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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 choride 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, $CaCl_2$ 2.5, $MgCl_2$ 1.0, Na_2HPO_4 1.2, NaH_2PO_4 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.

The amino acid concentration in the superfusate was analyzed using reversed phase HPLC and fluorimetric detection following automatic precolumn o-phthaldialdehyde (OPA) derivatization. The HPLC system consisted of a solvent gradient delivery pump (L-6200, Merck-Hitachi, Tokyo, Japan), an autosampler (AS-4000, Merck-Hitachi, Tokyo, Japan) and an analytical column (RP 18 Lichrosphere, 250 × 4 mm, 5 μm, Merck, Darmstadt, FRG) protected by a guard column (RP 18 Lichrosphere, 4 × 4 mm, 5 μm, Merck, Darmstadt, FRG). The mobile phase consisted of a sodium acetate buffer (50 mM) containing NaN_3 (1 mM) adjusted with 0.1 N HCl to pH 6.9 (eluent A). This solution was mixed in a stepwise gradient with eluent B (methanol/H_2O = 95/5) starting at 26% eluent B. The gradient was changed as follows: from 0–5 min 26–30% eluent B, from 5–31 min isocratic run, from 31–36 min 30%–100% eluent B, from 36–39 min 50% eluent B mixed with 50% acetonitrile. This was followed from 39–41 min by 50–100% eluent B and for another 4 min 100% eluent B. Over the next 5 min the gradient returned to its initial composition. For the derivatizing reagent 5 mg of OPA were dissolved in 0.5 ml of methanol and 5 ml of bicarbonate buffer (0.5 mM, pH 9.5). Six μl 2-mercaptoethanol were added. On the day of analysis, this solution was diluted 1 : 10 with bicarbonate buffer (0.5 mM, pH 9.5). Derivatization and injection were carried out automatically with a Merck-Hitachi autosampler by mixing 100 μl of superfusate with 50 μl of derivatizing reagent. After 90 s, 50 μl of 0.1 N HCl were added and 100 μl of the derivatized sample were injected. The fluorescence detector used (Merck-Hitachi F1050, Tokyo, Japan) had an excitatory filter of 330 nm and a emission filter of 450 nm. Evaluation of amino acids was carried out by comparison of peak areas of samples with external standard solutions using an integrator (Merck-Hitachi D 2500, Tokyo, Japan). The amino acids analyzed were glutamate, arginine, taurine and GABA.

The statistical significance was calculated by Friedman's test, followed by Wilcoxon's signed-rank test for paired data. As controls, the mean release rates of amino acids and the mean arterial blood pressure in the three samples preceding a blood pressure change or superfusion with a drug were used.

Results

Collection of superfusate started 60 min after commencement of superfusion (Fig. 1). Continuous collection of the superfusate in time periods of 10 min for 320 min revealed that the overall release rates of the four amino acids remained fairly constant. Nevertheless, during superfusion oscillations appeared in the release rates of the amino acids. In this time period, 5 periodical fluctuations appeared in the release of GABA, thus indicating the existence of ultradian oscillations with a frequency of approximately 1 cycle per 65 min. The release rate of taurine also showed phasic decreases at 150 min and 300 min but these fluctuations were less pronounced than those of GABA. In the time period of 320 min, about 8 peaks of increased glutamate release rate appeared, thus pointing to the existence of an ultradian rhythm with a frequency of about 1 cycle per 45 min. Slight variations in the release rate of arginine also appeared but their amplitude was lower than those of the other amino acids.

Hypothalamic superfusion with the neurotoxin TTX (1 μM) for 20 min led to a pronounced decrease in the release rates of taurine, GABA and glutamate (Fig. 2). The release rates of taurine and GABA continued to be low even after termination of superfusion with TTX, while the release rate of glutamate was normalized rapidly. Superfusion with TTX tended to decrease slightly and transiently the outflow of arginine, but this effect was not statistically significant ($p = 0.07$).

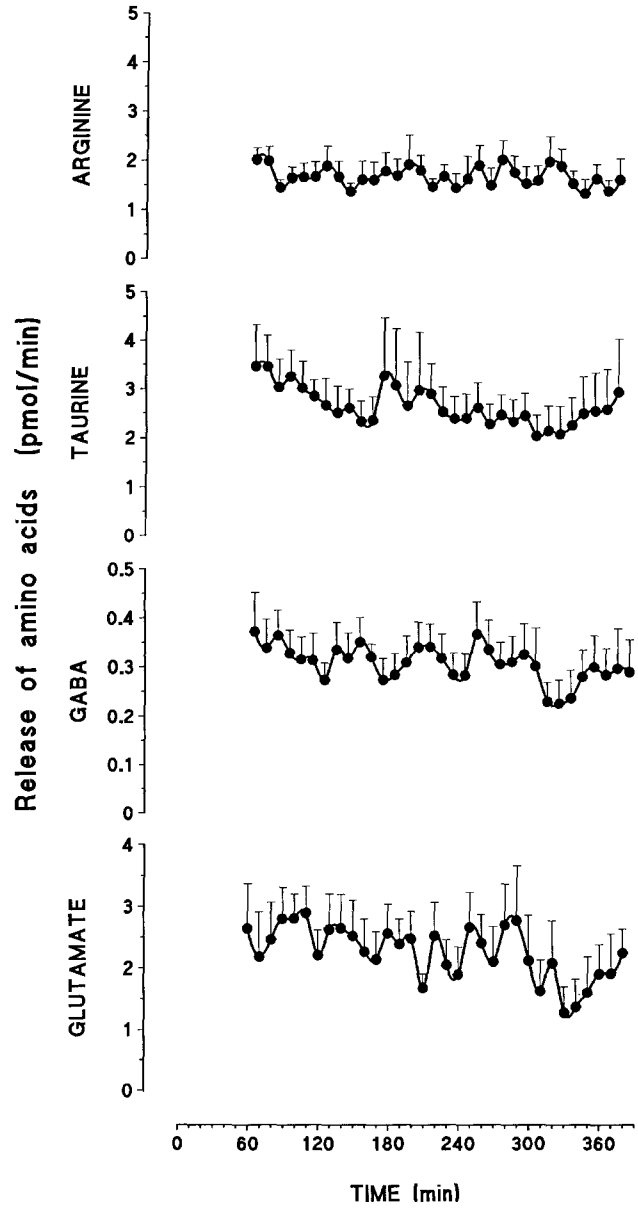

Fig. 1. Spontaneous release of arginine, taurine, GABA and glutamate in the posterior hypothalamus of the conscious, freely moving rat. Ordinate: release of amino acids as pmol/min. Abscissa: time in min. Mean values ± S.E.M.

Superfusion with two different concentrations of potassium chloride (50 or 90 mM) led to a very pronounced and concentration-dependent release of GABA (Fig. 3). The release of glutamate was also enhanced by potassium chloride, although to a lesser extent than that of GABA. Similarly, the release rate of taurine was enhanced by potassium chloride in a concentration-dependent way, while the release of arginine was not influenced. The efficacy of potassium chloride to enhance the release of amino acids was GABA > glutamate > taurine.

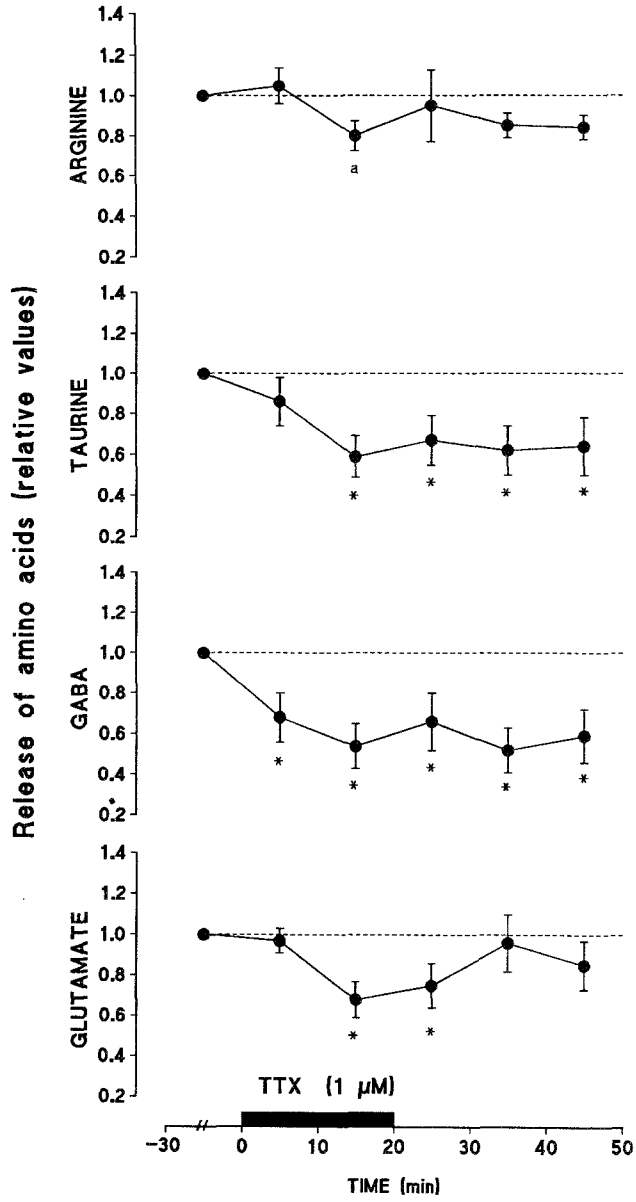

Fig. 2. Effects of superfusion with TTX on the release of amino acids. Ordinates: release as relative values. The release rates in the three samples preceding superfusion with TTX were taken as 1.0. Abscissa: time in min. Horizontal bar indicates beginning and duration of superfusion with TTX. Mean release rates preceding TTX were (pmol/min): arginine 2.7 ± 0.9 (n = 6), taurine 5.6 ± 1.1 (n = 8), GABA 0.50 ± 0.12 (n = 7), glutamate 3.3 ± 1.1 (n = 7). Mean values ± S.E.M. $*p < 0.05$, $^{a}p = 0.07$ (Wilcoxon's signed rank test for paired data)

Superfusion of the hypothalamus with veratridine (1 or 10 μM) also elicited a concentration-dependent release of taurine, GABA and glutamate. Again, the most pronounced effect concerned the release of GABA, while the release rates of taurine and glutamate were enhanced to a lesser degree than the release of GABA (Fig. 4).

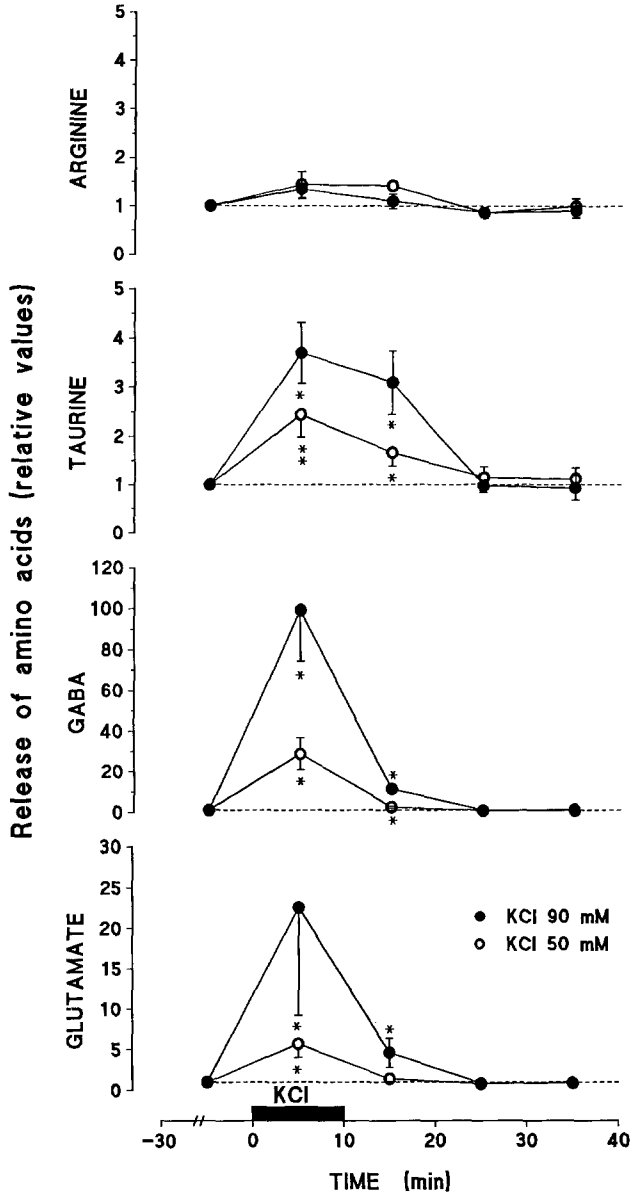

Fig. 3. Effects of superfusion with potassium-rich CSF on the release of amino acids. Ordinates: release of amino acids as relative values. The mean release rate in the three samples preceding superfusion with potassium-rich CSF was taken as 1.0. Abscissa: time in min. Horizontal bar indicates beginning and duration of superfusion with potassium-rich CSF. Release rates of amino acids preceding potassium-rich CSF were (pmol/min): arginine 2.4 ± 0.8 (n = 7), taurine 5.7 ± 1.2 (n = 9), GABA 0.38 ± 0.04 (n = 8), glutamate 4.2 ± 1.4 (n = 7) for 50 mM potassium chloride and arginine 2.3 ± 0.7 (n = 5) taurine 4.1 ± 0.8 (n = 5), GABA 0.32 ± 0.07 (n = 6), glutamate 4.5 ± 1.4 (n = 5) for 90 mM potassium chloride. Mean values ± S.E.M. $*p < 0.05$ (Wilcoxon's signed rank test for paired data)

A fall of blood pressure was elicited by an intravenous infusion of nitroprusside (30 μg/kg/min) for 10 min. The depressor response of approximately 30 mm Hg decreased the release rates of the inhibitory amino acids taurine and

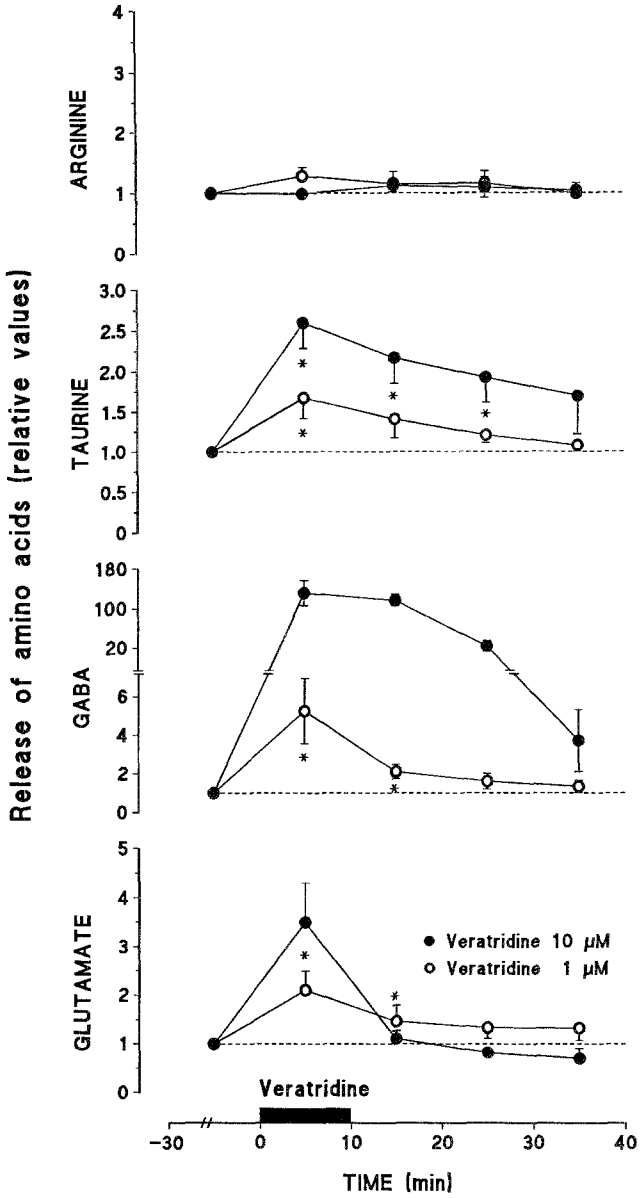

Fig. 4. Effects of superfusion with veratridine on the release of amino acids. Ordinates: release of amino acids as relative values. The release rates in the three samples preceding superfusion with veratridine were taken as 1.0. Abscissa: time in min. Horizontal bar indicates beginning and duration of superfusion with veratridine. Release rates of amino acids preceding veratridine were (pmol/min): arginine 2.2 ± 0.8 (n = 7), taurine 2.7 ± 1.1 (n = 6), GABA 0.31 ± 0.04 (n = 7), glutamate 3.5 ± 1.2 (n = 6) for 1 μM veratridine and arginine 1.8 ± 0.3 (n = 6), taurine 1.3 ± 0.2 (n = 5), GABA 0.25 ± 0.05 (n = 4), glutamate 1.6 ± 0.4 (n = 3) for 10 μM veratridine. Mean values ± S.E.M. *$p < 0.05$ (Wilcoxon's signed rank test for paired data)

GABA, while the release rate of the excitatory amino acid glutamate was enhanced (Fig. 5). On the other hand, a pressor response to intravenous infusion

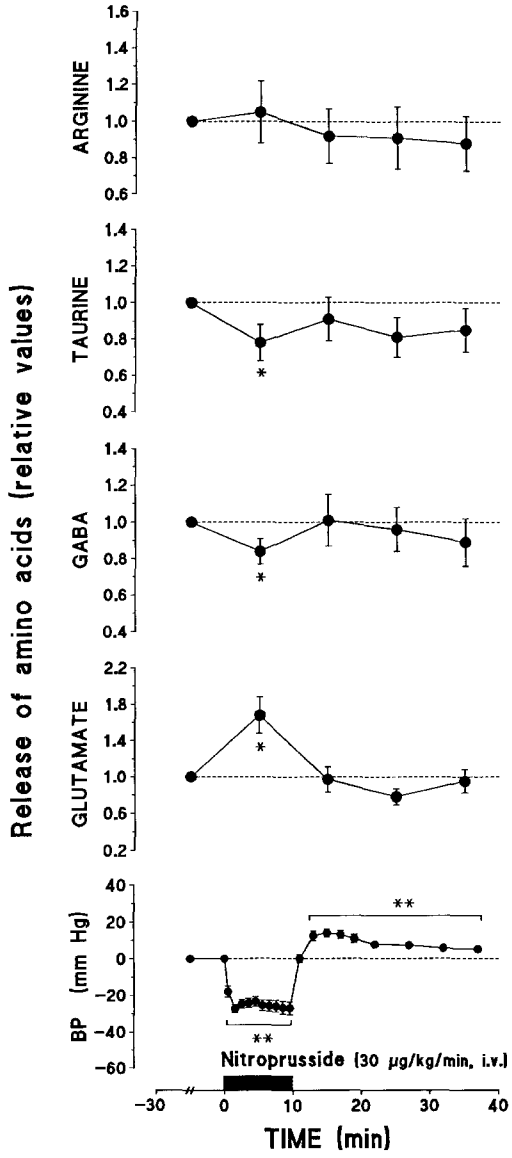

Fig. 5. Effects of nitroprusside infusion on the release rates of amino acids and on mean arterial blood pressure. Ordinates: release rates of amino acids as relative values or arterial blood pressure as mm Hg. Release rates of amino acids in the three samples preceding nitroprusside infusion were taken as 1.0. Abscissa: time in min. Horizontal bar indicates beginning and duration of intravenous infusion of nitroprusside. Release rates of amino acids preceding nitroprusside infusion were (pmol/min): arginine 1.8 ± 0.3 (n = 8), taurine 3.8 ± 1.1 (n = 11), GABA 0.30 ± 0.05 (n = 11), glutamate 3.6 ± 1.3 (n = 8). Arterial blood pressure preceding nitroprusside infusion was 107.2 ± 4.8 mm Hg (n = 11) Mean values ± S.E.M. $*p < 0.05$, $**p < 0.01$ (Wicoxon's signed rank test for paired data)

of noradrenaline (3 µg/kg/min) for 10 min enhanced the release rates of taurine and GABA, while the release of glutamate was not changed (Fig. 6).

Experimentally induced blood pressure changes did not influence the release of arginine (Figs. 5, 6).

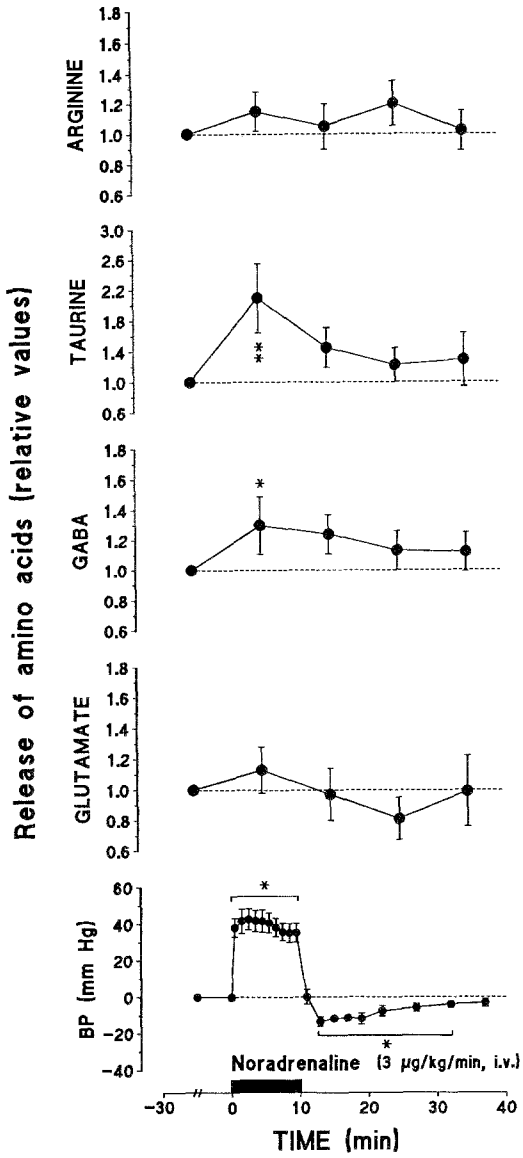

Fig. 6. Effects of noradrenaline infusion on the release rates of amino acids and on mean arterial blood pressure. Ordinates: release rates of amino acids as relative values or arterial blood pressure as mm Hg. Release rates of amino acids in the three samples preceding noradrenaline infusion were taken as 1.0. Abscissa: time in min. Horizontal bar indicates beginning and duration of intravenous infusion of noradrenaline. Release rates of amino acids preceding noradrenaline infusion were (pmol/min): arginine 2.6 ± 0.5 (n = 10), taurine 4.3 ± 1.2 (n = 10), GABA 0.25 ± 0.05 (n = 7), glutamate 6.1 ± 2.5 (n = 7). Arterial blood pressure preceding noradrenaline infusion was 109.3 ± 5.6 mm Hg (n = 10). Mean values ± S.E.M. $*p < 0.05$, $**p < 0.01$ (Wilcoxon's signed rank test for paired data)

Intravenous infusion of physiologic saline for 10 min changed neither blood pressure nor amino acid release in the posterior hypothalamus.

Discussion

Hypothalamic superfusion through a push-pull cannula over 320 min and determination of endogenous amino acids in the superfusate revealed the existence of fluctuations in the release rates. The most frequent oscillations were observed in the release rate of glutamate (45 min/cycle), followed by GABA (65 min/cycle) and taurine (160 min/cycle). In various brain regions, the release rates of histamine (Philippu et al., 1982; Prast et al., 1988, 1992) and catecholamines (Philippu et al, 1979; Lanzinger et al., 1989; Dietl et al., 1993) also fluctuate with similar, but not identical frequencies. In the cat hypothalamus, GABA is also released according to an ultradian rhythm (Dietl and Philippu, 1979). Because of the low number of experiments, a further analysis of the fluctuations in the releases of taurine, GABA and glutamate was not attempted.

To investigate the origin of the neurotransmitters released in the hypothalamus, this brain area was superfused through the push-pull cannula with neuroactive substances. Potassium chloride or veratridine enhanced greatly the release of taurine, GABA and glutamate. On the other hand, superfusion with the neurotoxin TTX led to a profound and sustained decrease in the release rates of these amino acids. Since superfusion with TTX reduced the release of taurine, GABA and glutamate by about 40 per cent, it seems that almost half of the amounts of these neurotransmitters appearing in the superfusate originates from neuronal sites. These compounds did not influence significantly the release of arginine in the hypothalamus.

The various frequencies of the ultradian fluctuations in the release of taurine, GABA and glutamate, together with the pronounced differences in the responses to potassium chloride and veratridine, might indicate that these amino acids are released from different neurons in the hypothalamus.

Central administrations of a plethora of compounds alter blood pressure. Some amino acids applied to the brain also lead to either pressor or depressor responses (review: Philippu, 1988). These cardiovascular effects are due to stimulation or inhibition of receptors located on neurons involved in central cardiovascular control. It is obvious that the change in blood pressure elicited by a compound applied centrally does not necessarily point to the involvement of this compound as a neurotransmitter in central cardiovascular control. If a central neurotransmitter were indeed involved in cardiovascular control, then it might be expected that experimentally induced blood pressure changes should modify the release rate of this neurotransmitter so as to counteract the alteration in blood pressure. To prove whether amino acids determined in the hypothalamic superfusate may be involved in central cardiovascular control, blood pressure was modified by drugs applied peripherally and the effects on the release of the amino acids were investigated.

A fall of blood pressure elicited by nitroprusside decreased the release rates of the inhibitory amino acids taurine and GABA. In contrast to this, the rise in blood pressure caused by noradrenaline enhanced the release rates of these amino acids. Since the releases of GABA and taurine are reduced when blood pressure is low and enhanced when blood pressure is high, it seems likely that the two amino acids, released from hypothalamic neurons, possess a tonic

hypotensive function and contribute to homeostasis of blood pressure. Indeed, intrahypothalamic injections of GABA agonists or i.c.v. injection of taurine decrease blood pressure (Wible et al., 1988, Bousquet et al., 1981), while GABA antagonists elevate blood pressure (Wible et al., 1988). On the other hand, the release rate of the excitatory amino acid glutamate was enhanced when arterial blood pressure was lowered by nitroprusside. Thus, glutamate seems to possess a hypertensive function in the hypothalamus. Nevertheless, the pressor response to noradrenaline did not influence the release rate of glutamate. Very probably, the activity of glutamatergic neurons of the hypothalamus is enhanced so as to counteract the fall of blood pressure, but a rise in blood pressure does not seem to modify the activity of these neurons. In agreement with this idea, microinjection of glutamate into the posterior hypothalamus of conscious rats also increases blood pressure (Ohta et al., 1985).

Besides GABA and glutamate, taurine has also been proposed to be a neurotransmitter or neuromodulator in the brain (Davison and Kaczmarek, 1971; Sieghart and Karobath, 1974; Kuriyama, 1980; review: Huxtable, 1989). Our results demonstrate that all three amino acids are released, at least to a great part, from hypothalamic neurons and that they are involved in central cardiovascular control.

In conclusion, the findings show that inhibitory and excitatory amino acids are released from hypothalamic neurons according to ultradian fluctuations with different frequencies. The inhibitory amino acids GABA and taurine possess a tonic, hypotensive function in the posterior hypothalamus, a known pressor area, while gutamate released seems to possess a hypertensive function in this brain area.

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