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In vivo Release of Endogenous Catecholamines, Histamine and GABA in the Hypothalamus of Wistar Kyoto and Spontaneously Hypertensive Rats*

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Summary. The release of dopamine, noradrenaline, adrenaline, histamine and GABA was studied in the posterior hypothalamus of conscious, freely moving Wistar Kyoto (WKy) rats and spontaneously hypertensive (SH) rats (Okamoto strain). A guide cannula was stereotaxically inserted under anaesthesia. Some days later a push-pull cannula was inserted into the guide cannula so that the tip of the push-pull cannula reached the posterior hypothalamic nucleus. The posterior hypothalamic nucleus of the freely moving animal was superfused with artificial CSF. The rates of release of the neurotransmitters were determined in the superfusate which was continuously collected in 10 min periods.

In SH rats, the rates of resting release of dopamine and histamine were higher than in WKy rats, while the rates of release of noradrenaline and adrenaline in SH rats were lower than those in WKy rats. No significant differences were found in the rates of resting release of GABA between SH and WKy rats. Superfusion of the posterior hypothalamic nucleus of WKy rats with KCl-rich CSF (60 mmol/l KCl) significantly increased the rates of release of noradrenaline and adrenaline, while those of dopamine and GABA tended to be enhanced. In SH rats, hypothalamic superfusion with KCl-rich CSF increased the rates of release of dopamine, noradrenaline, adrenaline and GABA. The KCl-induced release of neurotransmitters did not differ between SH and WKy rats. Superfusion with KCl-rich CSF did not influence the rates of release of histamine either in SH or in WKy rats.

The findings indicate that differences exist in the resting release of dopamine, noradrenaline, adrenaline and histamine between SH and WKy rats. Our experimental set-up seems to be useful in investigating neurochemical changes in distinct brain areas of animals at different physiopathologic stages under in vivo conditions.

Key words: SHR – Posterior hypothalamus – Release – Catecholamines – GABA – Histamine – Push-Pull cannula

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Introduction

Central mechanisms are involved in the regulation of cardiovascular functions and in the development of certain forms of hypertension (for review see: Juskevich and Lovenberg 1981). The mechanisms for increasing the sympathetic activity require the participation of several central putative neurotransmitters. Besides noradrenaline and adrenaline, serotonin, acetylcholine (for review see: Philippu 1981), dopamine, GABA and histamine have been proposed to participate in the central regulation of cardiovascular functions (for review see: Juskevich and Lovenberg 1981).

Among brain structures involved in the regulation of the sympathetic activity the posterior hypothalamus seems to be of prime importance. Electrical stimulation of the posterior hypothalamus leads to a pressor response which is more pronounced in SH than in WKy rats (Juskevich et al. 1978). Recently, we reported that short-lasting or long-lasting increases in the arterial blood pressure reduce and enhance the rates of release of the catecholamines dopamine, noradrenaline and adrenaline in the posterior and anterior hypothalamus, respectively. Furthermore, pronounced short-lasting or long-lasting decreases in blood pressure exert the opposite effects in the two hypothalamic regions: the rates of release of all three catecholamines are enhanced in the posterior, but they are diminished in the anterior hypothalamus (Dietl et al. 1981; Philippu et al. 1980, 1981; Sinha et al. 1980).

In the present work we investigated whether differences exist in the resting as well as in the KCl-induced release of dopamine, noradrenaline, adrenaline, GABA and histamine in the posterior hypothalamus of SH and WKy rats under in vivo conditions.

Methods

WKy and SH rats (Okamoto strain) were anaesthetized with chloral hydrate (300 mg/kg i.p.). The head was fixed in a stereotaxic frame (LPC) and a guide cannula (OD 1.25 mm, ID 0.9 mm) with its stylet was stereotaxically inserted according to the atlas of König and Klippel (1963) until the tip of cannula was 2 mm above the left posterior hypothalamic nucleus (AP 3.7 mm, V −0.4 mm, L 0.5 mm). The cannula was fixed with dental screws and cement. Three days after the operation the stylet of the guide cannula was removed and a push-pull cannula (outer needle: OD 0.7 mm, ID 0.5 mm, inner needle: OD 0.2 mm, ID 0.1 mm) was inserted which was 2 mm longer than the guide cannula thus reaching the upper surface of the posterior hypothalamic nucleus (V −2.4 mm).

The inner cannula of the push-pull cannula was 0.2 – 0.5 mm shorter than the outer cannula.

The freely moving animal was placed into a large cage (0.13 m^2) which could be rotated manually. The push-pull cannula was connected with tubing with two peristaltic pumps (Desaga: PLG 132100), one to push and one to pull the superfusing fluid. The tubing was supported on a swivel (for details see Philippu et al. 1982; Philippu 1983). The hypothalamus was superfused with artificial CSF pH 7.2 (Philippu et al. 1979) through the push-pull cannula (for details see Philippu 1983) at a constant rate of 150 µl/min. Since the upper end of the outer cannula was open and the tip of the inner cannula did not protrude, the pressure at the superfusion site remained constant throughout the experiment and virtually corresponded to the atmospheric pressure (Philippu 1983). The superfusate was continuously collected in 10 min periods in 3 separate tubes for the determination of catecholamines, GABA and histamine. When the hypothalamus was superfused with a high KCl-concentration (60 mmol/l) that of NaCl was reduced (80 mmol/l) to avoid hyperosmolarity. The tubes were kept on dry-ice until determination was carried out. The catecholamines were radioenzymatically determined in 200 µl of the superfusate after separation by thin layer chromatography (Philippu et al. 1979). GABA was determined in 500 µl by an adaptation (Dietl and Philippu 1979) of the radioreceptor assay (Enna and Snyder 1976), histamine in 100 µl by a slight modification (Philippu et al. 1982) of the radioenzymatic assay of Taylor and Snyder (1972). The sensitivity limits of the assays were as following: catecholamines 50 fmol/sample (Philippu et al. 1979), GABA 60 pmol/sample (Dietl and Philippu 1979), histamine 100 fmol/sample (Philippu et al. 1982). Mean arterial blood pressure was measured before operation and in the day after superfusion by an occlusion- and plethysmographic device in the tail of the unanaesthetized rat. There were no differences between the two measurements. For calculation of mean blood pressure the values of the measurement in the day after superfusion were considered.

At the end of the experiment the rats were killed by injection of sodium pentobarbitone. To render easy the localization of the tip of the cannula, a minute amount of cresyl violet was gently pushed through the inner needle of the cannula with a syringe. The brain was removed and immersed in 10% formaldehyde. The localization of the push-pull cannula was histologically verified in 200 µl slices stained with cresyl violet.

Statistical significance was calculated by Student's *t*-test. The mean values of the first four 10 min samples of each experiment were considered for the calculation of the resting rates of release. The rate of release on superfusion with KCl-rich CSF was compared with that in the preceding sample.

Results

In WKy and SH rats, the rates of release of all these endogenous compounds remained fairly constant for at least 3 h. The mean arterial blood pressure of WKy rats (age 60 ± 3 days, weight 103 ± 11 g, $n = 8$) and SH rats (age 60 ± 3 days, weight 121 ± 4 g, $n = 9$) was 95 ± 6 mm Hg and 124 ± 7 mm Hg, respectively ($P < 0.01$). The mean weight of WKy rats did not differ from that of SH rats ($P > 0.1$).

The hypothalamic area superfused with the cannula was 0.2 mm^2. The resting release of the endogenous neurotransmitters was determined over a period of 40 min in 10 min samples and expressed as fmol/10 min (dopamine, noradrenaline, adrenaline and histamine) or as pmol/10 min (GABA). Figure 1 shows the rates of resting release of these neurotransmitters in the hypothalamic superfusates of WKy and SH rats. The resting rates of release in WKy rats ($n = 7-8$) were: dopamine 1,109 ± 68, noradrenaline 782 ± 85, adrenaline 1,165 ± 139, histamine 328 ± 20 (fmol/10 min), GABA 1,674 ± 201 (pmol/10 min) and in SH rats ($n = 7-9$): dopamine 1,769 ± 317, noradrenaline 460 ± 61, adrenaline 599 ± 139, histamine 470 ± 27 (fmol/10 min), and GABA 1,311 ± 156 (pmol/10 min).

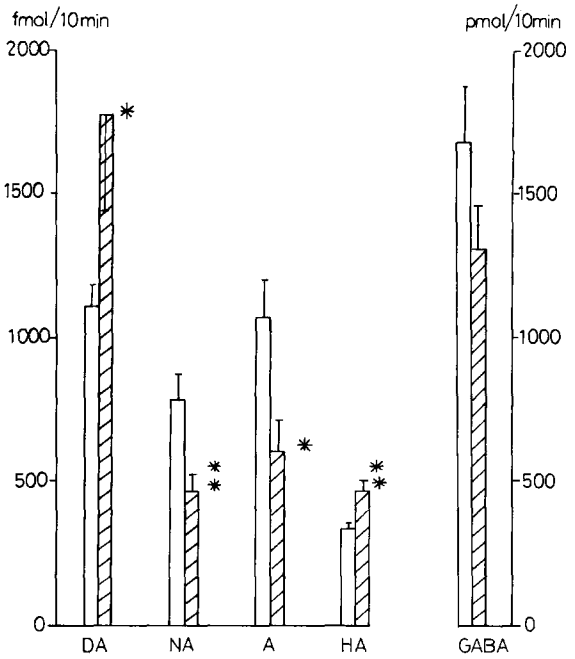

Fig. 1. Resting release of dopamine (*DA*), noradrenaline (*NA*), adrenaline (*A*), *GABA* and histamine (*HA*) in the posterior hypothalamic nucleus. Open columns: WKy rats ($n = 7-8$), striped columns: SH rats ($n = 7-9$). For differences between WKy and SH rats $^*P < 0.05$, $^{**}P < 0.01$. Mean values ± S.E.M. (Student's *t*-test for unpaired data)

The rates of release of dopamine and histamine were higher in the superfusate of SH rats than in that of their counterparts. On the other hand, the rates of resting release of noradrenaline and adrenaline were significantly lower in SH rats compared to WKy rats. There was no difference in the rates of release of GABA in the 2 groups of animals.

To investigate whether the resting release of the neurotransmitters may be modified by stimuli, the posterior hypothalamic nucleus was superfused with CSF which contained 60 mM KCl. Hypothalamic superfusion with KCl-rich CSF for 10 min led to a significant increase in the rates of release of noradrenaline and adrenaline in WKy rats (Fig. 2). The rates of release of dopamine and GABA also tended to be increased but they did not reach the threshold of statistical significance. The rate of release of histamine was not influenced. In SH rats, superfusion with KCl-rich CSF enhanced the rates of release of dopamine, noradrenaline, adrenaline and GABA without changing that of histamine. The KCl-induced increase in the rates of release of dopamine, noradrenaline, adrenaline and GABA in WKy rats did not statistically differ from that found in SH rats.

Recently, a similar push-pull cannula was used for superfusion of the hypothalamus of conscious, freely moving

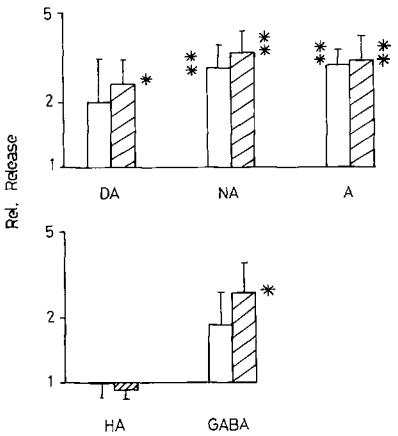

Fig. 2. Release of dopamine (*DA*), noradrenaline (*NA*), adrenaline (*A*), *GABA* and histamine (*HA*) by KCl-rich CSF. Open columns: WKy rats ($n = 7-8$), striped columns: SH rats ($n = 7-9$). The rates of release in the samples preceding superfusion with 60 mmol/l KCl were taken as 1. For differences between control samples and samples during superfusion with 60 mmol/l KCl $*P < 0.05$, $**P < 0.01$. Mean values ± S.E.M. (Student's *t*-test for paired data)

rabbits, in which the arterial blood pressure was recorded throughout the experiment (Philippu et al. 1981; Robinson et al. 1983). It was shown that the hypothalamic superfusion with CSF does not influence the arterial blood pressure. Thus, it is not likely that superfusion of the rat hypothalamus through the push-pull cannula elicited changes in the arterial blood pressure which in turn modified the rates of release of the various neurotransmitters. Moreover, there was no obvious correlation between alterations in the rates of release of the neurotransmitters and exogenous factors (e.g. noise) or behavioral changes of the animals, such as enhancement in their locomotor activity.

Discussion

The purpose of the present study was to investigate the release of endogenous dopamine, noradrenaline, adrenaline, histamine and GABA in the posterior hypothalamus of SH and WKy rats.

The rates of resting release of the catecholamines in the superfusate of the rat hypothalamus were similar with their rates of release in the conscious, freely moving rabbit, in which the hypothalamus was superfused with a cannula of the same diameter of the outer needle (Philippu et al. 1981). The rates of resting release of catecholamines and histamine were different between SH and WKy rats. In SH rats, the rates of release of noradrenaline and adrenaline were lower, those of dopamine and histamine were higher than in WKy rats.

The differences in the rates of release of the catecholamines seem to be causally related to the differences in the arterial blood pressure between SH and WKy rats, since experimentally induced changes in blood pressure alter the release of catecholamines in the hypothalamus. We recently reported that a pronounced rise in the arterial blood pressure decreases the rates of release of dopamine, noradrenaline and adrenaline in the posterior hypothalamus of the anaesthetized cat (Dietl et al. 1981). These findings were interpreted as indicating that a peripherally induced pressor response leads to a counteracting decrease in the release of the three catecholamines in this hypothalamic area. It seems justified to postulate that the increased blood pressure in SH rats also lowers the rates of release of noradrenaline and adrenaline in the posterior hypothalamic nucleus to counteract the blood pressure change. However, the resting rate of release of dopamine was found to be increased in the posterior hypothalamic nucleus of SH rats. The dissociation in the patterns of release of dopamine on the one hand and of noradrenaline and adrenaline on the other indicates that in the rat the various catecholamines are differently implicated with the hypertension: while the enhanced rate of release of dopamine in the posterior hypothalamus might be responsible for the rise in blood pressure, the reduced rates of release of noradrenaline and adrenaline seem to be the consequence of the hypertension rather than the cause for its development. However, it should be pointed out that the interpretation of the results is based on observations obtained in cats (see above). As yet nothing is known about effects of experimentally induced blood pressure changes on the release of catecholamines in the rat hypothalamus. It is also possible that the decreased rates of release of noradrenaline and adrenaline were secondary to the enhanced release of dopamine, because agonists of dopamine receptors reduce the stimulation-induced release of noradrenaline in the hypothalamus (Bryant et al. 1975). Moreover, in the spleen the inhibitory effect of dopamine agonists on the release of noradrenaline is diminished by drugs which block dopamine receptors, such as pimozide and sulpiride (Dubocovich and Langer 1980).

Blood levels of vasopressin have been found to be elevated in various forms of experimental hypertension including SH rats (Crofton et al. 1978). Since dopamine of the brain also plays a role in the regulation of the release of vasopressin (Bridges et al. 1976; Urano and Kobayashi 1978), the increased rate of release of dopamine in the hypothalamus might be causally related to the high blood levels of vasopressin in SH rats.

As already mentioned, the rate of resting release of histamine was found to be increased in the posterior hypothalamus of SH rats. Central application of histamine or histamine agonists elicits a rise in the arterial blood pressure (Trendelenburg 1965; White 1961; Tuomisto and Eriksson 1980) and enhances the pressor response to the electrical stimulation of the posterior hypothalamus (Philippu and Wiedemann 1981). Moreover, histamine is a potent releaser of vasopressin (Blackmore and Cherry 1955; Bhargava et al. 1973; Dogteron et al. 1976; Tuomisto et al. 1980). These results taken together indicate that an increased release of endogenous histamine in the hypothalamus might lead to a rise in blood pressure.

Conflicting results exist concerning the levels of histamine in the brains of SH rats. Spector et al. (1972) and Correa and Saavedra (1981) found elevated levels of histamine in various nuclei of the brain and in the median eminence of SH rats, while Taylor (1975) and Chalmers et al. (1979) failed to detect any differences in the levels of histamine in various brain regions between SH and WKy rats. However, it should be kept in mind that an increased rate of release of neurotransmitters does not necessarily need to be accompanied by an alteration in their tissue concentrations. A release of histamine from mast cells is not likely, because their number seems to be fairly low in the rat hypothalamus (Adam and Hye 1966; Dropp 1972; Brownstein et al. 1974; Edvinsson et al. 1977). In this connection it is of interest to mention that acute changes in the arterial blood pressure elicited by

intravenous injections of drugs, controlled bleeding, or transection of the spinal cord also enhance the release of endogenous histamine in the posterior hypothalamus (Philippu et al. 1983).

The resting release of GABA did not differ between SH and WKy rats thus rendering unlikely a relationship between increased arterial blood pressure and rate of release of this inhibitory neurotransmitter in the posterior hypothalamus. This finding was somewhat surprising, because hypothalamic superfusion with GABA enhances the release of noradrenaline and increases the pressor response to electrical stimulation of the hypothalamus as well (Philippu et al. 1973).

Superfusion with KCl-rich CSF did not affect the rate of release of histamine. The ineffectiveness of KCl in the rat is puzzling, because superfusion of the posterior hypothalamus of the anaesthetized cat with a similar concentration of KCl increases the release of endogenous histamine (Philippu et al. 1982). The failure of KCl to affect the release of histamine might indicate that histamine is released from mast cells rather than from neuronal sites. However, the low number of mast cells in this area and the constant rate of release of histamine for a long period of time do not support this idea. On the other hand, hypothalamic superfusion with KCl enhanced the rates of release of catecholamines and GABA. These findings are in accordance with previous results (Kant and Meyerhoff 1977; Dietl and Philippu 1979; Philippu et al. 1979; Elghozi et al. 1981).

In conclusion, the results show that spontaneous hypertension in rats is associated with changes in the rates of release of dopamine, noradrenaline, adrenaline and histamine in the posterior hypothalamus.

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