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DIFFERENTIAL DAMAGE BY HYPOXIA TO DOPAMINE AND SEROTONIN NERVE TERMINALS

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Serotonin and dopamine resemble each other in terms of their synthetic and degradative pathways, as well as in terms of structural features. However, responses to a low-oxygen environment differ between these two compounds. In this experiment, we studied the fluctuations of levels of serotonin, dopamine and their metabolites in the striatum of the rat brain under low-oxygen conditions using a microdialysis technique. Comparison of the extents of increases in extracellular levels of dopamine and serotonin, accompanied by decreases in levels of metabolites, indicates that the effects of hypoxia on the two types of neuronal terminal are different. This study suggests that dopamine plays a more important role than serotonin in the mechanism of irreversible destruction of neurons during moderate hypoxia.

Serotonin (5-HT) and dopamine (DA) act as neurotransmitters in the brain and their physiological roles are very important. Fluctuations in levels of these substances have long been recognized as problems under low oxygen conditions (1,2,3,4). Previously, using an in vivo microdialysis method we found that the rate of DA release increases transiently to 100 times the basal rate in the striatum of the rat brain, during inhalation of 5 % oxygen (PaO_2=23-24 mm Hg) (5). In this experiment, we studied fluctuations in the levels of 5-HT and its metabolite 5-HIAA, as well as of DA and related metabolites, in the striatum of the rat brain under low-oxygen conditions.

Catecholamine and 5-HT neurons exhibit many similarities. The cell bodies are predominantly located in the brainstem and project axons to the cerebrum (6). The high-affinity uptake of catecholamine and 5-HT are both mediated by energy-dependent processes and have similar Km in

Abbreviations used: 5-HT, 5-hydroxytriptamine(serotonin) ; DA, dopamine ; ECF, extracellular fluid ; HVA, homovanillic acid ; DOPAC, 3,4-dihydroxyphenylacetic acid ; 5-HIAA, 5-hydroxyindoleacetic acid ; MAO, monoamine oxidase.

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the same concentration range (7,8). Serotonin is synthesized through a process of decarboxylation and hydroxylation as is dopamine, but DA is synthesized from tyrosine while 5-HT is synthesized from tryptophan. Moreover, both metabolic pathways involve oxidation by monoamine oxidase. Therefore, the synthetic and metabolic pathways of these two compounds are very similar. However, their responses to a low-oxygen environment in terms of released, tissue and metabolites levels differ from one another.

METHODS

Male Wistar rats (weighing 250-300 g) were initially anesthetized with a mixture of 2% halothane, 30% oxygen and a balance of nitrous oxide in a chamber. Alcuronium chloride (1 mg/kg) was administered to the animals by intraperitoneal injection followed by intubation and rats were subsequently ventilated mechanically by a rodent respirator (HARVARD APPARATUS, U.S.A) with the same anesthetic gas during surgery. Animals were exposed to a mixture of 5% O_2-95% N_2 gas for hypoxic treatment. After 20 min of exposure to this mixture, reoxygenation was initiated. Halothane anesthesia was maintained during the hypoxic period to alleviate pain, but the anesthetic effects of halothane itself on the release of catecholamines had been investigated beforehand. The animals were mounted on a Kopf stereotaxic instrument with the incisor bar set 3.3 mm below the interaural line. The skull was exposed and a burr hole was drilled directly above the striatum. A dialysis probe (membrane length = 3 mm for the striatum, 2 mm for the hippocampus) was implanted into the left striatum with the following coordinates relative to bregma: A +0.2; L +2.5; V -6.0 A +4.8; L +4.6; V -8.5, for the hippocampus, according to the atlas of Paxinos and Watson (9). The probe was continuously perfused (2 µl/min) with a Ringer solution by means of a microinfusion pump. Sample collection was initiated after a stabilization period to avoid the effect of insertion of the probe. For two hours after the operation, samples were collected over the course of 10-min intervals. The localization of the tip of the probe was verified visually at the end of each experiment. Moreover, the levels of DA in striatal tissues was compared with that of 5-HT. At each time point, the rats were sacrificed by microwave irradiation. Brains were removed and dissected into seven regions on an ice-chilled sheet of glass by the method of Glowinski and Iversen (10).

The microdialysis probe was prepared using cellulose ester tubing (o.d., 270 µm; ASAHI Medical Co., Ltd, Tokyo, Japan), which was glued onto the frame of a microdialysis probe, obtained from Carnegie Medicin (Stockholm, Sweden) and replace for each experiment. Dialysates and extracts of brain tissue were directly injected into a reversed-phase ion-pair HPLC-ECD (high-performance liquid chromatography-electrochemical detection) system for measurement of 5-HT, DA, DOPAC, HVA and 5-HIAA. The mobile phase consisted of 0.05 M sodium acetate/0.035 M citric acid buffer (pH 3.9) containing 0.1 mM EDTA Na2 (ethylenediamine tetra-acetic acid disodium salt), 0.5 mM sodium octane sulfonate and 15% (v/v) methanol. Monoamines were separated at 30 °C on a 150 x 4.6 mm stainless-steel column prepacked with 5-µm particles of Yamamura ODS-AQ (Yamamura Chemicals Lab. Co., Ltd, Kyoto, Japan), at a flow rate of 1.0 ml/min. Monoamines were detected by a carbon-paste working electrode set at +0.75 V. The calculated limit for detection of DA and 5-HT with our methods was 5 fmol. Statistical differences were tested by the Mann-Whitney U test. All reagents for HPLC were of analytical grade and were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Synthetic monoamine standards were obtained from Sigma Chemical (St. Louis, MO, U.S.A.).

RESULTS

Baseline values (n = 9) were as follows: DA, 16.3 ± 3.8 fmol/min; 5HT 4.7± 0.4 fmol/min, DOPAC, 780.3 ± 29.1 fmol/min; HVA, 968.0 ± 38.5 fmol/min; and 5-HIAA, 282.1 ± 21.3 fmol/min. Extracellular concentrations of DA were markedly increased during the hypoxic episode. The concentrations of serotonin were also increased during hypoxia, but the extent of 5-HT increase was smaller than that of DA (Fig. 1). By contrast, hypoxia produced a considerable decrease in the extracellular concentrations of DOPAC, HVA and 5HIAA (Fig. 2) However, extracellular concentrations of 5-HIAA declined to a lesser degree (to 75.5 ± 5.6% of baseline) than did the levels of the metabolites of DA (DOPAC, ; 48.0 ± 8.8%, HVA, ; 44.5 ± 9.3%) during hypoxia (Fig. 2). The level of DA recovered rapidly, returning to normal within 10-20 min in room air after a hypoxic episode. The level of 5-HT also recovered similarly but with a slight time lag. During the 60-min recovery period, concentrations of DOPAC, HVA and 5-HIAA returned to control values.

In striatal tissue, there were also the increases in the content of DA after hypoxia as were the increase of extracellar levels (Table I). In rats pkilled immediately after 20 min of hypoxia, striatal concentrations of DA were 15.12 ± 0.78 pmol/mg of tissue, compared with 12.24 ± 0.91 pmol/mg of tissue in controls. By contrast, striatal concentrations of 5-HT were almost unchanged by hypoxia.

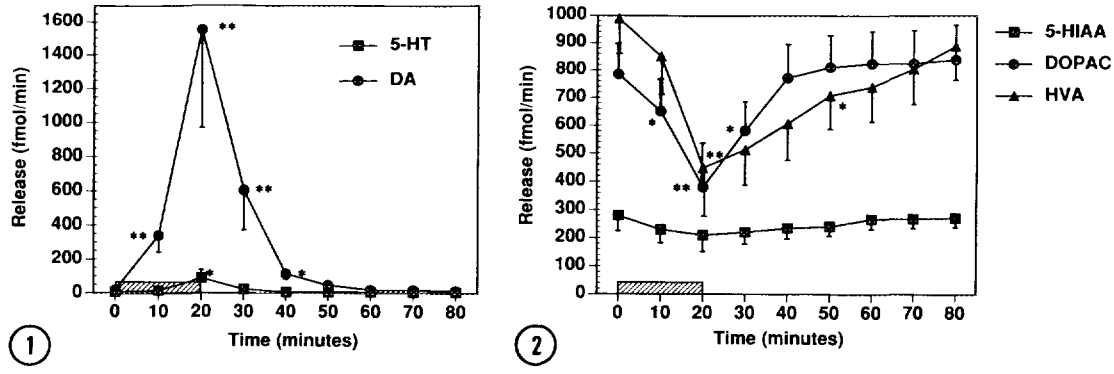

Fig. 1. Changes in extraneuronal levels of DA and 5-HT in the striatum under hypoxic conditions. Microdialysis sampling was performed at 10 min intervals starting at 2h after probe insertion and was collected at 10 min intervals. Animals were exposed to 5% oxygen-95% nitrogen mixed gas for a period of 20 min (shaded zone) followed by reoxygenation during subsequent recovery period. Data are mean + or - SEM. * P<0.05; ** P<0.01 vs control group (by the Mann-Whitney U test).

Fig. 2. Changes in extraneuronal levels of DA metabolites and 5-HT metabolite in the striatum under hypoxic conditions. Experimental procedures were as described in legend of Fig. 1.

Table I. Effects of hypoxia on tissue concentrations of DA, 5-HT and their metabolites

Compound	Concentration (pmol/mg of wet tissue)		
	Control	Hypoxia	Recovery
DA	12.24±0.91	15.12±0.78*	12.82±0.97
DOPAC	3.01±0.37	1.82±0.34*	2.87±0.38
HVA	2.09±0.18	1.92±0.39	2.11±0.23
5-HT	1.02±0.06	1.09±0.09	1.04±0.13
5-HIAA	0.95±0.06	0.72±0.08	0.88±0.08

Control (n=6) and hypoxic (n=6) rats were killed by microwave irradiation. In rats exposed to 5% oxygen-95% nitrogen mixed gas, tissue concentration of DA was higher than in controls (*; $p < 0.05$) After 60-min reoxygenation period, recovery animals (n=6) were killed.

The concentrations of the metabolite of 5-HT , namely, 5-HIAA and of the metabolites of DA, namely, DOPAC and HVA, were depressed immediately after hypoxia but gradually increased to 0.9-1.4 times the control level within 60 min.

This study suggests the probability of greater damage or loss of DA nerve terminals than of 5-HT terminals during hypoxia. In the region of the hippacampus, the difference in terms of hypoxic injury between DA and 5-HT nerve terminals was quite marked. Extracellular DA is not normally detectable in the hippocampus, so we cannot calculate the extent of the increase in this parameter. However, more DA was released than 5-HT under hypoxic conditions as clearly shown by the chromatogram in Figure 3. At postmortem (within 5 min after cardiac arrest), evidence of the maximum release of neurotransmitter was found, perhaps because of the destruction of reuptake mechanisms (11). In this situation, levels of DA and 5-HT were not significantly different. Therefore, the DA nerve terminals appear to be more vulnerable to cerebral hypoxia than the 5-HT nerve terminals (Fig. 3).

DISCUSSION

Our data demonstrate that, in rats exposed to moderate hypoxia, differential changes occur in the metabolism of striatal DA and 5-HT. However, our the observations do not explain why extraneuronal levels of DA are increased to a greater extent than those of 5-HT. It is not clear why 5-HT terminals would not also be subject to the same stress.

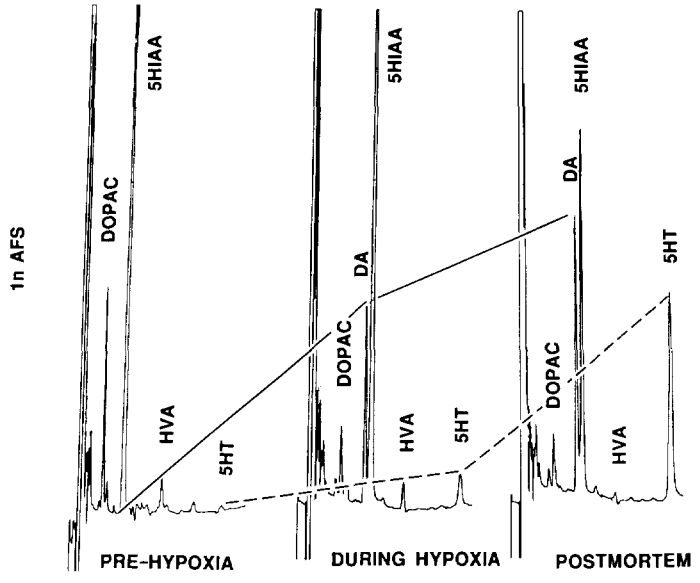

Fig. 3. Chromatogram showing the difference between DA and 5-HT fluctuations in the hippocampus. Dopamine is not detectable under normal conditions, and increased markedly by exposure to a hypoxic gas mixture. Solid line shows the fluctuation of levels of DA. Dotted line shows the fluctuation of levels of 5-HT.

Fluorescence-histochemical studies in the gerbil stroke model have demonstrated a decrease in intraneuronal stores of catecholamine. The catecholamine-specific fluorescence was noted in regions adjacent to catecholamine nerve terminals and also within glia (12). This result suggests the extensive release of catecholamines into the extraneuronal and extracellular space.

After 20 min of hypoxia, both levels of extracellular DA and tissue levels of DA rose. Although Tissue levels of neurotransmitter represent the combined extra- and intraneuronal concentrations but tissue levels reflect intraneuronal concentrations to a greater extent. Thus, discrepancy between levels in dialysates and in tissues of metabolites is probable. The elevation in tissue levels of DA indicates that total intracellular levels of DA are elevated. These changes could be explained by a decreased rate of release, a decreased rate of intracellular degradation, an increased rate of reuptake, or an increased rate of synthesis of DA during hypoxia.

Assays of [^{3}H]-DA uptake in synaptosomal preparations from the striatum of 7-day-old rats exposed to 8% oxygen for 2 h demonstrated the suppression of reuptake (13). Thus, reduced reuptake activity may well account for the increased levels of DA in the ECF (extracellular fluid) during hypoxia. Additionally, the differential reduction in the rate of uptake of tracer-labeled DA relative to the rate of 5-HT was observed in gerbil ischemic brains (1). However, Lavyne et al. (14) postulated

that the selective reduction of incorporation of [^{3}H]-DA and [^{3}H]-NE into synaptosomes during cerebral infarction represents greater leakage of catecholamines from the synaptosomes, rather than greater damage to the mechanism for uptake of catecholamines.

During exposure of rats to hypoxia, concentrations of HVA, DOPAC, and 5-HIAA in the striatum declined. Reduced concentrations of ECF metabolites levels of neurotransmitters could reflect changes in rates of production or removal of the metabolites.

The activity of the degradative enzyme, monoamine oxidase (MAO), may be impaired under hypoxia, and decreased rates of degradation could account for the observed reductions in levels of HVA and DOPAC. Serotonin is also metabolized by MAO to 5-HIAA, but levels of 5-HIAA were not depressed to the same extent as those of DOPAC or HVA during hypoxia. The reuptake of released 5-HT would result in its degradation by MAO in mitochondria at nerve terminals. MAO at serotonergic nerve terminals appears to remain active in our hypoxic model.

Similarly, changes in cerebral blood flow are unlikely to account for the decreased levels of metabolites of DA and 5-HT. Any increased washing out of metabolites as a result of increased blood flow would be expected to affect levels of metabolites of DA and 5-HT to the same extent. However, differences in the extent and temporal patterns of metabolite suppression and the subsequent recovery between DA metabolites and 5-HIAA were apparent.

It is possible that the increased extraneuronal levels of DA observed under moderate hypoxia may contribute to the pathogenesis of neuronal injury. Under lethal hypoxic conditions, levels of extraneuronal 5-HT increase markedly. In such cases, serotonin stimulates the influx of Ca^{2+} (15) and may be a major contributor to neuronal injury. In fact, Klisch et al. (16) demonstrated protective effect of an antagonist of 5-HT_2 receptors during forebrain ischemia. Whether a marked increase in levels of 5-HT is induced or not may determine the subsequent outcome.

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