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The effects of γ_2-melanocyte-stimulating hormone and nimodipine on cortical blood flow and infarction volume in two rat models of middle cerebral artery occlusion

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Abstract

We observed that the pro-opiomelanocortin-derived neuropeptide, γ_2-melanocyte-stimulating hormone (γ_2-MSH), has various peripheral and central hemodynamic effects in the rat, including a marked enhancing effect on cerebral blood flow. This hemodynamic profile might be of interest in the pharmacotherapeutic approach to acute cerebral ischemia. Being an adrenocorticotropin (ACTH) analogue, γ_2-MSH might also possess direct neuronal protective properties. Therefore, in two rat models of focal cerebral ischemia we studied the effects of γ_2-MSH, with nimodipine, a Ca^{2+} channel antagonist, as a reference compound, on parasagittal laser-Doppler-assessed cortical blood flow and infarction volume. In isoflurane-anesthetized Wistar and F344 rats i.v. bolus infusions (four in total) of γ_2-MSH or nimodipine or their vehicle controls were given 1 h before, 1 min after, and 1 h and 2 h after occlusion of the middle cerebral artery. We used both an intravasal and an extravasal middle cerebral artery occlusion technique because pilot experiments had shown differences in the severity of ischemia with the two techniques. γ_2-MSH (100 nmol/kg in 1 min) increased cortical blood flow significantly but transiently, both pre- and post-ischemically, whereas nimodipine (20 μg/kg in 1 min) increased cortical blood flow only pre-ischemically in both models of middle cerebral artery occlusion. γ_2-MSH had no effect on cortical and striatal infarction volume, while nimodipine caused a significant reduction of cortical infarction volume in the extravasal middle cerebral artery occlusion model. To conclude, despite its hemodynamic and possible neuroprotective properties, γ_2-MSH did not prevent ischemic neuronal damage after middle cerebral artery occlusion in rats. This might be partly due to the short half-life of the peptide, leading to a transient increase in cortical blood flow and short neuronal exposure time, suggesting that prolonged infusion of the neuropeptide might be required. The results with nimodipine support the notion that it attenuates cortical ischemic damage, independently of effects on cerebral hemodynamics.

Keywords: Cerebral ischemia; Laser-Doppler flowmetry; Middle cerebral artery occlusion; γ_2-MSH (γ_2-melanocyte-stimulating hormone); Nimodipine; (Rat)

1. Introduction

Recently, we observed that the pro-opiomelanocortin-derived neuropeptide, γ_2-melanocyte-stimulating hormone (γ_2-MSH), has strong peripheral and central hemodynamic effects in the rat, including a marked enhancing effect on cerebral blood flow (De Wildt et al., 1993, 1994, 1995; Versteeg et al., 1993). This hemodynamic profile might be of interest in the pharmacotherapeutic approach to acute cerebral ischemia. Furthermore, being an adrenocorticotropin (ACTH) analogue, γ_2-MSH might also possess direct neuronal protective properties (Hamers et al., 1993; Duckers et al., 1993, 1994, 1996; Gispen et al., 1994; Hol et al., 1994; Vos et al., 1996a,b). The Ca^{2+} entry blocker, nimodipine, that is hemodynamically active and has neuroprotective properties, increases cerebral blood flow through cerebrovasodilatation under non-ischemic conditions in rats (Harper et al., 1981; Mohamed et al., 1985a,b). On the other hand, there are conflicting data concerning the effects of nimodipine on cerebral blood flow during ischemia. Some authors have reported that nimodipine in-

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creases cerebral blood flow during ischemia (Mohamed et al., 1985a,b; Meyer et al., 1986; Jacewicz et al., 1990a,b), while others did not find any elevation in cerebral blood flow (Gotoh et al., 1986; Hakim, 1986; Berger and Hakim, 1988, 1989; Dirnagl et al., 1990). There is also controversy about the neuroprotective properties of nimodipine during experimental cerebral ischemia: some authors observed neuroprotection by nimodipine (Mohamed et al., 1985a; Sauter and Rudin, 1986; Germano et al., 1987; Jacewicz et al., 1990a,b; Lazarewicz et al., 1990), while others did not find any evidence for neuroprotective effects of nimodipine (Gotoh et al., 1986; Vibulsresth et al., 1989; Berger and Hakim, 1989). It appears that different experimental procedures were used for the various experiments. The controversy concerning the protective effects of nimodipine can, therefore, most likely be explained by the fact that nimodipine is most effective when administered before ischemia. For this reason we decided to administer nimodipine both before and after the induction of ischemia, and to measure both the pre-ischemic and ischemic effects of nimodipine (and γ_2-MSH) on cortical blood flow by laser-Doppler flowmetry. Parasagittal laser-Doppler flowmetry, a measuring method which we recently developed (see Herz et al., submitted), allows measurement of the collateral blood flow rate into the middle cerebral artery territory after occlusion of the middle cerebral artery. We also used the method to show that the effects of middle cerebral artery occlusion on the severity of cortical blood flow reduction are significantly different in middle cerebral artery occlusion after craniotomy ('extravasal middle cerebral artery occlusion'), and middle cerebral artery occlusion produced with an intraluminal thread ('intravasal middle cerebral artery occlusion') (see Herz et al., submitted).

The first aim of the present experiments was to evaluate, both pre-ischemically as well as during ischemia, the effects of the hemodynamically active neuropeptide, γ_2-MSH, on collateral blood flow, with as reference compound nimodipine, which produces, under physiological conditions, an increase in cerebral blood flow. The second aim of the present experiments was to evaluate whether this difference in the severity of cortical blood flow reduction also caused different effects of γ_2-MSH on collateral blood flow measured after either of the two middle cerebral artery occlusion methods, again with nimodipine as a reference compound. The final aim of these experiments was to assess whether, under our conditions, γ_2-MSH and nimodipine can exert protective effects on cerebral infarction volume.

2. Materials and methods

2.1. Animals

The experiments were carried out with male Fischer 344 (inbred: Iffa-Credo Broekman, Someren, Netherlands) rats and male Wistar rats (outbred: U:WU (CPB), Utrecht, Netherlands) all being 4–5 months old (350–400 g). The rats had free access to standard laboratory chow and water both before and after surgical intervention. Anesthesia was induced with 3% isoflurane in a mixture of 70% N_2O and 30% O_2, while the rat was in a flow chamber. Following intubation the rats were mechanically ventilated (Harvard Rodent Ventilator, Model 683) with 1.5% isoflurane in 70% N_2O/ 30% O_2. Polyethylene cannulas were introduced in the left jugular vein (PE-50), to allow intravenous bolus drug administration and continuous substitution of saline (1 ml/h) to counteract loss of fluid through mechanical ventilation, and into the left femoral artery (PE-10) for assessment of blood pressure (Viggo-Spectramed DT-XX disposable transducer (Viggo-Spectramed, Bilthoven, Netherlands) and sampling of arterial blood for measurements of P_aCO_2 and P_aO_2, respectively. The P_aCO_2 and P_aO_2 were held in the normal range (P_aCO_2: 32–40 mm Hg; and P_aO_2: 95–150 mm Hg; Ciba-Corning 288 Blood Gas System). Body temperature was measured by means of a rectal probe, and kept at 37 ± 0.50°C (Harvard, Homeothermic Blanket Control Unit).

2.2. Middle cerebral artery occlusion

2.2.1. Extravasal occlusion

The middle cerebral artery was occluded by a modification of the technique of Tamura et al. (1981), as described in Herz et al. (1996). Briefly, with the animal placed in the lateral position in a stereotactic apparatus, a standardized craniotomy was made (exposing a long proximal middle cerebral artery segment) and the dura was opened. A 10-0 ethilon ligature was used to occlude the middle cerebral artery trunk as proximally as possible. Thereafter, with bipolar thermocoagulation, a long proximal segment of the middle cerebral artery was occluded starting at the place where the middle cerebral artery originates from the internal carotid artery and ending at the place where the middle cerebral artery crosses the inferior cerebral vein.

2.2.2. Intravasal occlusion

The middle cerebral artery was occluded using the intraluminal thread technique (Koizumi et al., 1986; Longa et al., 1989) as modified by Kawamura et al. (1991). Briefly, after ligation of the left external carotid artery and the left pterygopalatine artery, the left carotid artery was severed, a thread was introduced in its distal stump and advanced into the left internal carotid artery towards the cerebral circulation until a faint resistance was felt. At that moment, the tip of the thread occludes the lumen of the left anterior cerebral artery, so that it cannot be introduced further and the entrance of the left middle cerebral artery out of the internal carotid artery is also occluded by the thread. The occluding thread was a 3-0 Surgipro monofilament polypropylene thread (Auto Suture Nederland, Zeist, Netherlands), the tip (10 mm) of which had been dipped in

boiling xylol. This procedure not only weakens the tip, thus minimizing the risk of perforation of the intracranial part of the internal carotid artery, but also creates a small increase in the diameter of the tip so that the possibility that blood can leak along the occluding thread into the middle cerebral artery will diminish.

2.3. Laser-Doppler flowmetry

We used two PeriFlux PF3 (Perimed, Stockholm, Sweden) flowmeters, equipped with a 2 mW helium-neon laser with a wavelength of 632.8 nm. Blood flow oscillations were recorded using the 12 kHz low-pass filter setting and a 0.2 s time constant. Flow values are expressed in arbitrary units (perfusion units, PU). The needle probe (tip diameter of 0.45 mm; PF 302) was mounted on a micromanipulator. A rectangle was carefully drilled through the left parietal bone, with its corners on the coordinates (1P, 2L), (1P, 6L), (8P, 2L) and (8P, 6L) in relation to the bregma. The dura was left intact. The position of this rectangle was chosen to expose an area of the parietal cortex that was lateral to the place where the location of the anastomoses between branches of the middle and anterior cerebral artery could be expected (Coyle and Jokelainen, 1982, see also Fig. 1). The probe was positioned just above the dural surface, saline was applied to moisten the dura and fill the space between dura and probe. We developed the technique of parasagittal laser-Doppler flowmetry, in which two laser-Doppler flowmetry probes (tip diameter of 0.45 mm; PF 302) are placed on the cerebral cortex, to measure the dynamics of collateral blood flow through leptomeningeal anastomoses after middle cerebral artery occlusion (Herz et al., submitted). For this purpose probe number one was placed near the coordinates (3P, 3L) in relation to the bregma, that is near the leptomeningeal anastomoses, and probe number two near the coordinates (3P, 5L), that is 2 mm deeper in middle cerebral artery territory (and thus further away from the leptomeningeal anastomoses). For all laser-Doppler flowmetry recordings, positioning of the probes was such that the baseline flow in the probes had to be between 75–100 PUs. Values at these levels are known to represent microcirculation (Iadecola and Reis, 1990). Once a suitable placement of both probes was obtained, the probes were left in this position for the duration of the experiment.

2.4. Experimental procedure

Bolus infusions (four in total) of γ_2-MSH (100 nmol/kg in 1 min) or nimodipine (20 μg/kg in 1 min; or their vehicle controls (saline and polyethylene glycol 400) were administered into the left jugular vein 1 h before, 1 min after, and 1 h and 2 h after occlusion of the middle cerebral artery. The dosage of γ_2-MSH was chosen because it has a maximal increasing effect on cerebral blood

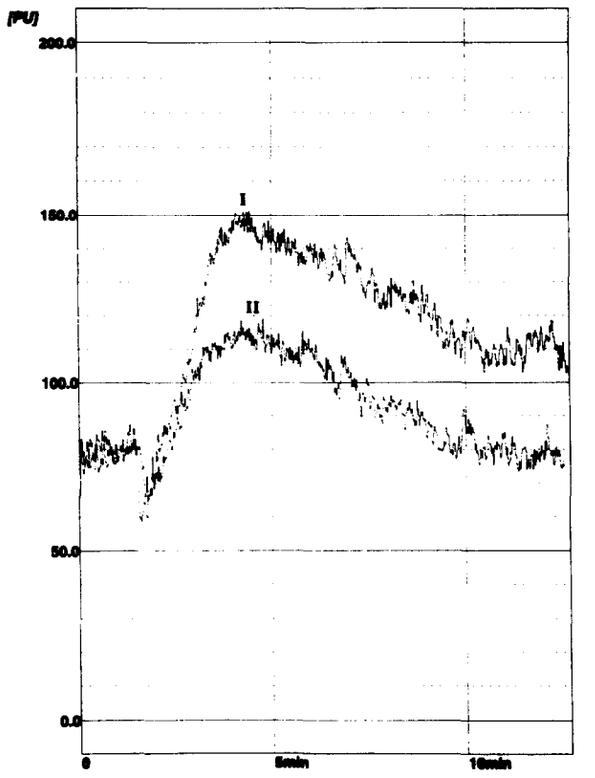

Fig. 1. Recordings of parasagittal laser-Doppler assessed cerebrocortical microcirculatory flow in a Wistar rat (in Perfusion Units (PU)) with two probes: probe 1 (I) near the coordinate 3 mm posterior and 3 mm lateral to bregma, probe 2 (II) near the coordinate 3 mm posterior and 5 mm lateral to bregma. Effects of intravenous administration of nimodipine (20 μg/kg in 1 min) before middle cerebral artery occlusion by means of an intraluminal thread (intravasal occlusion) are shown. Absolute flow increases, as measured in both probes as areas under the curve, were not significantly different between probes.

flow; it also produces a transient (lasting for less than 1 min) increase in blood pressure (De Wildt et al., 1995). The dosage of nimodipine was adopted from Germano et al. (1987), and, in pilot experiments, produced the highest cerebral blood flow increase together with the smallest and most transient (lasting for 1–2 min) blood pressure decrease. For the extravasal middle cerebral artery occlusion experiments F344 rats were used (γ_2-MSH and saline: $n = 6$, $n = 6$ respectively; nimodipine and polyethylene glycol: $n = 7$, $n = 7$ respectively). For the intravasal middle cerebral artery occlusion experiments we used, for logistic reasons, Wistar rats for the nimodipine/polyethylene glycol group ($n = 7$, $n = 8$ respectively). F344 rats were used for the γ_2-MSH/saline group ($n = 6$, $n = 7$ respectively) because pilot experiments had shown that the increases in cortical blood flow produced by γ_2-MSH in this strain were much more pronounced than those in Wistar rats. From the moment of administration of the first bolus infusion ($t = -1$ h) on, in all cases the laser-Doppler-assessed cerebral cortical microcirculatory flow and blood pressure were monitored for 4 h, using a computer-

Table 1
Physiological variables in nimodipine/polyethylene (PEG) experiments using extravasal middle cerebral artery occlusion

	Time after middle cerebral artery occlusion							
	−1 h		0 h		1 h		2 h	
	PEG	Nimodipine	PEG	Nimodipine	PEG	Nimodipine	PEG	Nimodipine
MABP (mm Hg)	98 ± 2	95 ± 3	95 ± 4	99 ± 3	97 ± 1	98 ± 3	95 ± 2	93 ± 3
Arterial pH (U)	7.43 ± 0.01	7.43 ± 0.01	7.41 ± 0.01	7.41 ± 0.01	7.41 ± 0.01	7.41 ± 0.01	7.40 ± 0.01	7.41 ± 0.01
P_aCO_2 (mm Hg)	37.8 ± 1.4	36.7 ± 0.8	37.0 ± 1.5	37.4 ± 1.3	39.0 ± 1.2	39.3 ± 1.0	36.9 ± 1.3	36.8 ± 1.1
P_aO_2 (mm Hg)	129.2 ± 3.5	121.2 ± 4.8	132.7 ± 3.9	122.0 ± 2.9	125.5 ± 3.0	117.6 ± 4.4	124.5 ± 2.2	118.0 ± 2.8

Values are means ± S.E.M. In all groups of F344 rats, the physiological variables were measured 1 h before ($t = -1$ h), $t = 0$ h, $t = 1$ h and $t = 2$ h after extravasal occlusion of the middle cerebral artery. No significant differences were found between values for parameters within and between experimental groups in time.

ized biosignal processing and analysis system (DiSys System, Mediware, Groningen, Netherlands). After the 4 h signal monitoring period the polyethylene cannulas were removed, and the wounds were closed. The rats were then returned to their cages, and after 48 h injected intraperitoneally with 90 mg/kg pentobarbital. Following thoracotomy, a blunt metal cannula was placed in the ascending aorta via the left ventricle, and secured. The right atrium was incised, the abdominal aorta was clamped, and the animal was perfused with 100–150 ml of saline and then with 200 ml of fixative (4% formalin in a 0.1 M phosphate buffer). The animals were decapitated, and their brains were placed in the same fixative for at least 1 week. The brain was frozen, and coronal sections, 25 μm thick, were cut throughout the rostrocaudal extent of the brain with a cryostat. Every 10th coronal section was selected, stained with a conventional hematoxylin/eosin staining method, and mounted on slides under coverslips. A computerized Image Processing System, IBAS (Kontron, Munich, Germany) was used to measure the area of infarction in all sections, both in absolute numbers and as a percentage of total slice area. The total volume of infarction was determined by integration of the infarction areas of the sections and the distance between them (250 μm).

2.5. Quantitative laser-Doppler flow analysis

For both probe signals the area under the curve (AUC) in both probes during the time of elevation of cortical blood flow after drug administration was calculated in PUs. From this value, we subtracted the AUC that was calculated by multiplying the baseline PU value (before administration of the drug) by the time that cortical blood flow elevation had lasted (see above). In this way only the absolute 'effect-AUC' of cortical blood flow increase could be determined. Finally we calculated the effect-AUCs of probe 2 as a fraction of that of probe 1 as an indication of the efficacy of the substance to raise collateral blood flow rate into the middle cerebral artery territory after middle cerebral artery occlusion (see Herz et al., submitted).

2.6. Statistics

Values are expressed as means ± S.E.M. Differences between data sets were evaluated statistically (SPSS for

Table 2
Effects on cortical blood flow produced by nimodipine, γ_2-MSH and their vehicle controls (polyethylene glycol (PEG) and saline) in rats with intravasal or extravasal middle cerebral artery occlusion

	Time after middle cerebral artery occlusion							
	−1 h		0 h		1 h		2 h	
	Probe 1	Probe 2	Probe 1	Probe 2	Probe 1	Probe 2	Probe 1	Probe 2
PEG (intra)	−21 ± 33	30 ± 15	−7 ± 18	8 ± 23	−8 ± 11	8 ± 13	18 ± 18	21 ± 15
Nimo (intra)	4977 ± 292 [a]	4311 ± 487 [a]	174 ± 148	142 ± 73	89 ± 56	53 ± 71	52 ± 46	91 ± 68
PEG (extra)	2 ± 21	39 ± 17	47 ± 14	54 ± 18	5 ± 17	−4 ± 14	28 ± 20	20 ± 14
Nimo (extra)	2822 ± 595 [a]	2627 ± 438 [a]	82 ± 102	55 ± 60	217 ± 132	149 ± 98	237 ± 140	296 ± 152
Saline (intra)	15 ± 8	1 ± 7	16 ± 14	21 ± 8	16 ± 9	21 ± 9	2 ± 10	5 ± 10
γ_2MSH (intra)	2087 ± 204 [a]	1815 ± 145 [a]	1027 ± 208 [a]	1076 ± 135 [a]	1044 ± 275 [a]	660 ± 158 [a]	1023 ± 99 [a]	636 ± 60 [a]
Saline (extra)	49 ± 22	98 ± 51	44 ± 38	58 ± 36	80 ± 37	6 ± 13	21 ± 13	50 ± 19
γ_2MSH (extra)	1325 ± 113 [a]	923 ± 146 [a]	1803 ± 546 [a]	668 ± 175 [a,b]	779 ± 134 [a]	447 ± 93 [a,b]	858 ± 163 [a]	549 ± 84 [a,b]

Values: means ± S.E.M. In both laser-Doppler probes (1 and 2) increases in cortical blood flow measured as areas under the curve in PUs at time of administration: 1 h before ($t = -1$ h), $t = 0$ h, $t = 1$ h and $t = 2$ h after extravasal (extra) (i.e. after craniotomy) or intravasal (intra) middle cerebral artery occlusion. [a] Denotes significant increases in cortical blood flow at $t = -1$ h for nimodipine (Nimo) after intra- and extravasal middle cerebral artery occlusion in both probes, and at all time points for γ_2-MSH (intra and extra) in both probes; [b] Denotes significant differences between probes 1 and 2 in CBF increase following γ_2-MSH after extravasal middle cerebral artery occlusion. PEG (intra): $n = 8$; Nimo (intra): $n = 7$; PEG (extra): $n = 7$; Nimo (extra): $n = 7$; Saline (intra): $n = 7$; γ_2MSH (intra): $n = 6$; Saline (extra): $n = 6$; γ_2MSH (extra): $n = 6$.

Windows) by analysis of variance (ANOVA). A $P < 0.05$ was considered to indicate a significant difference.

3. Results

3.1. Physiological variables

Table 1 lists the mean arterial blood pressure, P_aO_2, P_aCO_2 and arterial pH ($\pm$S.E.M.) measured just before the administration of the bolus infusions 1 h before ($t = -1$ h), 1 min after ($t = 0$ h), and 1 h ($t = 1$ h) and 2 h ($t = 2$ h) after middle cerebral artery occlusion in the nimodipine/polyethylene glycol-treated F344 group with extravasal middle cerebral artery occlusion (as an example). No significant differences in the values of these physiological parameters were observed, either between the different rat groups, or compared to the other experimental groups (nimodipine/polyethylene glycol extravasal middle cerebral artery occlusion and γ_2-MSH/saline intra- and extravasal middle cerebral artery occlusion; data not shown). Two rats from the intravasal middle cerebral artery occlusion group were excluded. The intracranial part of the internal carotid artery had been perforated when the occluding thread was advanced into the left internal carotid artery towards the cerebral circulation, which resulted in a subarachnoid hemorrhage.

3.2. Parasagittal laser-Doppler flowmetry recordings

Table 2 shows the effects on cortical blood flow of administration of nimodipine and γ_2-MSH (and their vehicles, polyethylene glycol and saline, respectively) before and after intra- or extravasal middle cerebral artery occlusion.

3.2.1. Intravasal middle cerebral artery occlusion

In the Wistar rats, intravenous administration of nimodipine (20 μg/kg in 1 min) before middle cerebral artery occlusion resulted in significant transient (approximately 6 min; see Fig. 1) increases of cortical blood flow of 4977 $\pm$ 715 PUs (as measured by probe 1 on coordinates 3P, 3L) and of 4311 $\pm$ 1194 PUs (as measured by probe 2 on coordinates 3P, 5L). The magnitude of these cortical blood flow increases was not significantly different in probe 1 and in probe 2. In contrast, administration of nimodipine after intravasal middle cerebral artery occlusion (at $t = 0$ h, $t = 1$ h and $t = 2$ h) did not produce a significant increase in cortical blood flow in any of the two laser-Doppler probes. The vehicle, polyethylene glycol, never increased cortical blood flow in the Wistar rats, whether before and or after middle cerebral artery occlusion.

In the F344 rats, intravenous administration of γ_2-MSH (100 nmol/kg in 1 min) before middle cerebral artery occlusion resulted in a significant transient (approximately 1 min; see Fig. 2) increase of cortical blood flow of 2087 $\pm$ 500 PUs (as measured by probe 1 on coordinates 3P, 3L) and of 1815 $\pm$ 354 PUs (as measured by probe 2 on coordinates 3P, 5L). Administration of γ_2-MSH at $t = 0$ h, $t = 1$ h and $t = 2$ h after intravasal middle cerebral artery occlusion evoked significant increases in cortical blood flow in both laser-Doppler probes: 1027 $\pm$ 510, 1044 $\pm$ 673 and 1023 $\pm$ 243 PUs (probe 1) and 1076 $\pm$ 330, 660 $\pm$ 386 and 636 $\pm$ 246 PUs (probe 2), respectively. The magnitude of these cortical blood flow increases was not significantly different in probes 1 and 2, either before or after middle cerebral artery occlusion. The vehicle, saline, never increased cortical blood flow in the F344 rats, whether before or after middle cerebral artery occlusion.

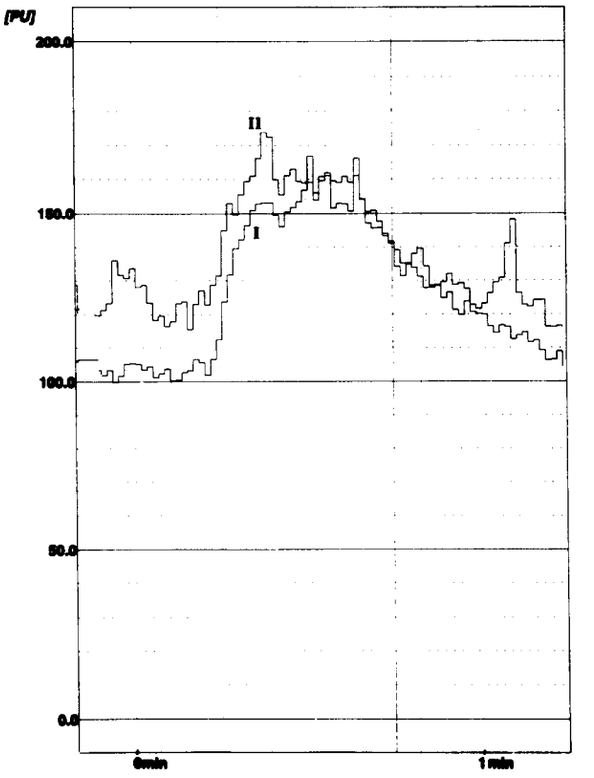

Fig. 2. Recordings of laser-Doppler assessed cerebrocortical microcirculatory flow in an F344 rat (in perfusion units (PU)) with two probes: probe 1 (I) near the coordinate 3 mm posterior and 3 mm lateral to bregma, probe 2 (II) near the coordinate 3 mm posterior and 5 mm lateral to bregma. Effects of intravenous administration of γ_2-MSH (100 nmol/kg in 1 min) before middle cerebral artery occlusion by means of an intraluminal thread (intravasal occlusion) are shown. Absolute flow increases, as measured in both probes as areas under the curve, were not significantly different between probes.

3.2.2. Extravasal middle cerebral artery occlusion

In the F344 rats, intravenous administration of nimodipine (20 μg/kg in 1 min) before middle cerebral artery occlusion resulted in a significant transient (approximately 6 min; see Fig. 3) increases of cortical blood flow of

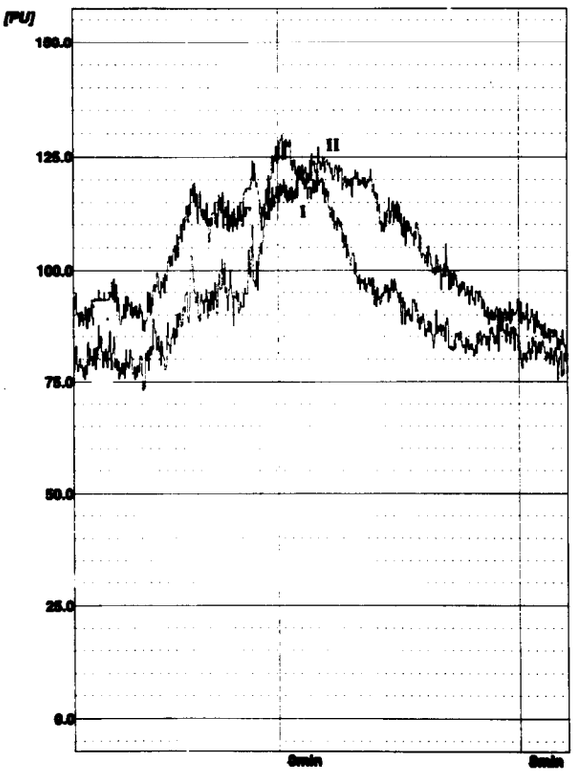

Fig. 3. Recordings of laser-Doppler assessed cerebrocortical microcirculatory flow in an F344 rat (in perfusion units (PU)) with two probes: probe 1 (I) near the coordinate 3 mm posterior and 3 mm lateral to bregma, probe 2 (II) near the coordinate 3 mm posterior and 5 mm lateral to bregma. Effects of intravenous administration of nimodipine (20 μg/kg in 1 min) before middle cerebral artery occlusion under visual control after craniectomy (extravasal occlusion) are shown. Absolute flow increases as measured in both probes as areas under the curve were not significantly different between probes.

2822 ± 595 PUs (as measured by probe 1 on coordinates 3P, 3L) and of 2627 ± 438 PUs (as measured by probe 2 on coordinates 3P, 5L). The magnitude of these cortical blood flow increases was not significantly different in probes 1 and 2. In contrast, administration of nimodipine after extravasal middle cerebral artery occlusion (at $t = 0$ h, $t = 1$ h and $t = 2$ h) did not produce a significant increase in cortical blood flow in any of the two laser-Doppler probes. The vehicle, polyethylene glycol, never increased cortical blood flow in F344 rats, whether before and or after middle cerebral artery occlusion.

In the F344 rats, intravenous administration of γ_2-MSH (100 nmol/kg in 1 min) before middle cerebral artery occlusion resulted in significant transient (approximately 1 min) increases of cortical blood flow of 1325 ± 113 PUs (as measured by probe 1 on coordinates 3P, 3L) and of 923 ± 146 PUs (as measured by probe 2 on coordinates 3P, 5L). Administration of γ_2-MSH at $t = 0$ h, $t = 1$ h and $t = 2$ h after extravasal middle cerebral artery occlusion evoked significant increases in cortical blood flow in both laser-Doppler probes: 1803 ± 546, 779 ± 134 and 858 ± 163 PUs (probe 1) and 668 ± 175, 447 ± 93 and 549 ± 84 PUs (probe 2) respectively. The magnitudes of these cortical blood flow increases were significantly different in probes 1 and 2 only after extravasal middle cerebral artery occlusion ($P = 0.022$; MANOVA paired measurements), with the highest increase in cortical blood flow consistently in probe 1 (see Fig. 4). The vehicle, saline, never increased cortical blood flow in F344 rats, either before or after middle cerebral artery occlusion.

3.3. Volume of cerebral infarction

Fig. 5 shows the volumes of cerebral infarction in the eight experimental groups. The extravasal middle cerebral artery occlusion experiments yielded no evidence for neuroprotectivity of γ_2-MSH. Nimodipine, however, produced a significant ($P = 0.002$; ANOVA) decrease in cortical infarction volume of 35%, but had no effect on the volume of striatal infarction. In the intravasal middle cerebral artery occlusion experiments the volumes of cortical in-

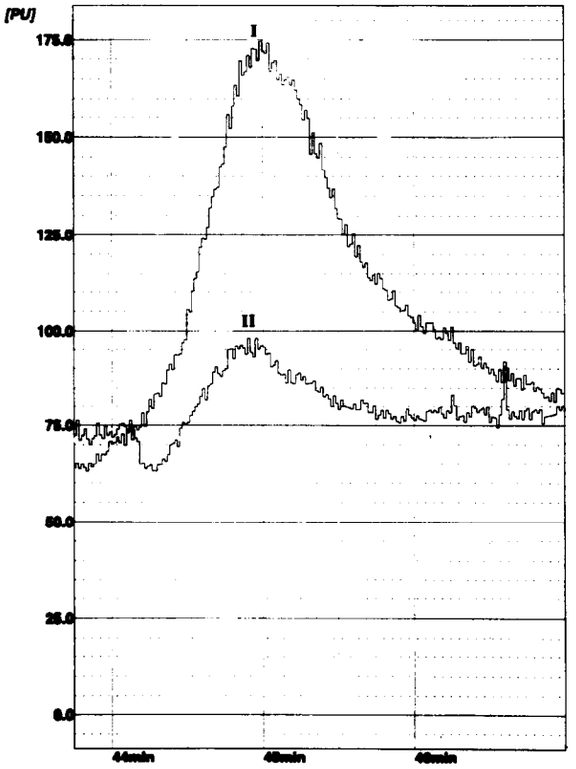

Fig. 4. Recordings of laser-Doppler assessed cerebrocortical microcirculatory flow in an F344 rat (in perfusion units (PU)) with two probes: probe 1 (I) near the coordinate 3 mm posterior and 3 mm lateral to bregma, probe 2 (II) near the coordinate 3 mm posterior and 5 mm lateral to bregma. Effects of intravenous administration of γ_2-MSH (100 nmol/kg in 1 min) after middle cerebral artery occlusion under visual control after craniectomy (extravasal occlusion) are shown. Absolute flow increases as measured in probe 1 were consistently and significantly ($P = 0.022$, MANOVA paired measurements) higher than flow increases measured in probe 2 (flow increases were measured as areas under the curve).

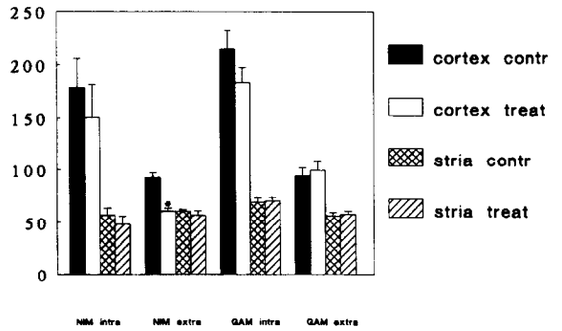

Fig. 5. Effects of nimodipine (NIM) and γ_2-MSH (GAM) on infarction volume after middle cerebral artery occlusion in mm^3 (y axis). Values: means ± S.E.M. Cortex contr, cortex treat: cortical infarction volume in control vehicles or drug treated animals respectively; Stria contr, stria treat: striatal infarction volume in control vehicles or drug treated animals respectively. Intra, extra: intravasal or extravasal middle cerebral artery occlusion respectively. * Denotes significant difference in cortical infarction volume after extravasal middle cerebral artery occlusion ($P < 0.05$, ANOVA) between nimodipine treated and vehicle controls.

farction were larger than in the extravasal middle cerebral artery occlusion experiments, while neither nimodipine nor γ_2-MSH produced a significant decrease in cortical or striatal infarction volume.

4. Discussion

4.1. Effects on cortical blood flow

Using parasagittal laser-Doppler flowmetry, we observed that nimodipine was only effective to produce an increase in cortical blood flow when it was administered prior to middle cerebral artery occlusion, irrespective of the technique used for middle cerebral artery occlusion (intra- or extravasal occlusion). A number of reports have shown that nimodipine does not produce an increase in cerebral blood flow after middle cerebral artery occlusion (Hakim, 1986; Gotoh et al., 1986; Berger and Hakim, 1988, 1989; Dirnagl et al., 1990), although others have observed similar increases evoked by nimodipine during ischemia (Mohamed et al., 1985a,b; Meyer et al., 1986; Jacewicz et al., 1990a,b). The absence of increases in cerebral blood flow following nimodipine (being a potent cerebral vasodilator) during ischemia can be explained by the already maximal vasodilatation in ischemic brain tissue (Waltz and Sundt, 1967; Garcia, 1984). Just after middle cerebral artery occlusion this vasodilatation is an active process, but, as ischemia proceeds, vasodilatation is more and more a result of vasomotor paralysis (Lassen and Christensen, 1976; Garcia, 1984).

It is of interest that, notwithstanding the fact that the effects were short-lasting (± 1 min), the neuropeptide γ_2-MSH was able to produce increases in cortical blood flow before as well as after middle cerebral artery occlusion. We observed that, after extravasal middle cerebral artery occlusion, the average increase in cortical blood flow, as measured in probe 1, that was near the anastomoses between branches of the anterior and middle cerebral artery, was significantly higher than the flow increase measured in probe 2. After intravasal middle cerebral artery occlusion, the increases in cortical blood flow as produced by γ_2-MSH were not significantly different in probes 1 and 2. The observation that greater increases in flow could be measured near the above mentioned anastomoses (probe 1) than deeper in middle cerebral artery territory (probe 2) indicates that it is possible to increase local cerebral blood flow pharmacotherapeutically through these anastomoses after middle cerebral artery occlusion. Our results with the intravasal middle cerebral artery occlusion technique show that the effects of γ_2-MSH after middle cerebral artery occlusion were higher on average, though not statistically significantly so, than those after extravasal middle cerebral artery occlusion. It was surprising that γ_2-MSH causes a comparable increase in cortical blood flow in both occlusion models, because we had expected, on the basis of a compromised collateral blood flow after intravasal middle cerebral artery occlusion (Herz et al., submitted) to observe in this model an increase in cortical blood flow elicited by γ_2-MSH smaller than that after extravasal middle cerebral artery occlusion. Because of the fact that, after intravasal middle cerebral artery occlusion, the flow increases following γ_2-MSH were not significantly different in probes 1 and 2, we conclude that leakage of blood along the occluding filament may occur when an intraluminal thread is used to occlude the middle cerebral artery (Herz et al., submitted) both into the middle cerebral artery and into the anterior cerebral artery. Our experiments with the intravasal middle cerebral artery occlusion technique also showed that leakage into the middle cerebral artery and anterior cerebral artery can be substantial when hemodynamically active substances (as for example γ_2-MSH) are administered. In this way, both the cortical blood flow reduction after intravasal middle cerebral artery occlusion (Herz et al., submitted) and the pharmacotherapeutically induced flow increases after intravasal middle cerebral artery occlusion are more evenly distributed over the cerebral cortex than after extravasal middle cerebral artery occlusion. After extravasal occlusion of the middle cerebral artery, the only substantial attribution to the local blood flow can enter the middle cerebral artery territory through the leptomeningeal anastomoses, producing a gradient in blood flow reduction with severity increasing as the middle cerebral artery territory is entered more deeply and the distance to the leptomeningeal anastomoses thereby increased.

Prior to intravasal middle cerebral artery occlusion, we observed significantly greater increases in cortical blood flow pre-ischemically for both nimodipine and γ_2-MSH than prior to extravasal middle cerebral artery occlusion. Two factors might contribute to this difference: the fact that the craniotomy had already been made and the dura

opened at the moment of pre-ischemic drug administration in the extravasal middle cerebral artery occlusion experiments, and the fact that we used Wistar rats for the intravasal middle cerebral artery occlusion experiments and F344 rats for all other experimental groups.

4.2. Effects on infarction volume

Using the experimental protocols described here we found no evidence for a neuroprotective effect of γ_2-MSH. Obviously, despite its favourable hemodynamic profile and its possible neuroprotective properties, γ_2-MSH did not prevent ischemic neuronal damage after either intra- or extravasal middle cerebral artery occlusion in rats. This might have been partly due to the short half-life of the peptide, leading on the one hand to only a transient increase in cortical blood flow, too short-lasted to exert a beneficial effect, and, on the other hand to a short neuronal exposure time, that may not have been long enough to exert direct neuroprotective properties. This suggests that sustained infusion of the neuropeptide might be a better approach to render γ_2-MSH possibly more effective in terms of neuroprotectivity. Concerning a future possible sustained increase in cerebral blood flow after middle cerebral artery occlusion elicited by γ_2-MSH, one should realize that such a therapy should be initiated in the first few minutes after vascular occlusion, before tissue necrosis occurs. Should this therapy be initiated later, it should be combined with measures to block complex post-ischemic biochemical perturbations (Grotta, 1987).

When the extravasal middle cerebral artery occlusion technique was used, nimodipine produced a significant (35%) decrease in cortical infarction volume, whereas it had no effect on the volume of striatal infarction. Because nimodipine, administered post-ischemically, did not produce any increases in cortical blood flow, these results support the findings that this drug attenuates cortical ischemic damage independently of effects on cerebral hemodynamics (Hakim, 1986; Berger and Hakim, 1988; Dirnagl et al., 1990). Although intravasal middle cerebral artery occlusion resulted in a larger volume of cortical infarction than did extravasal middle cerebral artery occlusion, nimodipine did not produce a significant decrease in cortical (or striatal) infarction volume after intravasal middle cerebral artery occlusion. The mean volume of cortical infarction in the nimodipine group was less (150.5 ± 31.5 mm^3) than that of the vehicle group (179.1 ± 26.6 mm^3), but the standard deviations of the volumes of cortical infarctions in these groups were too large to yield a significant difference. Obviously the extravasal middle cerebral artery occlusion technique produces a much smaller variability in infarction volume than does the intravasal middle cerebral artery occlusion technique, making it a more appropriate model for measuring small neuroprotective effects of drugs. Recently, Kiyota et al. (1993) and Zhao et al. (1994) have observed that the hyperthermia that is observed during occlusion of the rat middle cerebral artery with an intraluminal thread blunts or obliterates the effect of drugs which normally have a beneficial effect on brain damage due to focal ischemia. We cannot exclude this possibility, because we did not monitor body temperature in our experiments.

In conclusion, despite the fact that we found that the neuropeptide γ_2-MSH was able to transiently increase cortical blood flow pre- as well as post-ischemically, the peptide did not prevent the occurrence of neuronal damage after intra- or extravasal middle cerebral artery occlusion in rats. Further experiments are needed to test the possibility that sustained infusion of the neuropeptide might have a neuroprotective effect. Nimodipine, which we observed to increase cortical blood flow only prior to middle cerebral artery occlusion, significantly reduced cortical infarction volume after extravasal middle cerebral artery occlusion, while the effect of the compound on infarction volume after intravasal middle cerebral artery occlusion did not reach significance. These results are consistent with findings that nimodipine attenuates cortical ischemic damage independently of effects on cerebral hemodynamics. Parasagittal laser-Doppler flowmetry allowed us to show that significant differences exist between the effects of cortical blood flow increases elicited by γ_2-MSH after intravasal or extravasal middle cerebral artery occlusion, again pointing to differences in pathophysiology after the two occlusion models.

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