Methionine enkephalin as a possible neuromodulator of regional cerebral blood flow

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Summary. In swine, cerebral blood flow was documented by a left ventricular injection of radiolabeled 15-micron spheres. Utilizing this procedure, the effect of the putative neurotransmitter methionine-enkephalin on regional cerebral blood flow was systemically evaluated. Our results revealed that a peripheral infusion of methionine enkephalin into miniature swine significantly increased cerebral blood flow in the basal ganglia, cerebellum, pons, inferior parietal cortex, superior parietal cortex and frontal cortex. Non-significant increases were observed in the hippocampus, occipital cortex and medulla oblongata while no effect on blood flow was observed in the pituitary gland. Significance of these results reside in the potential role of methionine enkephalin as a modulator of blood flow to the brain.

Key words. Methionine-enkephalin; cerebral blood-flow; putative-neurotransmitter; swine; brain regions; neuromodulator.

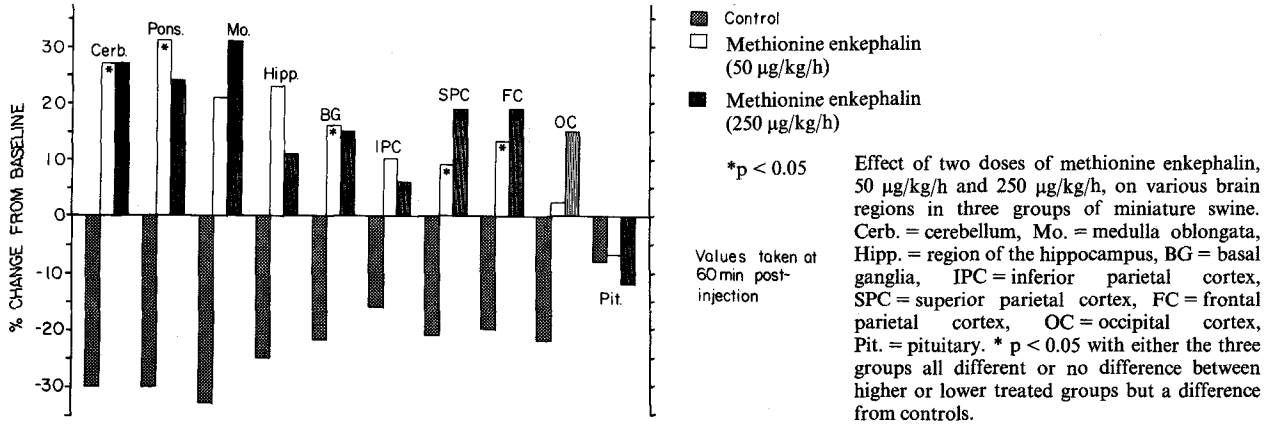
The peptidyl opiates, β-endorphin and enkephalins, being putative neurotransmitters, neuromodulators or hormones have been shown to modulate pain-sensation, mood and mental function, blood pressure, consumption of various psychoactive drugs and other important physiological and behavioral functions[1–4]. Methionine enkephalin (ME) is present in neurons in the brain[5] and its calcium-dependent release by depolarizing stimuli suggests that it may be a neurotransmitter centrally[6]. Our laboratory was interested in the neurogenic control of mental function. Since brain function is altered significantly during cerebral blood flow impairment as in the case of senile[7] or even alcohol dementia[8,9], we decided to investigate the possible role of peptidyl opiates, in particular ME in control of regional cerebral blood flow. This is the first report showing that ME increases blood flow in miniature swine.

Fifteen miniature swine on a 14:10 light-dark cycle (University of Texas Facilities, Bastrop, Texas, 10–12 kg) were anesthetized with chloralose, intubated and mechanically ventilated. Catheters were than advanced retrograde into the left cardiac ventricle via the right carotid artery (microsphere injection) and the distal aorta via the right femoral artery (reference withdrawals, pressure measurements). The right jugular vein was cannulated for drug infusion. After a 45-min period of stabilization, cerebral blood flow was determined as pretreatment and 30, 60 and 90 min by a bolus left ventricular random injection of either ^{125}I, ^{141}Ce, ^{85}Sr or ^{48}Sc radiolabeled microspheres[10]. Blood flow was calculated from the equation:

$$\text{Tissue blood flow} = \frac{\text{tissue counts} \times \text{withdrawal rate}}{\text{withdrawal counts}}$$

Three groups of five swine each were continuously infused with either 50 µg ME/h/h, 250 µg ME/h/kg or saline (control)[11]. The animals were sacrificed with potassium chloride and the skull removed using a rotary saw. The dura mater was removed and nerves were excised to facilitate removal of the brain. The pituitary was then removed with a small forcep. Using the left hemisphere only (a right carotid catheter was used which may have influenced blood flow in the right hemisphere), the following regions were removed in the following sequence: cerebellum, medulla oblongata, pons, basal ganglia, hippocampus, frontal cortex (1.5 cm section), occipital cortex (1.5 cm section), inferior parietal cortex and superior parietal cortex. The various regions were then weighed, minced and placed in vials for counting in a gamma counter. Resolution of isotopic spectra and determination of blood flow was performed by standard techniques[12]. All blood flow values were expressed as percent change from baseline within each group. The significance of differences among the three groups at each time period was determined by analysis of variance and Duncan's multirange test. (Duncan's multirange test provided a reasonable post hoc ANOVA test since the number of conditions was small.)

Our experiments revealed that ventricular infusion of 50 µg ME/kg/h and 250 µg ME/h/h significantly increased blood flow in five and six brain regions, respectively (fig.). At 50 µg ME/kg/h (60 min), blood flow was found to significantly increase when compared to controls by the following amounts: basal ganglia 40.75%, cerebellum 57.90%, pons 64.36%, superior parietal cortex 31.93% and frontal cortex 33.17%. However, at the higher infusion dose of 250 µg ME/kg/h (60 min), more regions were significantly effected: basal ganglia 40.19%, cerebellum 55.41%, pons 55.23%, inferior parietal cortex 25.25%, superior parietal cortex 40.67%, and frontal cortex 40.08%. Non-significant blood flow increases were seen in the hippocampus, occipital cortex and medulla oblongata. Generally, blood flow increases were time-dependent with a peak occurring at 60 min of infusion and are not dose-dependent but vary depending on region (fig.). Although not all statistically significant, each brain region tested (except the pituitary) did show blood flow increases at the higher infusion concentration.



Effect of two doses of methionine enkephalin, 50 µg/kg/h and 250 µg/kg/h, on various brain regions in three groups of miniature swine. Cerb. = cerebellum, Mo. = medulla oblongata, Hipp. = region of the hippocampus, BG = basal ganglia, IPC = inferior parietal cortex, SPC = superior parietal cortex, FC = frontal parietal cortex, OC = occipital cortex, Pit. = pituitary. * $p < 0.05$ with either the three groups all different or no difference between higher or lower treated groups but a difference from controls.

No changes were noted in cardiac output, heart rate, left ventricular stroke work or total peripheral resistance. A gradual decrease in mean systemic arterial pressure during the experiment was seen in all three groups. This is not uncommon in anesthetized swine and may explain the decreases in cerebral blood flow seen in the control group. There were no significant differences in mean arterial pressure among the three groups at any time period.

These results demonstrate that intravenous ME produces dose related increases in regional cerebral blood flow. Furthermore, these increases do not appear to correlate with changes in systemic hemodynamic parameters reported by others[13,14]. The present study does not address the question of whether the increases in blood flow seen are the result of a direct affect on cerebral vasculature or a physiologic response to a primary affect on neural tissue. It is of interest, however, that the areas most responsive to ME infusion also contain the greatest concentration of potential ME receptors.

Also unanswered is the question of whether this is a physiologic or pharmacologic response. If indeed, the enkephalinergic system plays a physiologic role in the regulation of intracerebral blood flow, failure of this system may be a component of diseases characterized by cerebral hypoperfusion such as senile dementia.

Additionally, although the question as to whether this effect in miniature swine is through endogenous enkephalinergic receptors is not resolved, future studies with opiate antagonists will help decide this point. This information, coupled with our results, may provide a rationale to stimulate research concerned with the potential therapeutic use of peptidyl opiates (analogs) as a treatment modality in conditions of reduced cerebral blood flow.

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1 Kastin, A.J., Olson, R.D., Schally, A.V. and Coy, D.H., *Life Sci. 25* (1979) 401.
2 Loh, H.H., Tseng, L.R., Wei, E., and Li, C.H., Proc. natn. Acad. Sci. USA *73* (1976) 2895.
3 Graf, L., Szekely, J.I., Ronai, A.Z., Dunai-Kovacs, Z., and Bajusz, S., Nature, Lond. *263* (1976) 240.
4 Bloom, F., Segal, D., Ling, N., and Guillemin, R., Science *194* (1976) 630.
5 Heymann, M.A., Payne, B.D., Hoffman, J.I.E., and Abraham, M.R., Prog. cardiovasc. Dis. *20* (1977) 1.
6 Henderson, G., Hughes, J., and Kosterlitz, H.W., Nature *271* (1978) 677.
7 Braun, A., Gustafson, L., and Inguar, D.H., Proc. of VII Int. Cong. of Neuropath., Budapest, Hungary. Excerpta med. (1975) 101–105.
8 Bergland, M., and Risberg, J., Acta neur. scand. *56*, Suppl. 64 (1977) 480.
9 Golden, C.J., Quaife, M., and Graber, B., Int. J. Neurosci. *17* (1982) 145.
10 Levine, B.A., Gaskill, H.V., and Sirinek, K.R., Surgery *90* (1981) 631.
11 Koturek, S.J., Tasler, J., Cieszkowski, M., Jaworek, J., Coy, D., and Schally, A., Gastroenterology *74* (1978) 851.
12 Heymann, M.A., Payne, B.D., Hoffman, J.I.E., and Abraham, M.R., Prog. cardiovasc. Dis. *20* (1977) 1.
13 Yukimura, T., Stock, G., Stumpf, H., Unger, Th., and Ganter, D., Hypertension *3* (1981) 528.
14 Willette, R.W., Krieger, A.J., and Sapru, N.N., J. cardiovasc. Pharm. *4* (1982) 1006.

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Use of three specific radioimmunoassays in measuring neurohypophysial hormone content and plasma concentrations of vasopressin, oxytocin and DDAVP in rats after prolonged infusion of DDAVP

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Summary. Specific radioimmunoassays (RIA) were employed for measuring plasma and neurohypophysial concentrations of oxytocin (OT) and vasopressin (AVP) after administration of 1-deamino-8-D-Arg-vasopressin (DDAVP). DDAVP concentrations were measured by a newly-developed specific RIA. Through the use of minipumps, DDAVP was infused i.p. over a period of 3 days in normally hydrated rats. Despite decreased urine production and increased urine osmolality no changes could be observed in neurohypophysial and plasma hormone concentrations.

Key words. Radioimmunoassay; oxytocin; vasopressin; neurohypophysis.

The use of the long-acting arginine vasopressin analogue 1-deamino-8-D-arginine vasopressin (DDAVP) in the treatment of central diabetes insipidus is widespread[1,2]. This hormone analogue also promotes the release of Factor VIII[3], making it useful for the treatment of mild hemophilia. Furthermore, DDAVP has been suggested for therapy of enuresis nocturna[4].

The purpose of this study was to investigate whether sustained antidiuresis obtained through long-term infusion of DDAVP would alter the neurohypophysial content of vasopressin (AVP) and oxytocin (OT) or plasma levels of these hormones in the rat.

Materials and methods. Peptide standards. DDAVP, AVP and OT were synthesized by Ferring, Malmö. The purity of these peptides was assessed by high performance liquid chromatography (HPLC) and was found to be greater than 95%.

Animal studies. Male Sprague-Dawley rats, weighing 180–220 g were placed under light Barbital® anesthesia. Osmotic minipumps (Alza®, model 2001) filled with a solution of DDAVP in bacteriostatic 0.9% NaCl were placed in the peritoneal cavity through a small upper abdominal incision. In preliminary experiments an infusion rate of 1 nmole/kg/day was found to give the desired plasma concentrations of DDAVP. The animals were placed in metabolic cages 3 days before surgery to acclimatize. Controls given 0.9% NaCl only, and experimental animals, were divided into groups of 10. The animal room was lighted from 06.00 to 18.00 h. 24-h urine collections were made for determination of urine volume (U_{vol}) and urine osmolality (U_{osm}). After 3 days the rats were decapitated and blood was collected in heparinized plastic tubes. After centrifugation at +4 °C for 15 min at 800 g_{av}, plasma was aspirated and stored at −70 °C until extraction. The dissected neurohypophyses were placed in an ice bath in 2 ml 0.1 M HCl, homogenized in a Tenbroeck glass homogenizer and centrifuged at 1600 g_{av} for 10 min. The supernatants were stored frozen at −70 °C until assayed.

Preparation of iodinated peptides. All peptides were iodinated with the iodogen method[5]. A polypropylene tube was coated with a thin layer of Iodogen (Pierce Co, USA). 5 nmoles peptide