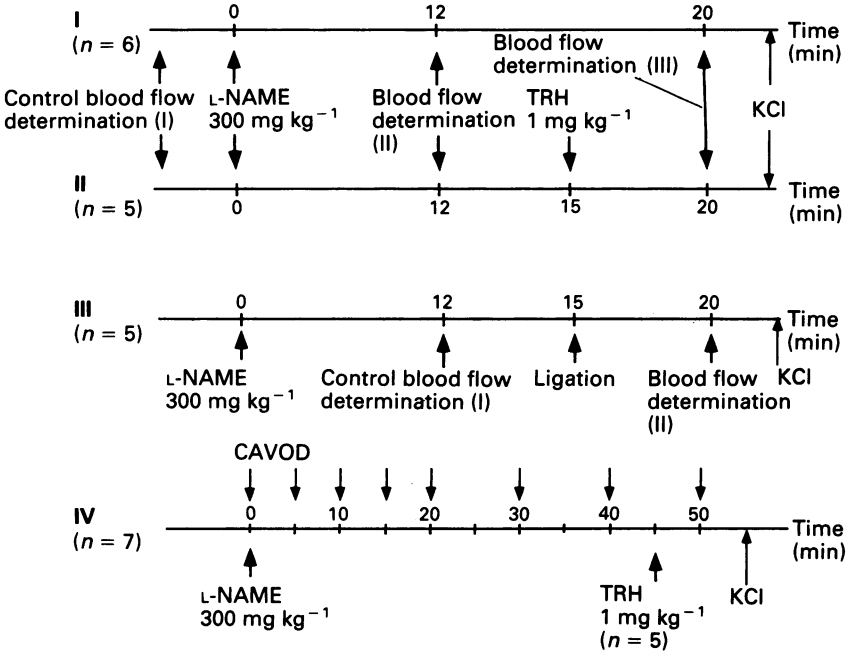

Figure 1 Experimental protocols in experiments with blood flow determination (I-III) and in experiments with measurements of the cerebral arteriovenous oxygen saturation difference (CAVOD) (IV).

resistance (VR) was calculated as $VR = MAP \times Q_t^{-1}$ where MAP is given in mmHg and Q_t = tissue blood flow in g min^{-1} 100 g^{-1} tissue and values expressed as % of control value.

(IV) In the fourth series of experiments ($n = 7$) the arteriovenous O_2 saturation difference in the brain (CAVOD) was measured in order to investigate whether the vasoconstriction observed after the L-NAME injection was due to a decrease in the O_2 demand of the brain. The animal was placed in a stereotaxic instrument (David Kopf Instrument, Tujunga, CA, U.S.A.). A hole was drilled in the midline of the skull between the coronal and lamboid suture. Indomethacin (Sigma Chemical Company, St. Louis, MO, U.S.A.) was administered i.v. in a dosage of 20 mg kg^{-1} in order to inhibit the cyclo-oxygenase activity and thus reduce the adhesion of platelets to the vessel wall. The superior sagittal sinus was cannulated under visual control with a thin polyethylene catheter (pp50). The tip of the cannula was inserted into the vein in the downstream direction. The arterial and venous O_2 saturations were determined with an ABL 300 acid-base analyser (Radiometer, Copenhagen, Denmark) in both a control situation and at different times after the administration of 300 mg kg^{-1} L-NAME (Figure 1). In 5 animals, 1 mg kg^{-1} TRH was given i.v. at the end of the experiment and the CAVOD was measured 5 min after the administration of the peptide.

The following drugs were used: TRH, lot no. 125F-59201 (Sigma Chemical Company, St. Louis, MO, U.S.A.) and N^G-nitro-L-arginine methyl ester (L-NAME) lot no. 3640692 (Sigma Chemical Company, St. Louis, MO, U.S.A.). Drugs were freshly dissolved in saline.

A statistical evaluation of the results was made with the two-tailed Student's t test for paired or unpaired observations or with an analysis of variance (ANOVA) with repeated measurements. Fischer's PLSD test was used as a post ANOVA test for comparison within treatments. The level of significance was set at $P < 0.05$ and was adjusted with Bonferroni's correction. The results are expressed as means ± s.e.

Results

There was no difference in CBF between the side with an intact sympathetic supply and the sympathectomized side (in both the control situation and after drug administration); therefore only the values for the side with an intact sympathetic supply are presented.

I. Effects of N^G-nitro-L-arginine methyl ester (L-NAME)

There were no major changes in arterial blood gases after the administration of L-NAME. The injection of L-NAME caused an increase in MAP from 84.0 ± 4.5 to 95.3 ± 7.5 mmHg (11.2 ± 0.6 to 12.7 ± 1.0 kPa) ($P < 0.05$) ($n = 11$). There was also a decrease in HR from 289 ± 7 to 277 ± 8 beats min^{-1} ($P < 0.05$) and in CO_W from 230 ± 22 to 122 ± 11 g min^{-1} kg^{-1} ($P < 0.001$). The administration of L-NAME caused an increase in total VR to 216 ± 12% of the control ($P < 0.001$). In several peripheral organs such as heart, gastric mucosa, duodenum, triceps muscle, kidney and adrenal gland, L-NAME caused an increase in VR ranging between 80–200% (data not shown). In the spleen a marked increase in VR of 627 ± 179% ($P < 0.01$) was noted after the administration of L-NAME. In all organs investigated the increases in VR were sustained 20 min after the L-NAME injection.

In the uvea, administration of L-NAME caused a reduction in blood flow. Basal values in the choroid were 1101 ± 257 mg min^{-1} on the intact side and 1256 ± 332 mg min^{-1} on the sympathectomized side. Corresponding values in the iris were 39 ± 10 mg min^{-1} and 69 ± 20 mg min^{-1} and in the ciliary body 99 ± 23 mg min^{-1} and 97 ± 19 mg min^{-1}, respectively. There were no significant differences in control situation between the intact and the sympathectomized side. The reduction in blood flow was greater on the side with an intact sympathetic supply and this difference in blood flow reduction was statistically significant, measured 12 min after the L-NAME injection, in both the choroid ($P < 0.01$) and the iris ($P < 0.05$). In the retina the blood flow decreased from 18 ± 2 to 8 ± 1 mg min^{-1} on the intact side and from 23 ± 3 to 10 ± 1 on the sympathectomized side (Figure 2).

The administration of L-NAME caused a decrease in blood flow ranging between 10–30% (Figure 3) in several cerebral regions investigated. Total cerebral blood flow (CBF_{tot}) decreased from 65 ± 4 to 48 ± 3 g min^{-1} 100 g^{-1} ($P < 0.05$). This decrease in CBF was maintained 20 min after the administration of L-NAME (Figure 3). No significant effects were found in gray matter, the hippocampal region and the caudate nucleus.

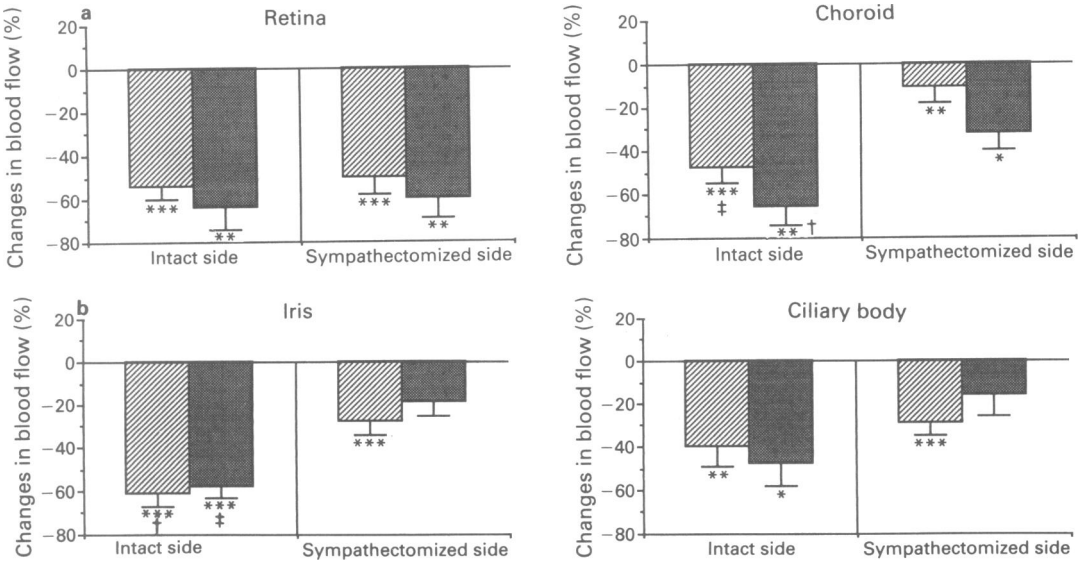

Figure 2 Ocular blood flow at 12 min (hatched columns, $n = 11$) and 20 min (stippled columns, $n = 6$) after the administration of 300 mg kg^{-1} N^G-nitro-L-arginine methyl ester on the intact side and on the sympathectomized side; (a) retina and choroid; (b) iris and ciliary body. Values are given as % of controls. $*P < 0.05$; $**P < 0.01$, $***P < 0.001$ as compared to controls. $\dagger P < 0.05$; $\ddagger P < 0.,01$ as compared with sympathectomized side (Student's t test, paired data).

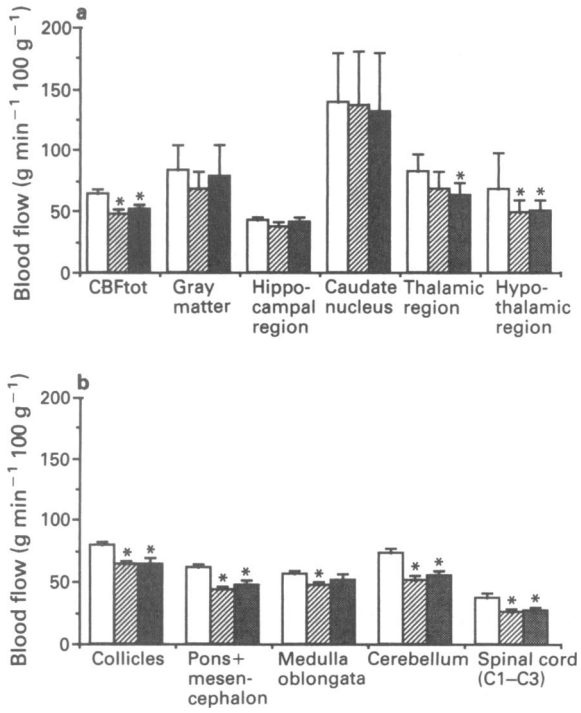

Figure 3 Total (CBF_{tot}) and regional cerebral blood flow ($n = 6$) in control situation (open columns), 12 min (hatched columns) and 20 min (stippled columns) after the administration of 300 mg kg^{-1} N^G-nitro-L-arginine methyl ester. Values are given in g min^{-1} 100 g^{-1} tissue. *$P<0.05$, as compared to controls (ANOVA).

II Effects of TRH in L-NAME pretreated animals

In this series of experiments the cardiovascular parameters measured 12 min after the administration of L-NAME are referred to as controls. The arterial blood gases and pH were not affected during the experiments. After the administration of TRH there was an increase in MAP from 87.8 ± 5.3 to 123.8 ± 11.3 mmHg ($P<0.05$). HR and CO_W were not significantly affected by TRH.

The administration of TRH caused an increase in cerebral blood flow in several regions investigated (Table 1). Blood flow in the hippocampal region increased to 208 ± 28% of the control and in the caudate nucleus the corresponding value was 209 ± 17%. CBF_{tot} increased to 189 ± 20% of the control ($P<0.05$) (Figure 4).

Table 1 Regional cerebral blood flows in animals pretreated with 300 mg kg^{-1} N^G-nitro-L-arginine methyl ester (L-NAME) before and after the administration of 1 mg kg^{-1} of thyrotropin-releasing hormone (TRH) ($n = 5$)

	12 min post-L-NAME	*L-NAME + TRH*
Gray matter	41 ± 7	90 ± 12**
Hippocampal region	38 ± 2	79 ± 12*
Caudate nucleus	74 ± 9	152 ± 19**
Thalamic region	47 ± 6	108 ± 14**
Hypothalamic region	48 ± 4	91 ± 11**
Collicles	60 ± 7	75 ± 5*
Pons + mesencephalon	43 ± 2	65 ± 3***
Medulla oblongata	44 ± 2	61 ± 5**
Cerebellum	54 ± 4	64 ± 7
Spinal cord (C1-C3)	23 ± 2	28 ± 2

Values are given in g min^{-1} 100 g^{-1} tissue.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ as compared with blood flow determination after L-NAME pretreatment (Student's t test, paired data).

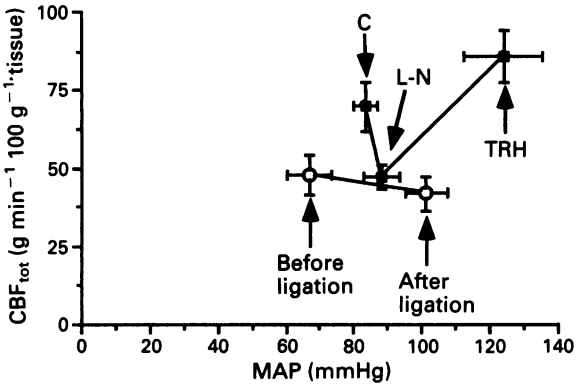

Figure 4 Total (CBF_{tot}) cerebral blood flow and mean arterial blood pressure (MAP) (mmHg) in (■) control situation (C), after the administration of 300 mg kg^{-1} N^G-nitro-L-arginine methyl ester (L-NAME, L-N) and after the administration of 1 mg kg^{-1} thyrotropin-releasing hormone (TRH) ($n = 6$), and (○) before and after aortic ligation ($n = 5$) in animals pretreated with 300 mg kg^{-1} L-NAME.

III Effects of increased mean arterial blood pressure in L-NAME pretreated animals

In these experiments the blood flow after the administration of L-NAME is referred to as the control. Control values for blood gases and arterial pH were in the same range as in the earlier experiments. After the ligation of the abdominal aorta there was a decrease in PCO_2 in arterial blood from 4.56 ± 0.21 to 3.72 ± 0.27 kPa ($P<0.01$). The blood pressure, measured through an ear artery, was increased from 66.8 ± 6.8 to 101.3 ± 6.0 mmHg ($P<0.01$) by ligation of the abdominal part of the aorta. In these experiments only cerebral tissues were analysed. Total (CBF_{tot}) (Figure 4) and regional cerebral blood flows were not affected in these experiments.

IV Cerebral arteriovenous oxygen saturation difference (CAVOD)

There were no significant changes in arterial pH or PCO_2 during the experiments. The CAVOD in the brain increased

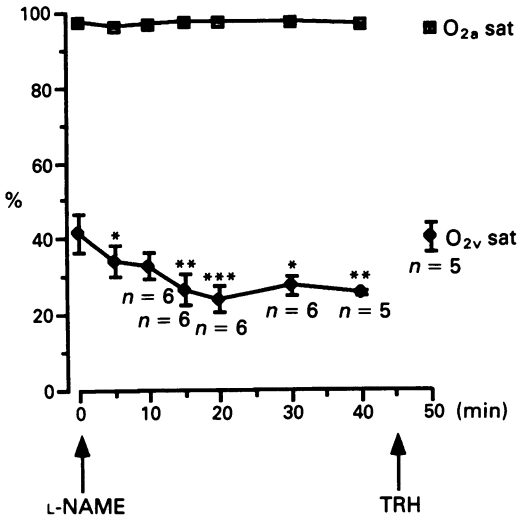

Figure 5 Arterial (O_{2a}) and venous (O_{2v}) oxygen saturation in the brain in control situation and after the administration of 300 mg kg^{-1} N^G-nitro-L-arginine methyl ester (L-NAME) ($n = 7$) and 5 min after the administration of 1 mg kg^{-1} thyrotropin-releasing hormone (TRH) ($n = 5$). *$P<0.05$; **$P<0.01$; ***$P<0.001$ (Student's t test).

from 56.0 ± 5.2% to 62.3 ± 4.0% ($P<0.05$) 5 min after the injection of L-NAME and then gradually increased to 73.6 ± 3.5 20 min after the L-NAME delivery (Figure 5). Measured 40 min after the administration of L-NAME, the CAVOD was 71.0 ± 0.7%. The injection made in 5 experiments of 1 mg kg^{-1} of TRH caused a decrease in the CAVOD from 71.3 ± 0.6 to 54.6 ± 4.6% ($P<0.05$).

Discussion

Effects of L-NAME and TRH

In the present study, the inhibition of the NO synthesis by administration of L-NAME caused a reduction in blood flow in many tissues including the eye as well as several cerebral regions. This indicates an involvement of endogenous NO in the regulation of regional blood flow in rabbits under urethane anaesthesia. There was also an increase in MAP after the administration of L-NAME, a decrease in CO and a concurrent decrease in heart rate.

Several mechanisms may be involved in the vascular effects of L-NAME. NO is released under basal physiological conditions and in response to receptor stimulation (Moncada *et al.*, 1991) and the L-arginine:NO pathway is among the mechanisms whereby cells regulate their own function or communicate with others (Moncada *et al.*, 1991). The importance of this L-arginine:NO pathway in the nervous system is not yet fully clarified. However, NO synthase is distributed in the CNS in various cell types, including brain neurones, retina and vascular endothelial cells (Bredt *et al.*, 1990) and it seems likely that this pathway is associated with other mediators in the central nervous system. In the cardiovascular system, basal release of NO seems to play an important role and acts as a general adaptive mechanism whereby the vascular endothelium responds to changes in its environment and regulates blood flow and blood pressure through an action on the vascular smooth muscle.

Lacolley *et al.* (1991) recently showed that the pressor response induced by L-arginine analogues was diminished after ganglionic blockade. This indicates an interaction between the sympathetic nervous system and the system using NO as a mediator. Interestingly, in some tissues in our study the effect on the sympathetically intact side was greater than on the sympathetically deprived side. Taken together, these results suggest that NO modulates vascular resistance both by a peripheral action and by an effect on the central regulation of sympathetic tone, which is in agreement with recent results by Sakuma *et al.* (1992). The reduction in blood flow in the brainstem caused by L-NAME is one factor that may activate the sympathetic nervous system.

TRH has an influence on the autonomic nervous system and interacts with both its sympathetic and parasympathetic parts (McCann *et al.*, 1989; Koskinen & Bill, 1984; Seligsohn, 1992a). There is evidence for the involvement of endogenous TRH in the regulation of blood pressure in spontaneously hypertensive rats (Nurminen, 1992). In the rabbit, TRH elicits both vasoconstriction in the gastric mucosa, pancreas and spleen and vasodilatation in the brain (Koskinen, 1986b; Seligsohn, 1992a). The vasoconstriction in the rabbit seems mainly to be due to an α_1-adrenoceptor mechanism (Seligsohn & Koskinen, 1991). However, the mediator of the vasodilatation is unknown.

Effects of L-NAME and TRH in the eye

In the eye the administration of L-NAME caused a reduction in blood flow of about 50–60%. In the rabbit the blood flow through the uvea is not autoregulated and is affected by changes in blood pressure as well as in intraocular pressure (Bill, 1974). Sympathetic stimulation reduces blood flow through all parts of the rabbit uvea, although retinal blood flow is unaffected (Bill, 1984). The results of the present study show that the reduction in blood flow in the uvea was more prominent on the side with an intact sympathetic supply. This could indicate an activation of sympathetic vasoconstrictor fibres as discussed above or could be due to an enhanced vasoconstrictor effect of noradrenaline. Another possibility is that there is a facilitation of the release of noradrenaline due to removal of NO-induced prejunctional inhibition (Lacolley *et al.*, 1991). Interestingly, in the retina, where there are no sympathetic nerves, there was no difference in response to L-NAME between the intact side and the sympathetically deprived side. The importance of NO formation in the ophthalmic artery has recently been shown in *in vitro* experiments (Yao *et al.*, 1991; Haefliger *et al.*, 1992). Our results support the concept that the ophthalmic circulation is in a state of vasodilatation as a result of NO production (Haefliger *et al.*, 1992; Granstam *et al.*, 1993). In this study TRH did not have any major effects on the ocular blood flow, which is in agreement with previously reported results (Seligsohn & Koskinen, 1991).

Effects of L-NAME and TRH in the brain

There is evidence for the involvement of NO or a NO-like substance in the transmission of information from vasodilator nerves to cerebral artery smooth muscle (Toda & Okamura, 1991). In addition, inhibition of NO synthesis with L-NMMA has been shown to cause a constriction of bovine cerebral arteries *in vitro* (Gonzáles & Estrada, 1991). *In vivo*, it was recently shown by Ko'zniewska *et al.* (1992) that inhibition of endogenous NO caused a decrease in cerebral blood flow in the rat. These results also suggest that there might be an involvement of NO in the cerebral autoregulatory response. However, no evidence was found in the experiments reported here for a major involvement of NO in the autoregulation of CBF; the results indicate rather that autoregulation is established at a lower level of CBF after L-NAME. This is in agreement with recent observations in the rat, made by Wang *et al.* (1992).

It seems clear that several peptide mechanisms are involved in the regulation of cerebral and spinal blood flow (Uddman & Edvinsson, 1989). TRH has been shown to cause a cerebral vasodilatation in various species (Koskinen, 1989; Hugoson-Seligsohn & Koskinen, 1989; Seligsohn, 1992c). The cerebrovasodilator effect of the peptide does not seem to be due to an increase in cerebral metabolism (Koskinen & Bill, 1984; Koskinen & Sperber, 1986; Hugoson-Seligsohn & Koskinen, 1989). TRH seems to elicit its effects by activation of an intracerebral vasodilator pathway (Koskinen, 1986a). The cerebrovasodilator effect of TRH was abolished by yohimbine pretreatment (Seligsohn & Koskinen, 1991), indicating that α_2-adrenoceptors are involved. Anatomical details of the pathway are unknown. However, it has recently been shown that stimulation of a medullary region elicits cerebral vasodilatation and that this effect was abolished in animals pretreated with the α_2-receptor antagonist, yohimbine (Saeki *et al.*, 1989). This pathway might be identical to that activated by TRH. The demonstration of NO synthase in the endothelial layers of various cerebral vessels and in nerve fibres surrounding the adventitia of brain vessels suggests that regulation of cerebral circulation might involve release of NO. However, the results presented here suggest that the vasodilator mechanisms involving TRH do not operate via release of NO.

After administration of TRH there was an increase in MAP in the animals pretreated with L-NAME. This made it difficult to interpret the increase in CBF observed. It might be due to increased MAP if autoregulation was affected by L-NAME or it might be a 'normal' response to TRH. The experiments with ligation of the aorta were performed to elucidate this problem and demonstrated intact autoregulation.

The arteriovenous difference in oxygen saturation (CAVOD) increased after L-NAME administration. This increase was

about 25%, which is similar to the decrease in CBF found at the same time. This result indicates that the vasoconstriction could not be explained, at least entirely, by a decrease in oxygen demand in the brain due to changes in cerebral metabolism. The administration of TRH in L-NAME-pretreated animals caused a marked increase in cerebral blood flow in the same range as has previously been reported and the arteriovenous difference in oxygen saturation decreased towards the control level (Koskinen & Bill, 1984; Seligson, 1992a). The increase in CBF is not likely to be due to the rise in MAP since, as discussed above, L-NAME seems to leave autoregulatory mechanisms intact.

Interestingly, TRH seemed to have a pronounced cerebrovasodilator effect in regions where the arginine:NO pathway appeared to play a relatively smaller role.

In conclusion, the results of the present study indicate that endogenous nitric oxide is involved in the regulation of regional blood flow and blood pressure in the rabbit. There seems to be an interaction between the NO-mediated mechanism and the sympathetic nervous system, at least in the control of uveal blood flow. No evidence was found for any major involvement of endogenous nitric oxide in the cardiovascular response to TRH. However, TRH restored CBF and CAVOD to normal levels in animals given L-NAME.

We wish to thank Ms Kristina Andersson for excellent technical assistance. This study was supported by Grant No. B90-14x-00147 from the Swedish Medical Research Council and Grant No. 5 RO1 Ey 00475 from the National Eye Institute, U.S. Public Health Service.

References

ALM, A. & BILL, A. (1972). The oxygen supply to the retina. II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. *Acta Physiol. Scand.*, **84**, 306–319.

BILL, A. (1974). Effects of acetazolamide and carotid occlusion on the ocular blood flow in unanesthetized rabbits. *Invest. Ophthamol.*, **13**, 954–958.

BILL, A. (1984). Circulation in the eye. In *Microcirculation*, Part 2, ed. Renkin, E.M. & Michel, C.C. pp. 1001–1034. *Handbook of Physiology, The Cardiovascular System*, vol. IV. U.S.A.: The American Physiological Society.

BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, **347**, 768–770.

FEUERSTEIN, G., HASSEN, A.H. & FADEN, A.I. (1983). TRH: cardiovascular and sympathetic modulation in brain nuclei of the rat. *Peptides*, **4**, 617–620.

GRANSTAM, E., WANG, L. & BILL, A. (1993). Vascular effects of endothelin-1 in the cat: modification by indomethacin and L-NAME. *Acta Physiol. Scand.* (in press).

GONZÁLEZ, C. & ESTRADA, C. (1991). Nitric oxide mediates the neurogenic vasodilation of bovine cerebral arteries. *J. Cereb. Blood Flow. Metab.*, **11**, 366–370.

HAEFLIGER, I.O., FLAMMER, J. & LÜSCHER, T.F. (1992). Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery. *Invest. Ophthamol. Vis. Sci.*, **33**, 2340–2343.

HÖKFELT, T., FUXE, K., JOHANSSON, O., JEFFCOATE, S. & WHITE, N. (1975). Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. *Eur. J. Pharmacol.*, **34**, 389–392.

HORITA, A., CARINO, M.A. & LAI, H. (1986). Pharmacology of thyrotropin-releasing hormone. *Annu. Rev. Pharmacol. Toxicol.*, **26**, 311–332.

HUGOSON-SELIGSOHN, E.E. & KOSKINEN, L.-O.D. (1989). TRH-Induced blood flow and mean arterial pressure changes in the rabbit are not dependent on the anaesthetic used. *Br. J. Pharmacol.*, **97**, 190–196.

KOSKINEN, L.-O.D. (1986a). Effects of TRH on cerebral and peripheral blood flow: role of submesencephalic brain steam centres. *Acta Physiol. Scand.*, **128**, 277–288.

KOSKINEN, L.-O.D. (1986b). Effects of low intravenous doses of TRH, acid-TRH and cyclo(His-Pro) on cerebral and peripheral blood flow. *Br. J. Pharmacol.*, **87**, 509–519.

KOSKINEN, L.-O.D. (1989). Cerebral and peripheral blood flow effects of TRH in the rat – a role of vagal nerves. *Peptides*, **10**, 933–938.

KOSKINEN, L.-O.D. & BILL, A. (1984). Thyrotropin-releasing hormone (TRH) causes sympathetic activation and cerebral vasodilation in the rabbit. *Acta Physiol. Scand.*, **122**, 127–136.

KOSKINEN, L.-O.D. & SPERBER, G. (1986). Regional glucose metabolism in the rabbit brain in control and TRH-treated animals. *Acta Physiol. Scand.*, **126**, 349–353.

KO'ZNIEWSKA, E., OSEKA, M. & STY'S, T. (1992). Effects of endothelium-derived nitric oxide on cerebral circulation during normoxia and hypoxia in the rat. *J. Cereb. Blood Flow Metab.*, **12**, 311–317.

LACOLLEY, P.J., LEWIS, S.J. & BRODY, M.J. (1991). Role of sympathetic nerve activity in the generation of vascular nitric oxide in urethane-anesthetized rats. *Hypertension*, **17**, 881–887.

LEPPÄLUOTO, J., KOIVUSALO, F. & KRAAMA, R. (1978). Thyrotropin-releasing factor: distribution in neural and gastrointestinal tissues. *Acta Physiol. Scand.*, **104**, 175–179.

MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.

McCANN, M.J., HERMANN, G.E. & ROGERS, R.C. (1989). Thyrotropin-releasing hormone: effects on identified neurons of the dorsal vagal complex. *J. Auton. Nerv. Syst.*, **26**, 107–112.

NURMINEN, M.-L. (1992). Intracerebroventricular immunization with TRH-antiserum lowers blood pressure in spontaneously hypertensive rats. *Acta Physiol. Scand.*, **144**, 75–81.

OKUDA, C., MIZOBE, T. & MIYAZAKI, M. (1988). Involvement of endogenous thyrotropin-releasing hormone in central regulation of the cardiovascular system after bleeding in conscious rats. *Brain Res.*, **474**, 399–402.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*, **333**, 664–666.

PERSSON, M.G., WIKLUND, N.P. & GUSTAFSSON, L.E. (1991). Nitric oxide requirement for vasomotor nerve-induced vasodilation and modulation of resting blood flow in muscle microcirculation. *Acta Physiol. Scand.*, **141**, 49–56.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S., (1990). Characterization of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

SAEKI, Y., SATO, A., SATO, Y. & TRZEBSKI, A. (1989). Stimulation of the rostral ventrolateral medullary neurons increases cortical cerebral blood flow via activation of the intracerebral neural pathway. *Neurosci. Lett.*, **107**, 26–32.

SAKUMA, I., TOGASHI, H., YOSHIOKA, M., SAITO, H., YANAGIDA, M., TAMURA, M., KOBAYASHI, T., YASUDA, H., GROSS, S.S. & LEVI, R. (1992). N^G-methyl-L-arginine, an inhibitor of L-arginine-derived nitric oxide synthesis, stimulates renal sympathetic nerve activity *in vivo*. A role for nitric oxide in the central regulation of sympathetic tone? *Circ. Res.*, **70**, 607–611.

SELIGSOHN, E.E. (1992a). Adrenergic and non-adrenergic cardiovascular effects of thyrotropin-releasing hormone (TRH) in the anaesthetized rabbit. *Acta Physiol. Scand.*, **146**, 107–117.

SELIGSOHN, E.E. (1992b). Effects of endogenous nitric oxide and thyrotropin-releasing hormone (TRH) on the cardiovascular system in the anaesthetized rabbit. *Acta Physiol. Scand.*, **146**, suppl. 608, 55.

SELIGSOHN, E.E. (1992c). Effects of thyrotropin-releasing hormone on the cardiovascular system in cats and monkeys. *Neuropeptides*, **22**, 61.

SELIGSOHN, E.E. & KOSKINEN, L.-O.D. (1991). Effects of alpha$_2$-adrenoceptor blockade and thryotropin-releasing hormone (TRH) on the cardiovascular system in the rabbit. *Acta Physiol. Scand.*, **143**, 187–194.

SIRÉN, A.-L., LAKE, C.R. & FEUERSTEIN, G. (1988). Hemodynamic and neural mechanisms of action of thyrotropin-releasing hormone in the rat. *Circ. Res.*, **62,** 139–154.

TODA, N. & OKAMURA, T. (1991). Role of nitric oxide in neurally induced cerebroarterial relaxation. *J. Pharmacol. Exp. Ther.*, **258,** 1027–1032.

TANAKA, K., GOTOH, F., GOMI, S., TAKASHIMA, S., MIHARA, B., SHIRAI, T., NOGAWA, S. & NAGATA, E. (1991). Inhibition of nitric oxide synthesis induces a significant reduction in local cerebral blood flow in the rat. *Neurosci. Lett.*, **127,** 129–132.

UDDMAN, R. & EDVINSSON, L. (1989). Neuropeptides in the cerebral circulation. *Cerebrovasc. Brain Metab. Rev.*, **1,** 230–252.

WANG, Q., PAULSON, O.B. & LASSEN, N.A. (1992). Is autoregulation of cerebral blood flow in rats influenced by nitro-L-arginine, a blocker of the synthesis of nitric oxide? *Acta Physiol. Scand.*, **145,** 297–298.

YAO, K., TSCHUDI, M., FLAMMER, J. & LÜSCHER, T. (1991). Endothelium-dependent regulation of vascular tone of the porcine ophthalmic artery. *Invest. Ophthamol. Vis. Sci.*, **32,** 1791–1798.

YARBROUGH, G.G. (1979). On the neuropharmacology of thyrotropin releasing hormone (TRH). *Prog. Neurobiol.*, **12,** 291–312.

(Received September 4, 1992
Revised March 3, 1993
Accepted April 6, 1993)