In vivo release patterns and cardiovascular properties of inhibitory and excitatory amino acids in the hypothalamus*

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Summary. The posterior hypothalamus of conscious, freely moving rats was superfused with artificial cerebrospinal fluid through a push-pull cannula and the release of amino acids was determined in the superfusate. Under basal conditions, the release rates of taurine, GABA and glutamate fluctuated according to ultradian rhythms with different frequencies. Hypothalamic superfusion with veratridine or high concentrations of potassium chloride enhanced the release rates of taurine, GABA and glutamate in a concentration-dependent way. Tetrodotoxin decreased the basal release rates of the three amino acids. The release of arginine was not influenced significantly by these compounds. A fall of blood pressure elicited by intravenous infusion of nitroprusside decreased the release rates of GABA and taurine and enhanced the release of glutamate. Infusion of noradrenaline increased blood pressure and release rates of GABA and taurine, while the release of glutamate was not influenced. Neither the pressor, nor the depressor responses to drugs influenced the release of arginine in the hypothalamus. It is concluded that the inhibitory amino acids taurine and GABA released from hypothalamic neurons possess a tonic hypotensive function. The excitatory amino acid glutamate, released from glutamatergic neurons of the hypothalamus, seems to possess a hypertensive function in counteracting a fall of blood pressure.

Keywords: Amino acids – Taurine – GABA – Glutamate – Arginine – Blood pressure – Push-pull cannula

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**Introduction**

Cardiovascular homeostasis is centrally regulated by a complex network of mechanisms involving several brain areas and neurotransmitter systems. GABAergic neurons of the brain have also been implicated in central cardiovascular control. There is evidence that in the hypothalamus, GABA acts hypotensive, while in the nucleus of the solitary tract GABA released from GABAergic neurons possesses a hypertensive function (Klausmair and Philippu, 1989; review: Philippu, 1988). Little is known about the importance of other endogenous amino acids for blood pressure regulation. Although the central administration of some amino acids alters arterial blood pressure (Philippu, 1988), the role of endogenous amino acids in cardiovascular control is still obscure.

In an attempt to study the possible involvement of inhibitory and excitatory amino acids of the brain in cardiovascular control, we superfused the posterior hypothalamus of conscious, freely moving rats through a push-pull cannula with artificial cerebrospinal fluid (CSF) and determined the release of taurine, GABA and glutamate in the superfusate before, as well as during experimentally induced blood pressure changes. To investigate the origin of the amino acids found in the superfusate, effects of the neuroactive drugs tetrodotoxin (TTX), veratridine and potassium chloride on the release of the amino acids were studied. To prove the relevance of these results, the release of arginine, which is thought to possess no neurotransmitter function, was also determined.

**Materials and methods**

Male Sprague-Dawley rats (200–280 g) were stereotaxically implanted under ketamine (50 mg/kg, i.p.) and sodium pentobarbital (40 mg/kg, i.p.) anaesthesia with a guide cannula (o.d. 1.25 mm, i.d. 0.9 mm) for push-pull superfusion. The stereotaxic coordinates were (mm) A.P. -3.9, L 0.7, V -6.2 according to the atlas of Paxinos and Watson (1986). For measurement of arterial blood pressure and for infusion of drugs, respectively, the iliolumbar artery and jugular vein were permanently catheterized with PE 50 and PE 20 tubings. The catheters were filled with saline and heparin, tunneled under the skin and exteriorized on the neck. Two days after surgery, the stylet of the guide cannula was removed and a push-pull cannula (Philippu, 1984) (outer needle o.d. 0.7 mm, i.d. 0.5 mm; inner needle: o.d. 0.2 mm, i.d. 0.1 mm), which was 2 mm longer than the guide cannula, was inserted thus reaching the area of the posterior hypothalamus (V -8.2 mm). Superfusion was performed in the conscious, freely moving rat with artificial cerebrospinal fluid (CSF) pH 7.2 by means of two pumps: a CMC/100 (CMC, Stockholm, Sweden) microinjection pump and a Desaga (Heidelberg, FRG) peristaltic pump. The perfusion rate was 30 μl/min. CSF consisted of (mM): NaCl 140, KCl 3.0, CaCl₂ 2.5, MgCl₂ 1.0, Na₂HPO₄ 1.2, NaH₂PO₄ 0.3, glucose 3.0.

Veratridine or tetrodotoxin (TTX) (Sigma, München, FRG) were dissolved in CSF and were applied to the hypothalamus through the push-pull cannula for 10 min. When potassium-rich CSF was used, the concentration of NaCl was reduced appropriately, so as to maintain isoosmolarity. (−)-Noradrenaline and sodium nitroprusside were dissolved in physiologic saline and infused intravenously. In each animal, drugs were applied 3–4 times, the time interval between two adjacent superfusions with drugs or peripherally induced blood pressure changes being at least 70 min. Superfusate was continuously collected in 10 min time periods at −50 °C in a methanol bath. The samples were stored at −80 °C until the determination of amino acids was carried out. During the experimental trials, animals were deprived of food and water. At the end of the superfusion experiment, the brain was removed and the appropriate localization of the cannula was verified histologically.