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Serotonin outflow in the hypothalamus of conscious rats: origin and possible involvement in cardiovascular control

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Abstract

The push-pull technique was used to investigate the effects of neuroactive compounds and experimentally induced blood pressure changes on the release of endogenous serotonin in the posterior hypothalamic area of the rat. Hypothalamic superfusion with artificial cerebrospinal fluid which contained 80 mM K^+ or 1 μM veratridine enhanced the rate of serotonin release. Superfusion with tetrodotoxin (5 μM) led to a pronounced decrease in the serotonin release rate. Increases in blood pressure elicited by intravenous infusions of noradrenaline (3–4 μg/kg/min) or phenylephrine (10 μg/kg/min) enhanced the release of serotonin in the hypothalamus. Similarly, the serotonin release rate was enhanced by hypervolaemia. Decreases in blood pressure elicited by intravenous administration of nitroprusside (30–40 μg/kg/min) or chlorisondamine (3 mg/kg) reduced the release of serotonin. Likewise, the serotonin release rate was decreased by hypovolaemia. With one exception (hypothalamic superfusion with tetrodotoxin) neither neuroactive drugs, nor experimentally elicited blood pressure changes modified the release rate of the metabolite 5-hydroxyindoleacetic acid (5-HIAA). These findings show that changes in blood pressure lead to counteractive alterations in the release of serotonin. Thus, serotoninergic neurons of the posterior hypothalamus seem to be involved in the homeostasis of blood pressure by exerting a hypotensive function. At least in the hypothalamus, the concentration of 5-HIAA in the superfusate does not seem to be a reliable marker for the activity of serotoninergic neurons.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HIAA (5-hydroxyindoleacetic acid); Blood pressure; Veratridine; Tetrodotoxin; Hypothalamus; Noradrenaline; Hypervolemia; Hypovolemia; Push-pull cannula

1. Introduction

The highest density of serotonin-containing perikarya is found in the raphe nuclei (Dahlström and Fuxe, 1964; Fuxe, 1965; Hillegaart, 1991). In the cat, the posterior hypothalamus receives serotoninergic input from the cell groups B6, B7 and B8 of the rostral raphe nuclei (Sakai et al., 1990).

Serotonin applied centrally influences blood pressure and heart rate albeit the cardiovascular effects of the amine depend on animal species, concentration, as well as site of drug injection (Philippu, 1988). In anaesthetized rats, serotonin microinjected into the anterior (Smits and Struyker-Boudier, 1976; Sukamoto et al., 1984; Dreteler et al., 1991) or medial (Sukamoto et al., 1984; Dreteler et al., 1991) hypothalamus increases arterial blood pressure.

The ability of serotonin injected into the hypothalamus to influence blood pressure does not predict that serotoninergic neurons of this brain structure are involved in central cardiovascular control. In the present study, we used the push-pull technique to investigate in a direct way the influence of experimentally induced blood pressure changes on the release of serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the posterior hypothalamus. In the rat, this 'pressor' area possesses a serotoninergic innervation which derives from cell bodies located in the raphe nuclei (Dahlström and Fuxe, 1964; Steinbusch, 1981). Additionally, we studied the origin of serotonin appearing in the superfusate by superfusing the hypothalamus with the neuroactive compounds KCl, veratridine or tetrodotoxin.

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2. Materials and methods

Male Sprague-Dawley rats weighing 230–280 g were housed in a light-, temperature- and humidity-controlled environment and provided with food and water ad libitum. Before surgery for placement of the guide cannula, rats were anaesthetized with sodium pentobarbital (40 mg/kg i.p.) and ketamine (50 mg/kg i.p.). The head was fixed in a stereotaxic frame and the guide cannula (o.d. 1.25 mm, i.d. 0.9 mm) with its stylet was stereotaxically inserted at an angle of 3° with respect to the midsagittal plane until the tip of the cannula was 2 mm above the right posterior hypothalamic area. With respect to bregma, the stereotaxic coordinates were (mm) A.P. −3.9, L 0.7, V −6.2 according to the atlas of Paxinos and Watson (1986). The guide cannula was fixed with 3 stainless steel screws as anchors and dental cement. The iliolumbar artery and jugular vein were permanently catheterized with PE 50 and PE 20 tubings for measurement of arterial blood pressure (Recommed Hellige, Freiburg, Germany) and infusion of drugs, respectively. Rats were allowed to recover from anaesthesia for at least 2 days. The superfusion experiments started at 8 a.m. 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 posterior hypothalamic area (V −8.2 mm from skull surface). After an adaptation time of 2 h, the hypothalamus of the conscious, freely moving rat was superfused with artificial cerebrospinal fluid (CSF) pH 7.2 at a constant rate of 15 μl/min. CSF consisted of (mmol/l): NaCl 140, KCl 3.0, $CaCl_2$ 2.5, $MgCl_2$ 1.0, $NaHPO_2$ 1.2, NaH_2PO_4 0.3, glucose 3.0, pargyline 0.5.

Drugs were dissolved in CSF and applied for 10 min through the push-pull system. When K^+-rich CSF was used, the concentration of NaCl was reduced appropriately, so as to maintain isoosmolarity. Experimental blood pressure changes were elicited by intravenous infusions (50 μl/min) of noradrenaline, phenylephrine and nitroprusside or by alterations in blood volume. Blood volume was estimated as 7% of the body weight (Waynforth and Flecknell, 1992). In each animal, 3–4 experiments were carried out, the time interval between two adjacent superfusions with drugs or peripherally induced blood pressure changes being at least 80 min. The superfusate was continuously collected as 10 min fractions at 0°C into tubes containing 4 μl of the following solution to protect serotonin from decomposition (mmol/l, final concentrations): $HClO_4$ 164, HCl 1.6, $Na_2S_2O_5$ 0.4, EDTA 0.9, EGTA 1.4. The samples were stored at −80°C until determinations of serotonin and 5-HIAA were 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 localization of the cannula was verified histologically. Data from animals with cannula localizations outside the posterior hypothalamic area were discarded. Macroscopically, no infection of brain or other tissues was observed.

The concentrations of serotonin and 5-HIAA were determined by high performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system consisted of an ESA 580 pump (ESA, Bedford, USA), a degasser (CMA 260, Stockholm, Sweden), an injector (Rheodyne 7125), an analytical column (PhaseSep Sherisorb S3 ODS2, 12.5 cm × 3 mm, 3 μm) and an electrochemical detector (Coulochem 5100A, ESA, Bedford, USA) with a sensitive analytical cell (ESA 5014). Pump and injector were interconnected with a guard cell (ESA 5020). The electrode potentials were set as follows: guard cell: +200 mV, first cell: +20 mV, second cell: +180 mV (typical background currents: first cell: 0–2 nA, second cell: 0–3 nA). The mobile phase consisted of NaH_2PO_4 (0.15 mol/l), Na-octanesulfonic acid (2.8 mmol/l), EDTA (50 μmol/l) (pH adjusted with *o*-phosphoric acid (85%) to pH 2.9) and 15% (v/v) methanol. The flow rate was 0.3–0.4 ml/min. Aliquots (100 μl) of the superfusates were injected onto the column. Evaluations of serotonin and 5-HIAA were carried out by comparing peak heights of samples of external standard solutions with serotonin and 5-HIAA at various concentrations by using an integrator (SIC Chromatocorder 12, Merck, Germany). The minimum detection limit for serotonin was 1–2 pg/sample at a signal-to-noise ratio of 3.

Data were analysed by Friedman's test followed by Wilcoxon's signed rank test for paired data.

3. Results

3.1. Basal release of serotonin and 5-HIAA in the posterior hypothalamus

Superfusion of the posterior hypothalamic area started immediately after cannula insertion. Preliminary experiments had shown that the release rates of serotonin and its metabolite 5-HIAA declined rapidly during the first 30–40 min and reached a steady state after another 10–30 min (not shown). Hence, collection of the superfusate started 80 min after cannula insertion.

The superfusate was collected continuously as 10-min fractions. Release rates of serotonin and 5-HIAA over 80 min are shown in Table 1. The overall release rates of serotonin and 5-HIAA remained rather constant over 360 min and amounted to 8.9 ± 1.2 and 197.3 ± 39.0, respectively (fmol/min; mean values ±

Table 1
Release rates of serotonin (5-HT) and 5-HIAA during control superfusion with CSF

Time (min)	5-HT (fmol/min)	5-HIAA (fmol/min)
10	7.8±2.7	60±16
20	8.2±1.9	82±33
30	8.3±1.9	77±42
40	6.7±1.7	75±34
50	7.3±1.9	88±43
60	7.5±2.5	84±31
70	8.9±2.8	63±43
80	8.3±2.1	60±22

Means of 4–5 experiments ± S.E.

S.E., $n = 30$). The ratio of the release rates of 5-HIAA/serotonin was 22 ± 5.

3.2. Effects of neuroactive substances on the release of serotonin and 5-HIAA

Hypothalamic superfusion for 10 min with CSF which contained 80 mM KCl led to a pronounced increase in the release rate of serotonin, while the release of 5-HIAA was not influenced (Fig. 1). Superfusion with 1 μM veratridine also enhanced the release of serotonin without influencing that of 5-HIAA (Fig. 2). Superfusion of the posterior hypothalamus with the neurotoxin tetrodotoxin for 20 min elicited a pronounced and sustained decrease in the release rate of serotonin. The release rate of the amine reached its initial level approximately 30 min after termination of superfusion with tetrodotoxin. The release of 5-HIAA also tended to decrease, but the level of statistical significance was reached only in the second sample after the start of superfusion with the neurotoxin (Fig. 3).

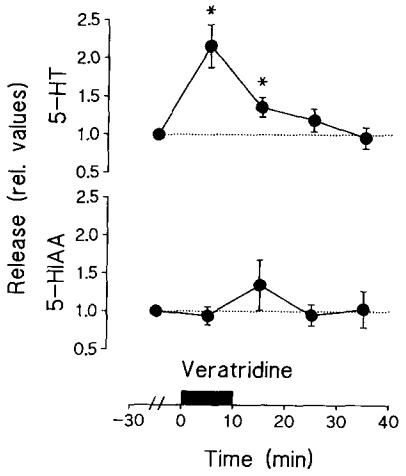

Fig. 2. Effects of veratridine on the release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding superfusion with veratridine were taken as 1.0. Horizontal bar denotes begin and duration of superfusion with veratridine (1 μM). Mean values ± S.E. * $P < 0.05$.

3.3. Effects of experimentally induced blood pressure changes on the release of serotonin and 5-HIAA

To elicit a sustained pressor response, noradrenaline (3–4 μg/kg/min) was infused intravenously for 10 min. The rise in blood pressure of approximately 65 mm Hg was associated with a profound increase in the

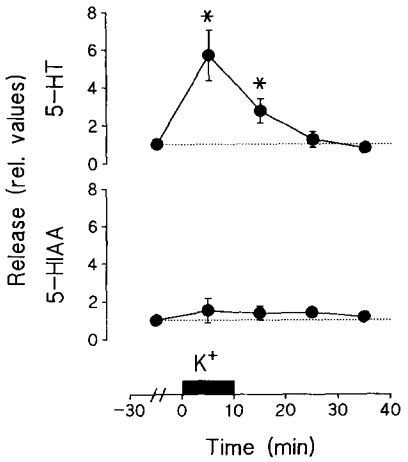

Fig. 1. Effects of hypothalamic superfusion with K^+-rich CSF on the release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding superfusion with K^+-rich CSF were taken as 1.0. Horizontal bar denotes begin and duration of superfusion with KCl (80 mM). Mean values ± S.E. * $P < 0.05$.

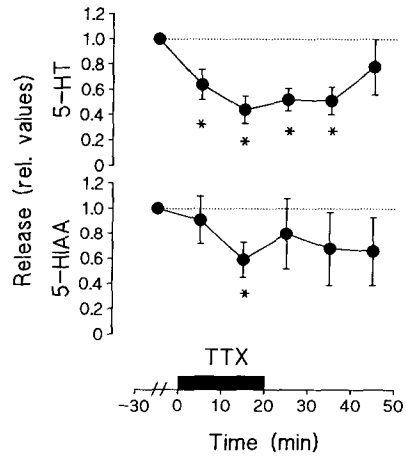

Fig. 3. Effects of tetrodotoxin (TTX) on the release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding superfusion with tetrodotoxin were taken as 1.0. Horizontal bar denotes begin and duration of superfusion with tetrodotoxin. Mean values ± S.E. * $P < 0.05$.

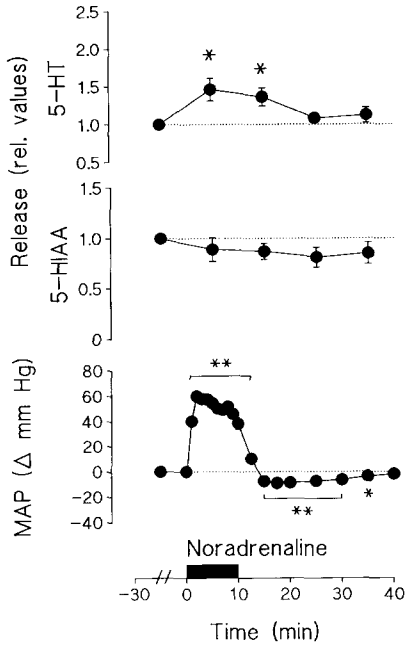

Fig. 4. Effects of noradrenaline on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean values preceding infusion with noradrenaline (3–4 μg/kg/min) were taken as 1.0. Horizontal bar denotes begin and duration of infusion with noradrenaline. Mean values ± S.E. * $P < 0.05$, ** $P < 0.01$.

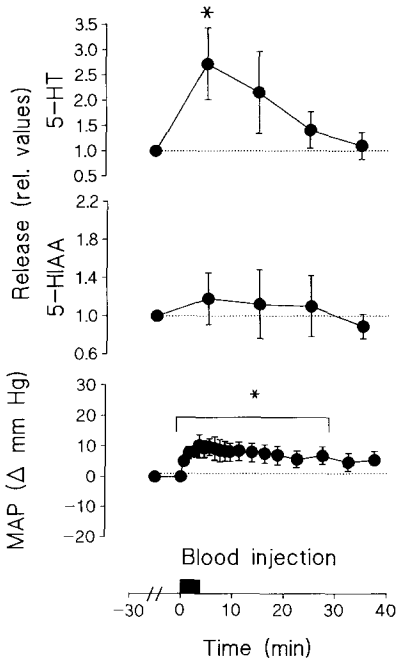

Fig. 6. Effects of blood injection on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding blood injection were taken as 1.0. Horizontal bar denotes begin and duration of blood injection. Mean values ± S.E. * $P < 0.05$, ** $P < 0.01$.

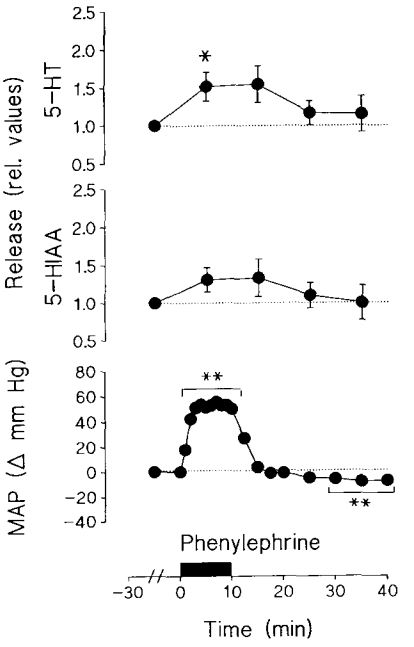

Fig. 5. Effects of phenylephrine on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding infusion with phenylephrine (10 μg/kg/min) were taken as 1.0. Horizontal bar denotes begin and duration of superfusion with phenylephrine. Mean values ± S.E. * $P < 0.05$, ** $P < 0.01$.

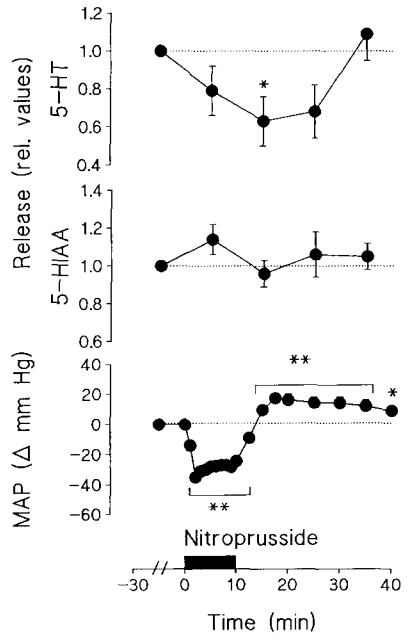

Fig. 7. Effects of nitroprusside on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding infusion with nitroprusside (30–40 μg/kg/min) were taken as 1.0. Horizontal bar denotes begin and duration of infusion with nitroprusside. Mean values ± S.E. * $P < 0.05$, ** $P < 0.01$.

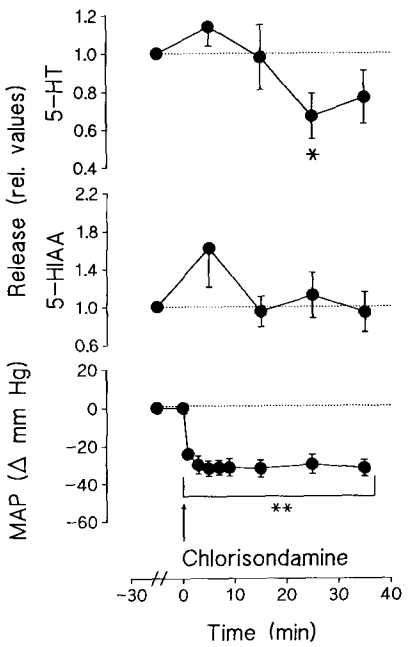

Fig. 8. Effects of chlorisondamine on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding injection of chorisondamine (3 mg/kg) were taken as 1.0. Arrow indicates injection of chlorisondamine. Mean values ± S.E. * $P < 0.05$, * * $P < 0.01$.

release rate of serotonin, while the release of 5-HIAA remained unchanged (Fig. 4). Intravenous infusion of phenylephrine (10 μg/kg/min) also led to a pressor response (Fig. 5). The rise in blood pressure was accompanied by an enhanced release of serotonin. The release rate of 5-HIAA was not changed significantly.

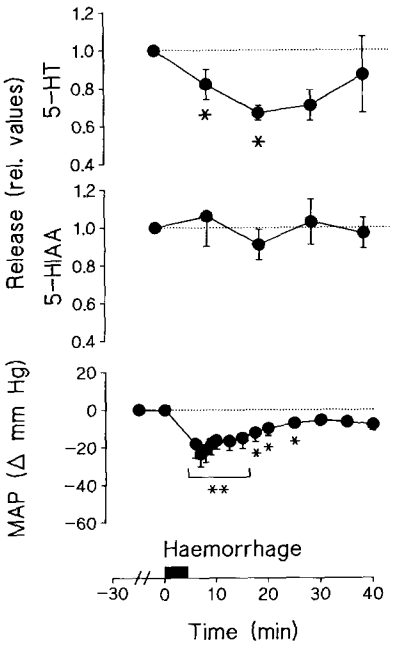

Fig. 9. Effects of haemorrhage on mean arterial blood pressure (MAP) and release rates of serotonin (5-HT) and 5-HIAA. The mean release rates in the three samples preceding haemorrhage were taken as 1.0. Horizontal bar denotes begin and duration of haemorrhage. Mean values ± S.E. * $P < 0.05$, * * $P < 0.01$.

A 35% hypervolaemia was evoked by injecting intravenously blood mixed with physiological saline. Blood injection led to a slight rise in blood pressure of about 12 mm Hg and to a pronounced increase in the release rate of serotonin. The release of the metabolite 5-HIAA was not influenced (Fig. 6).

A fall in blood pressure was elicited by nitroprusside (30–40 μg/kg/min) infused intravenously. This vasodilator lowered blood pressure by approximately 30 mm Hg. The fall in blood pressure was associated with a decrease in the serotonin release rate which lasted longer than the cardiovascular effect of nitroprusside. The release of 5-HIAA was not influenced (Fig. 7). A marked and sustained hypotension elicited by the ganglionic blocking agent chlorisondamine (3 mg/kg i.v.) did not change the release of 5-HIAA, while the release rate of serotonin was diminished significantly 30 min after injection of the drug (Fig. 8). A 17–20% hypovolaemia evoked by controlled haemorrhage led to a sustained decrease in the release of serotonin. Hypovolaemia did not influence the release of 5-HIAA in the posterior hypothalamus (Fig. 9).

4. Discussion

The basal release rates of serotonin and its metabolite 5-HIAA have been determined repeatedly in various areas of the hypothalamus. By using the microdialysis technique, the ratio 5-HIAA/serotonin is high and varies between 3000 and 168 (Adell et al., 1991; Matos et al., 1990; Schwartz et al., 1990; West et al., 1991; Shimizu et al., 1992). Under our experimental conditions, i.e. (1) by using the push-pull technique which makes it possible to superfuse directly a distinct brain area and to rapidly collect the superfusate, (2) by adding pargyline into the CSF, and (3) by collecting the superfusate in vials which contained a solution to protect serotonin from decomposition, the ratio 5-HIAA/serotonin was 22. In the hypothalamus, the ratio of the metabolite to the amine is approximately 1 (Adell et al., 1991; Fleckenstein et al., 1994). Obviously, the low ratio found under our experimental conditions reflects the apparent concentrations of the two compounds in the extracellular space more closely than the high ratios mentioned above.

To identify the sites from which serotonin is released in the superfusate, we superfused the hypothalamus with neuroactive compounds. K^+-rich CSF, as well as veratridine, led to a pronounced increase in the release of serotonin but the release of 5-HIAA was not influenced. On the other hand, hypothalamic superfusion with the neurotoxin tetrodotoxin decreased

the release of serotonin by about 60%. The release of 5-HIAA was also reduced transiently. These findings indicate that serotonin is released, to a great extent, from neuronal sites of the hypothalamus.

As mentioned in the Introduction, microinjections of serotonin into the anterior hypothalamus (Smits and Struyker-Boudier, 1976; Sukamoto et al., 1984; Dreteler et al., 1991) or medial hypothalamus (Sukamoto et al., 1984; Dreteler et al., 1991) increase blood pressure. The centrally induced pressor response to serotonin has been attributed to stimulation of 5-$HT_{2/1C}$ receptors (Pérgola and Alper, 1991). In the rat, the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) decreases blood pressure and heart rate when injected into the ventromedial hypothalamic area (Dreteler et al., 1991). The hypotensive effects of 8-OH-DPAT and flesinoxan have been attributed to stimulation of 5-HT_{1A} receptors within the ventrolateral medulla. Serotonin injected into the nucleus of the solitary tract also lowers blood pressure (review articles: Saxena and Villalón, 1990; Dabiré, 1991). If anything, serotonin injection into the posterior hypothalamus decreases blood pressure slightly (unpublished observations). Hence, central stimulation of various 5-HT receptors exerts opposite cardiovascular effects.

Experimentally induced blood pressor responses influenced profoundly the release of serotonin in the posterior hypothalamic area. The pronounced increase in blood pressure evoked by noradrenaline and phenylephrine enhanced the release of serotonin, but even a slight rise in blood pressure elicited by hypervolaemia also drastically enhanced the release of serotonin. Decreases in blood pressure evoked either by the vasodilator nitroprusside or by hypovolaemia were associated with decreases in the release rate of serotonin. Surprisingly, the decrease in the release rate of serotonin by chlorisondamine appeared 30 min after onset of hypotension. The delayed response of serotonin release to ganglionic blockade cannot be explained now.

Since experimentally induced increases in blood pressure elevate the release of serotonin, while decreases in blood pressure exert the opposite effect, it might be postulated that in the posterior hypothalamus serotonin released from serotoninergic nerve endings is involved in blood pressure homeostasis by having a counteractive, hypotensive function. Probably, serotonin released from serotoninergic nerve endings stimulates serotonin heteroreceptors located postjunctionally on descending neurons or interneurons of the posterior hypothalamus which, either directly or indirectly, lower blood pressure. Peripheral administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) enhances serotonin synthesis in the brain and lowers blood pressure (Freed et al., 1985). The hypotensive effect of 5-HTP might be due, at least partly, to enhanced release of serotonin in the posterior hypothalamus. The type of the hypothalamic 5-HT receptors responsible for the mediation of the depressor response to endogenous serotonin is not yet known. In the supraoptic nucleus of anaesthetized rats, hypovolaemia elicited by haemorrhage enhances the release of serotonin (Kendrick and Leng, 1988) indicating that, in this nucleus, serotonin has the opposite function to that in the posterior hypothalamic nucleus.

As mentioned above, neuroactive compounds and experimentally induced blood pressure changes altered the release rate of serotonin without influencing the concentration of 5-HIAA in the superfusate. Since neither increases nor decreases in serotonin release were associated with corresponding changes in the release of 5-HIAA, the extracellular concentration of the metabolite does not seem to be a reliable marker for the activity of serotoninergic neurons. Similar conclusions have been drawn by others (Wolf et al., 1985; Auerbach et al., 1989; Crespi et al., 1990; Celada and Artigas, 1993).

In conclusion, these findings show that in the posterior hypothalamus serotonin is released predominantly from serotoninergic nerve terminals. Changes in arterial blood pressure lead to counteractive alterations in the release of serotonin, which seems to have a hypotensive function in this brain structure. Finally, the 5-HIAA concentration in the hypothalamic extracellular space does not reliably reflect the activity of serotoninergic neurons.

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