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Influence of centrally administered α- and γ_2-melanocyte-stimulating hormone on hypothalamic blood flow autoregulation in the rat

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The effect of intracerebroventricularly (i.c.v.) administered α-melanocyte-stimulating hormone (MSH) and γ_2-MSH on hypothalamic blood flow autoregulation was studied in anesthetized rats at different levels of standardized arterial hypotension. Autoregulation was impaired upon i.c.v. administration of 5 μg/kg γ_2-MSH while α-MSH caused no changes. Since this effect of γ_2-MSH was identical to that produced by i.c.v. naloxone in the same model, γ_2-MSH may be a functional antagonist of central opioid mechanisms participating in the control of cerebral blood flow autoregulation.

We recently reported that blockade of centrally located β-endorphinergic mechanisms by i.c.v. injected naloxone or specific β-endorphin antiserum resulted in the same cerebrovascular effect: autoregulation of the hypothalamic blood flow (HBF) was abolished, HBF passively followed the changes of arterial pressure during stepwise hypotension, produced by bleeding[7]. Previous studies indicated that opioids and melanocortins might modulate in an often opposite way a variety of body functions in the central nervous system as well as in the periphery[2,3,4,9–11]. In the present study the effect of centrally administered melanocortins, α-melanocyte stimulating hormone (α-MSH) and γ_2-MSH, was examined on the autoregulation of hypothalamic blood flow and experimental evidence was obtained that γ_2-MSH has a naloxone-like, opiate-antagonistic effect on HBF autoregulation.

Experiments were carried out in anesthetized, relaxed, artificially ventilated male Wistar rats (280–320 g, Wu/CPB-TNO Zeist, The Netherlands). The skull was fixed in a stereotaxic head-holder.

Local cerebral blood flow was measured with 70 μm diameter Pt electrodes in the left mediobasal hypothalamic area by using the H_2-gas clearance method[1]. H_2-gas was administered by inhalation and the wash-out curves were analyzed with the initial-slope technique. Arterial blood pressure was continuously monitored through the right femoral artery. Cerebral blood flow autoregulation was tested by lowering the systemic arterial pressure by consecutive stepwise bleeding from the left femoral artery to 80, 60 and 40 mm Hg and then all shed blood was retransfused. In order to study the effects of melanocortins on steady-state control of HBF this parameter was repeatedly determined at the beginning of the experiments until a steady-state condition was reached. Then, 15 min after administration of either α-MSH, γ_2-MSH or vehicle into the right lateral cerebral ventricle through an implanted polyethylene cannula, the flow measurements were repeated. Each animal received only one dose of one peptide. Following this procedure blood from the left femoral artery was withdrawn in the above-mentioned steps.

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HBF measurements were repeated at each of the 3 levels of arterial hypotension, as well as after retransfusion of the shed blood. Brains were removed for histological identification of the electrode sites and position of the ventricular cannula at the end of each experiment. Statistical evaluation of the data was performed by using variance analysis (ANOVA) as well as Student's two-tailed *t*-test with Bonferroni's modification.

α-MSH as well as γ_2-MSH (Organon, Oss, The Netherlands) were dissolved in 0.001 N HCl, and were administered in 2 and 5 μg/kg doses, in 10 μl/kg volume, i.c.v.; administration of the same volume of vehicle served as control. The rats were anesthetized with 1.5 g/kg urethane (Urethanum, OPG, Utrecht, The Netherlands) intraperitoneally. Muscle relaxation was performed with 200 μg/kg intravenously administered pancuronium bromide (Pavulon, Organon, Oss, The Netherlands).

Effect on steady-state control hypothalamic blood flow. Mean arterial pressure value before the onset of the autoregulatory test for all animals in the study was 104 ± 2 mm Hg. After retransfusion of the shed blood mean arterial pressures of the vehicle treated groups, the 2 μg/kg and 5 μg/kg α-MSH-treated groups, and the 2 μg/kg and 5 μg/kg γ_2-MSH-treated groups were, respectively, 124 ± 4, 114 ± 5, 116 ± 4, 123 ± 6, 124 ± 2 mm Hg. The peptide-treated groups were not significantly different from the control group.

Steady-state control hypothalamic flow values are shown in Table I. As can be seen, these values varied between 0.58 and 0.89 ml/g/min in the different experimental groups. This range of control flow values, however, corresponds well to our previous data obtained with the same method in anesthetized rats[7,8]. Other authors obtained an even broader range of regional CBF values in steady-state conditions in the same species[5]. In spite of the variations of control flow data in the different groups, no significant difference was present. Because of the variations in control flow data, HBF alterations during the autoregulatory test were expressed not only in absolute terms but also in percent of the prebleeding control value (Table I). Local HBF showed no significant alterations following either vehicle or peptide (α-MSH, γ_2-MSH) administration. HBF values before and after vehicle administration were 0.65 ± 0.04, 0.58 ± 0.05 ml/g/min; before and after 5 μg/kg α-MSH administration 0.93 ± 0.11, 0.89 ± 0.08 ml/g/min; before and after 5 μg/kg γ_2-MSH administration 0.93 ± 0.08, 0.89 ± 0.11 ml/g/min. Systemic arterial pressure showed no change following administration of the two peptides.

Effect on hypothalamic blood flow autoregulation. As shown in Table I HBF autoregulation functioned

TABLE I

Effect of stepwise hemorrhagic hypotension on hypothalamic blood flow following i.c.v. administration of α-MSH and γ_2-MSH in anesthetized rats

Values represent means ± S.E.M.; abs, absolute flow values in ml/g/min; %, percentual flow values compared to the steady state control value as 100%.

Treatment	*Abs or %*	*Control*	*After bleeding to*			*After retransfusion*
			80 mm Hg	*60 mm Hg*	*40 mm Hg*	
Vehicle						
10 μl/kg (n = 8)	abs	0.58 ± 0.05	0.57 ± 0.05	0.50 ± 0.06	0.39 ± 0.05	0.63 ± 0.08
	%	100 ± 9	98 ± 9	86 ± 10*	67 ± 9*	109 ± 14
α-MSH						
2 μg/kg (n = 6)	abs	0.81 ± 0.05	0.74 ± 0.06	0.65 ± 0.05	0.49 ± 0.04	0.76 ± 0.10
	%	100 ± 6	91 ± 7	80 ± 6*	60 ±5*	94 ± 12
5 μg/kg (n = 6)	abs	0.89 ± 0.08	0.84 ± 0.09	0.75 ± 0.08	0.57 ± 0.06	0.86 ± 0.06
	%	100 ± 9	94 ± 10	84 ± 9*	64 ± 7*	97 ± 7
γ_2-MSH						
2 μg/kg (n = 6)	abs	0.71 ± 0.07	0.68 ± 0.05	0.62 ± 0.06	0.52 ± 0.05	0.70 ± 0.07
	%	100 ± 9	96 ± 7	87 ± 8	73 ± 7*	99 ± 9
5 μg/kg (n = 6)	abs	0.89 ± 0.11	0.76 ± 0.07	0.69 ± 0.07	0.48 ± 0.07	0.72 ± 0.05
	%	100 ± 12	85 ± 8*	77 ± 8*	53 ± 8*	80 ± 6*

*$P < 0.05$.

normally in the vehicle-treated as well as in the α-MSH-treated animals. Steady-state control HBF showed no significant alterations in these groups until systemic arterial pressure was lowered below 80 mm Hg by hemorrhage. Following stepwise lowering of the pressure to 60 and 40 mm Hg (i.e. below the lower limit of HBF autoregulation) local blood flow was further reduced in the hypothalamic region. The same picture was observed after 2 μg/kg i.c.v. γ_2-MSH injection. In contrast, 5 μg/kg i.c.v. administered γ_2-MSH caused impairment of HBF autoregulation: hypothalamic flow decreased to 85% of the control value ($P < 0.05$) at 80 mm Hg arterial pressure. HBF followed passively the pressure changes throughout the autoregulatory test, and did not return to the control value following retransfusion of the shed blood.

The present results demonstrate that steady-state control HBF showed no change upon i.c.v. administration of either α-MSH or γ_2-MSH in anesthetized, ventilated rats. Autoregulation of HBF, on the other hand (i.e. the steadyness of the flow during stepwise lowering of the arterial pressure) was impaired following 5 μg/kg γ_2-MSH administration into the lateral cerebral ventricle since blood flow followed passively the arterial pressure changes after this treatment. This effect is not a consequence of increased bleeding volume (i.e. the amount of blood which has to be withdrawn in order to decrease the arterial pressure to 80, 60, 40 mm Hg). There was no statistical difference between the bleeding volumes of the vehicle-treated control group (0.48 ± 0.10, 0.92 ± 0.17, 1.50 ± 0.22 ml/100 g) and those of the γ_2-MSH-treated group (0.55 ± 0.11, 0.86 ± 0.08, 1.25 ± 0.07 ml/100 g) at any step of the standardized hemorrhagic hypotension.

These results suggest that using the same experimental procedure and protocol, centrally administered γ_2-MSH exerts a similar effect as naloxone or β-endorphin antiserum, and an opposite effect as β-endorphin on local cerebral blood flow autoregulation in the hypothalamus of the rat. One may conclude that γ_2-MSH (which is the hydroxylated sequence of the NH_2 terminal of the pro-opiocortin molecule, Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-OH) at least in some respects may operate as a functional antagonist of β-endorphin. This has been suggested before by Van Ree et al.[10] from a number of experiments in which the influences of γ_2-MSH were compared with that of naloxone. Oki et al.[6] showed binding affinity of γ-MSH and structurally related peptides using [^{3}H]naloxone as a ligand for rat brain opiate receptors. The effect of γ_2-MSH was different in our model from that of α-MSH (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-OH) which had no influence on HBF autoregulation.

The present results are in agreement with the hypothesis — which has been put forward by several authors — that melanocortins and opioid peptides constitute a co-ordinated and balanced system modulating different brain functions[11,12]. Substantial experimental evidence shows that adrenocorticotropic hormone–MSH peptides and opioids affect several functions in the central nervous system as well as in the periphery, like neuronal firing, adenylate cyclase activity, Ca^{2+} uptake into synaptosomes, pain sensitivity, body temperature, sexual behavior, memory, opiate tolerance, intestinal motility, posture and locomotion[2]. On the basis of our previous data as well as of the presented results there is a chance that such a modulatory system might operate also in the mechanisms involved in cerebral blood flow autoregulation.

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