Characterization of Norepinephrine Release in Rat Posterior Hypothalamus Using in vivo Brain Microdialysis

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In the present study, we used the microdialysis technique combined with high performance liquid chromatography (HPLC) and electrochemical detection to measure the extracellular levels of norepinephrine (NE) in the posterior hypothalamus in vivo, and to examine the effects of various drugs, affecting central noradrenergic transmission, on the extracellular concentration of NE in the posterior hypothalamus. Microdialysis probes were implanted stereotactically into the posterior hypothalamus (coordinates: posterior 4.3 mm, lateral 0.5 mm, ventral 8 mm, relative to bregma and the brain surface, respectively) of rats, and dialysate collection began 2 hr after the implantation. The baseline level of monoamines in the dialysates were determined to be: NE 0.17 ± 0.01, 3,4-dihydroxyphenylacetic acid (DOPAC) 0.94 ± 0.07, homovanillic acid (HVA) 0.57 ± 0.05 pmol/sample (n=8). When the posterior hypothalamus was perfused with 90 mM potassium, maximum 55% increase of NE output was observed. Concomitantly, this treatment significantly decreased the output of DOPAC and HVA by 35% and 28%, respectively. Local application of imipramine (50 μM) enhanced the level of NE in the posterior hypothalamus (maximum 200%) compared to preperfusion control values. But, DOPAC and HVA outputs remained unchanged. Pargyline, an irreversible monoamine oxidase inhibitor, i.p. administered at a dose of 75 mg/kg, increased NE output (maximum 165%), while decreased DOPAC and HVA outputs (maximum 13 and 12%, respectively). These results indicate that NE in dialysate from the rat posterior hypothalamus were neuronal origin, and that manipulations which profoundly affected the levels of extracellular neurotransmitter had also effects on metabolite levels.

Key Words: Hypothalamus, Microdialysis, Norepinephrine, Rat

INTRODUCTION

There is considerable evidence demonstrating that neurons in the posterior hypothalamus play an important role in the autonomic regulation, especially cardiovascular regulation (Dampney, 1994; Sun et al, 1995; Kim et al, 1997). The posterior hypothalamic neurotransmitters which are involved in the control of autonomic function include GABA (Lisa et al, 1989; Singewald et al, 1993), histamine (Manning & Uhrlrich, 1983) and acetylcholine (Buccafusco, 1996; Kim et al, 1997). In addition, it is anticipated that norepinephrine (NE) functions as one of the major neurotransmitters in the posterior hypothalamus, because the posterior hypothalamus are richly innervated by noradrenergic nerve terminals, originated mainly from locus coeruleus (Dahlstrom & Fuxe, 1965; Hickfelt et al, 1973; Sawchenko & Swanson, 1982). However, the specific pharmacological characterization of neuronal NE in the posterior hypothalamus has not been completely defined.

Understanding of local neurotransmitter release and metabolism is important for the understanding of function of the specific brain area. But, most of our knowledge in this field were gained from postmortem status. Recently, the development of brain microdialysis technique has enabled the measurement of extracellular concentrations of endogenous neurotransmitters and their metabolites within a restricted brain area of living animals (Ungerstedt, 1981). This method causes less tissue damage as compared with other in vivo perfusion technique, such as push-pull perfusion, because of the lack of direct contact of the perfusion fluid with brain tissue (Benveniste, 1989). Using a brain microdialysis, extensive studies have been performed on the release of NE in the regional brain areas, including the locus coeruleus, frontal cortex, hippocampus and striatum (Matsumoto et al, 1983; Thomas et al, 1984; Florin-Lechmer et al, 1996; Carter, 1997; Kiss et al, 1989). However, there are only few evidence suggesting that NE released into the extracellular fluid play a role in the neurotransmission in the posterior hypothalamus (Nakata et al, 1990; Chave et al, 1996; Hashiguchi et al, 1997).

Therefore, in the present study, we have used the technique of microdialysis combined with high performance liquid chromatography (HPLC) and electrochemical detection (ECD) to measure extracellular levels of NE in the posterior hypothalamus in vivo. We also attempted to examine the effects of various drugs, affecting central nor-

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ABBREVIATIONS: DOPAC, 3,4-dihydroxyphenylacetic acid; ECD, electrochemical detection; HPLC, high performance liquid chromatography; HVA, homovanillic acid; NE, norepinephrine.
adrenergic transmission, on the extracellular concentration of NE in the posterior hypothalamus.

METHODS

General procedure

Male Wistar rats (250–300 g) were anesthetized with urethane (1.2 g/kg, i.p.) and maintained with α-chloralose (20 mg/kg, i.v.), when required. After the trachea was cannulated, polyethylene catheter was placed in the left femoral vein for the administration of drug. The animal was paralyzed with d-tubocurarine (0.5 mg/kg, i.m.) and artificially ventilated with room air enriched with O₂ using a roent ventilator (Harvard, Model 680, USA). Rectal temperature was maintained at 37 ± 0.5°C with a thermostatically controlled heating plate.

Brain microdialysis

In vivo microdialysis studies in the rat brain were performed using previously described technique (Sung et al., 1992). Briefly, the rat was placed in a stereotaxic instrument (David Kopf Instrument, USA) in a prone position with the upper incisor bar 3.3 mm below the interaural line and the skull were exposed. A small hole was drilled for the stereotaxic implantation of a microdialysis probe (Carnegie Medicine, CMA/10, Sweden) into the posterior hypothalamus (coordinates: posterior 4.3 mm, lateral 0.5 mm, ventral 8 mm, relative to bregma and the brain surface, respectively). The coordinates were chosen according to the stereotaxic atlas of Paxinos & Watson (1998). The probe was perfused during implantation and subsequent experimental period with Ringer’s solution (147 mM NaCl, 2.3 mM CaCl₂, 4 mM KCl, pH 7.4, flow rate 0.75 μl/min) via polyethylene tubing connected to a 1 ml syringe mounted on a microfusion pump (Carnegie Medicine, CMA/100, Sweden). Dialysate was not collected during the first 120 min of perfusion to allow recovery from the acute effects of implantation. Dialysis in 20-min fraction were then collected in 250 μl Eppendorf tubes containing 10 μl of 0.4 M perchloric acid using a fraction collector (Carnegie Medicine, CMA/140, Sweden). Usually three 20-min control dialysates were collected before drug infusion. All dialysates were analyzed for monoamines on the day of the experiment without any further preparation or freezing. Estimates of the recovery of the monoamines through microdialysis probe were conducted in vitro. The dialysis probe was placed in a 1.5 ml Eppendorf tube containing known amounts of relevant monoamines in Ringer’s solution (10⁻⁶ M concentration of each substance). In the same perfusion and chromatographic conditions stated below, the amount of each compound in the dialysate was compared with the amount in the Ringer’s solution and expressed as percentage recovery. At the perfusion rate of 0.75 μl/min, the in vitro recovery of compounds was as follows: NE 22.5±1.3%, 3,4-dihydroxyphenylacetic acid (DOPAC) 22.3±1.4%, homovanillic acid (HVA) 21.0±1.0% (n=8).

HPLC-ECD analysis of monoamines in dialysate

Twenty min samples of the dialysate were injected onto HPLC with a reverse phase column and an electrochemical detector. The monoamines and their metabolites were separated by ion pair reverse phase chromatography (μBondapak C₁₈, 30 cm x 3.9 mm, Waters, USA), which was maintained at 32°C with column heater (Waters, USA). The mobile phase consisted of 0.05 M citric acid, 0.05 M disodium phosphate (pH 3.1), 3.2 mM 1-octanesulfonic acid (sodium salt), 0.3 mM EDTA, 14% methanol, and was pumped at a flow rate of 1.0 ml/min using a solvent delivery system (Waters, M510, USA). Samples (20 μl) were injected with a Waters U6K injector and the compounds were detected coulometrically (ESA, Model 5200A, USA) with a analytical cell (EAS, Model 5010, USA). Potential for the first and second cells were set at +0.01 V and +0.32 V, respectively. A guard cell (EAS, Model 5020, USA; potential +0.45 V) was placed before injector. Chromatograms were displayed and analyzed on a chromatographic integrator (Spectra-Physics, Chromjet, USA). The concentrations of the monoamines in the dialysate samples were determined by comparison with standard solution of NE, DOPAC and HVA (0.4, 0.8, 0.8 pmol, respectively) injected onto the column immediately before and after each experiment.

Drug infusion

From 120 min after the implantation of the microdialysis probe, 3 basal samples were collected and then the posterior hypothalamus was perfused with one of the following solution: K⁺ (90 mM) or imipramine (50 μM). In some groups of rats, the effect of systemic pargyline (75 mg/kg, i.p.) was also studied. When K⁺ was increased, Na⁺ was reduced by an equivalent amount.

Histology

At the end of experiment, the microdialysis probe was removed and brain was perfused intracardially with saline, followed by 10% phosphate-buffered formalin. Coronal sections (50 μm) of the brain were made on a cryostat (Reichert-Jung, FRC) and stained with cresyl violet. Photographs of the sections on slides were made and histological verification of the dialysis probe implantation site was made using the stereotaxic atlas of Paxinos & Watson (1988).

Data presentation and statistical analysis

Data are expressed as mean±S.E. of the percentage increase or decrease over baseline values. Baseline values were calculated as mean of first 3 sequential samples. The effects of the drugs on the posterior hypothalamic monoamine release were analyzed using one way ANOVA coupled with Duncan’s multiple range test.

Drugs and reagents

NE and its metabolites (DOPAC and HVA), reagent grade of sodium chloride, potassium chloride, perchloric acid, disodium EDTA, calcium chloride and, 1-octanesulfonic acid sodium salt, imipramine hydrochloride, and pargyline hydrochloride were purchased from Sigma Chemical Company (St. Louis, USA). Citric acid monohydrate and HPLC grade of methanol were obtained from Fisher Scientific (Fair Lawn, USA), and sodium phosphate 12 H₂O was from Wako Chemicals (Osaka, Japan).
RESULTS

Histological verification of an implantation site of dialysis probe in the posterior hypothalamus

Dialysis probe implanted for up to 5 hr produced a probe tract in the brain area. Fig. 1 shows the brain section at the level of microdialysis probe implantation in one of the experiments. The area where the dialysis probe was implanted corresponded to the posterior hypothalamus, when compared with the rats brain atlas of Paxinos & Watson (1998).

Baseline monoamine levels in the posterior hypothalamus

Dialysate collection began 2 hr after the implantation of the dialysis probe into the posterior hypothalamus. NE and metabolite levels in the dialysate were invariably stable for 2 hr after probe implantation, however, the baseline dialysate concentrations of neurotransmitters and metabolites varied among rats. Therefore, the average amount of neurotransmitter output of the last three baseline samples was taken as 100% and all subsequent post-baseline samples were expressed relative to the baseline value. The baseline level of monoamines in the dialysates were determined to be: NE 0.17±0.01, DOPAC 0.94±0.07, HVA 0.57±0.05 pmol/sample (n=8).

Effect of potassium on hypothalamic neurotransmitters and metabolites

To determine whether the monoamines measured in the dialysate derived from neuronal activity, we studied the neuronal depolarizing effect of high potassium infusion through the dialysis probe. When the posterior hypothalamus was perfused with 90 mM potassium, maximum 555% increase of NE output was observed. Concomitantly, this treatment significantly decreased the output of DOPAC and HVA by 35% and 28%, respectively (Fig. 2).

Effect of imipramine on hypothalamic neurotransmitters and metabolites

Contribution of monoamine reuptake to the clearance of NE was examined, it was found that local application of a perfusate containing imipramine (50 μM) enhanced the level of NE in the posterior hypothalamus (maximum 200%) compared to preperfusion control values. But, DOPAC and HVA output remained unchanged (Fig. 3).

Effect of pargyline on hypothalamic neurotransmitters and metabolites

To characterize further the release of hypothalamic monoamine and to validate our experimental procedure, experiments involving systemic administration of drug known to interfere with monoamine metabolism were carried out. Pargyline, an irreversible monoamine oxidase inhibitor, i.p. administered at a dose of 75 mg/kg, increased NE output (maximum 165%), while decreased DOPAC and HVA outputs (maximum 13 and 12%, respectively) (Fig. 4).
DISCUSSION

In the present study, we demonstrated the release of endogenous NE as well as extracellular levels of metabolites (DOPAC and HVA) of its precursor, dopamine, in the rat posterior hypothalamus in vivo using brain microdialysis combined with HPLC with electrochemical detection. Furthermore, using drugs known to influence on the neurotransmitter release, inactivation and metabolic processes, we characterized the release of NE in the posterior hypothalamus.

Consistent with numerous studies, we found that baseline release of NE in the posterior hypothalamus was stable for 2 hrs after the implantation of dialysis probe (Routledge & Marsden, 1987b; Schwartz et al, 1989; Itoh et al, 1990). But the baseline release of endogenous NE varied depending on the experiment. The variability in the level of the neurotransmitter might be due either to depth of anesthesia or to localization of the probe within the posterior region of hypothalamus. The basal outflow of DOPAC and HVA was much greater than that of NE.

High concentrations of potassium increased the release of NE from the posterior hypothalamus. It is well known that elevated extracellular potassium produces a sustained depolarization of the neuronal membrane and consequently initiates the release of neurotransmitters (Blaustein et al, 1972; Paton, 1979). Therefore, in the present study, the 5-fold increase in extracellular NE during perfusion with 90 mM potassium was probably derived from noradrenergic neurons in the posterior hypothalamus.

NE can be taken up and stored by monoaminergic neurons in the peripheral and central nervous system. The reuptake process into the nerve terminals appears to be an important mechanism for the inactivation of the monoaminergic neurotransmitters released at the synapse. In the present study, inhibition of monoaminergic reuptake by perfusion with imipramine produced an increase in the extracellular NE level in the posterior hypothalamus. In addition, systemic administration of pargyline, a monoamine oxidase inhibitor, increased the extracellular levels of NE. Therefore, these results demonstrate that the neuronal reuptake and metabolizing mechanisms for NE are being operated in the rat posterior hypothalamus, indicating that neurons in the posterior hypothalamus receive tonic noradrenergic input.

The levels of major metabolites of dopamine, a precursor of NE, were also monitored in the present study. One of the most unexpected results in this study was the change
of the metabolites under a variety of conditions in which the extracellular level of NE was enhanced. The potassium-stimulated release of NE was associated with a decrease in extracellular DOPAC and HVA levels. This suggests that the extracellular levels of monoamine metabolites primarily reflect intraneuronal metabolism, and only to a minor degree the metabolism of monoaminergic neurotransmitters released from the nerve terminals. Although the reason for why the extracellular levels of DOPAC and HVA are reduced after high IC induction of depolarization is unclear, there are some possible mechanisms to explain this phenomenon. Under conditions of excessive neurotransmitter release, the synthesis of neuronal NE from dopamine must be accelerated. Consequently, less dopamine might be available to the monoamine oxidase in the axoplasm. There is good evidence for compartmentalization of neurotransmitters within the monoaminergic neurons a functional pool, where the neurotransmitter is synthesized at a fast rate and preferentially released, and a storage pool (Glowinski, 1975; McMillen et al., 1980) and these neurotransmitter pools are possible source of substrate for the metabolizing enzyme, monoamine oxidase. But, monoamine oxidase might metabolize monoaminergic neurotransmitters in the axoplasmic pools of newly synthesized, preferentially released neurotransmitters or neurotransmitter that leaked from the vesicular storage sites. Thus, the potassium-evoked release of NE in the neurons induced the marked depletion in the axoplasmic NE levels, thereby resulting in a decrease in the amine substrate for monoamine oxidase. Consequently, the outputs of DOPAC and HVA were decreased. Another explanation of these results is that potassium has the capacity to inhibit neuronal uptake of the transmitter. Increasing concentrations of potassium have been reported to inhibit neuronal uptake of [3H]-norepinephrine and [3H]-dopamine in a concentration-dependent manner (Colburn et al., 1968; Horn et al., 1971). In the present study, blocking the reuptake mechanism by imipramine did not change the extracellular levels of DOPAC and HVA. Westerink et al. (1987) & Zettenstrom et al. (1988) also reported that the potent DA reuptake inhibitor, nomifensin, when locally infused into striatum, did not change the level of extracellular striatal DOPAC, although the levels of extracellular DA were enhanced. As expected, the levels of metabolites dramatically decreased after systemic pargyline administration. Taken together, it is unlikely that the effect of high potassium concentration is related to the inhibition of neuronal reuptake of the neurotransmitters.

In conclusion, our results suggest that NE levels in dialysate from the rat posterior hypothalamus are of neuronal origin. These studies provide further support for the use of in vivo microdialysis to monitor the release of neurotransmitters from discrete brain areas of the anesthetized animal. We also found that manipulations which profoundly affected the levels of extracellular neurotransmitter had a concomitant effect on metabolite levels.

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