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Effects of Angiotensin II on Brain Monoamines in Nonhypoxic and Hypoxic Mice

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GEORGIEV, V., S. STANCHEVA, D. GETOVA, L. ALOVA AND M. OPITZ. *Effects of angiotensin II on brain monoamines in nonhypoxic and hypoxic mice.* PHARMACOL BIOCHEM BEHAV **50**(3) 457–461, 1995.—The effects of angiotensin II (ATII) administered intracerebroventricularly (ICV, acute) and subcutaneously (SC, acute and chronic) on acute hypoxia (asphyctic and hemic), and on the forebrain concentrations of monoamines dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in male mice were studied. ATII in both routes of administration exerted a decrease of the latency to hypoxia-induced convulsions. ATII slightly reduced the brain levels of DA and NE, and did not change those of 5-HT in hypoxic mice. ATII significantly reduced DA and 5-HT concentrations in nonhypoxic (normoxic) mice. Taken together, the results suggest that ATII-induced increase of susceptibility to hypoxia is accompanied by slight alterations in the brain monoamine metabolism.

Hypoxia (asphyctic, hemic) Mice Angiotensin II Brain Monoamines

ANGIOTENSIN II (ATII) has a large number of effects with considerable biologic significance. These include actions on membrane function, protein synthesis, cell growth, hormone synthesis, maintenance of salt and volume homeostasis, blood pressure control, stimulation of dipsogenic behavior (thirst and drinking), and stimulation of release of pituitary hormones (3,4,14,16,21). In recent years, it has been demonstrated that ATII markedly alters some types of behavior, including seizure susceptibility, exploratory behavior, and aversively motivated behavior (active and passive avoidance) (5,6).

Hypoxia (low oxygen) causes pronounced changes in mental function and is a model of metabolic encephalopathies. Tissue oxygen deficiency changes the whole cerebral biochemical process, and especially neurotransmitter metabolism. Monoamine neurotransmitters are vulnerable to hypoxia (2, 11,15).

ATII is known to enhance sympathetic neuroeffector transmission (19). Monoamines participate in the realization of some behavioral responses (learning and memory, exploratory behavior, seizure susceptibility, and stress) to centrally administered ATII (5–9).

The present study was undertaken to clarify the role of the monoamines dopamine (DA), norepinephrine (NE), and serotonin (5-hydroxytryptamine, 5-HT) in the action of ATII in nonhypoxic and hypoxic mice.

METHOD

Subjects

The experiments were carried out on 132 male albino mice (ICR strain), weighing 20–24 g, housed in cages and given food and water ad lib. All experiments were performed between 0900 a.m. and 1300 h.

Experimental Models of Hypoxia

Asphyctic hypoxia. The mice were placed individually in 250-cm^3 glass flasks, which were tightly closed with pressed plastic lids. The resulting hypoxia usually led to convulsions and death of the animals. Survival time (in minutes), or the interval between the moment the mouse was put into the flask and the occurrence of generalized clonic convulsions, was measured for each mouse.

Haemic hypoxia. Hypoxia was induced by 300 mg/kg sodium nitrite ($NaNO_2$) injected subcutaneously (SC). Survival time (in minutes), or the interval between the injection of sodium nitrite and the occurrence of generalized clonic convulsions, was measured for each mouse.

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Drugs

ATII (human form, synthetic; Sigma Chemical Co, St. Louis, MO) was dissolved in 0.01 N acetic acid (2.0 mg/ml) and kept as a stock solution at −40°C until used. From the stock solution, different concentrations of ATII (ICV administration: 0.5, 1.0, and 2.0 μg per mouse; and SC administration: 2.0 and 200 μg/kg body wt.) using saline as a solvent were prepared.

Vehicle contained 0.1 ml 0.01 N acetic acid and 9.9 ml saline. The respective controls received the vehicle, which was prepared in the same manner as an ATII solution.

Experimental Procedure

Mice were divided to the following groups (six mice per group): control nonhypoxic animals injected with vehicle ICV (single administration) and SC (single and 14-day administration); nonhypoxic animals injected with ATII ICV (0.5, 1.0, and 2.0 mμg per mouse, single administration) and SC (2.0 and 200 μg/kg body wt., single and 14-day administration); hypoxic animals (asphyctic and hemic) on the background of vehicle, which was injected ICV (single administration) and SC (single and 14-day administration); and hypoxic animals (asphyctic and hemic) on the background of ATII, which was injected ICV (0.5, 1.0, and 2.0 μg per mouse, single administration) and SC (2.0 and 200 μg/kg body wt., single and 14-day administration).

ATII was administered ICV in the right lateral cerebral ventricle 5 min and SC 15 min before exposure to asphyctic or hemic hypoxia. The volume of injections in the cerebral ventricle was 5 μl at a rate of 1 μl/5 s. Mice were briefly anaesthetized with ether. A midline incision was made along the scalp, and the skin and underlying fascia were removed bilaterally. A burr hole was drilled in the bone situated above the right lateral ventricle (0.3 mm posterior to bregma and 1 mm lateral to the midsagital suture). ATII was injected ICV 24 h after the operation through a 5-μl Hamilton syringe (the tip of the blunt needle of syringe was terminated in the ventricule, 2.75 mm below the dura mater; Hamilton, Reno, NV). A group of 20 mice was injected also ICV with methylene blue to confirm the injection site. The animals were sacrificed immediately after the injection, the brain were removed, and the diffusion of the dye via the third to the fourth ventricle was verified.

Tissue Preparations and Biochemical Determinations

Immediately after generalized clonic convulsions from asphyxia, the mice were decapitated. All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals (Bulgaria, 1991). The forebrains were rapidly removed on ice and were kept frozen at −40°C for about 24 h. The tissue samples were weighed and homogenized in 5 ml butanol and an appropriate amount of 0.01 N HCl (12). The homogenate was centrifuged at 1500 rpm for 10 min. A 2-ml aliquot of butanol supernatant was poured into the centrifuge tube containing 1.5 ml of 0.1 M phosphate buffer, pH 6.5. Another 2 ml of butanol supernatant, 5 ml heptane, and 0.5 ml of 0.1 N HCl were placed in a centrifuge tube for the analysis of 5-HT. Both mixtures were stirred on a vortex for 20 s. NE and DA were extracted into the phosphate buffer and 5-HT was extracted into the 0.1 N HCl. The samples were centrifuged at 3000 rpm to separate the organic and aqueous layers, and the top organic layer was aspirated using a vacuum with a liquid trap. NE and DA in phosphate extract and 5-HT in 0.1 N HCl extract were oxidized into fluorophores according to Jacobowitz and Richardson (12), and the levels of biogenic monoamines were expressed in micrograms of monoamine per gram of fresh brain tissue.

Data Analysis

Data were analyzed by a multifactor analysis of variance (ANOVA). Posthoc comparisons were performed by means of the two-tailed Student *t*-test, with statistical significance at $p \leq 0.05$.

RESULTS

Table 1 summarized the effects of acute (single) ICV and acute and chronic (for 14 days) SC administration of ATII on

TABLE 1

EFFECTS OF ANGIOTENSIN II INJECTED ICV AND SC ON ACUTE HYPOXIA IN MICE

Treatment	Dose	Route of Administration	Asphyctic Hypoxia (Mean ± SEM)	Hemic Hypoxia (Mean ± SEM)
Single administration of ATII				
Control vehicle		ICV	40.33 ± 1.66	22.83 ± 1.01
ATII	0.5 μg	ICV	26.33 ± 1.74*	18.50 ± 0.62†
ATII	1.0 μg	ICV	32.00 ± 1.53†	17.67 ± 0.61*
ATII	2.0 μg	ICV	28.66 ± 2.03*	27.00 ± 2.0
Single administration of ATII				
Control vehicle		SC	44.00 ± 0.73	29.50 ± 1.15
ATII	2.0 μg/kg	SC	32.50 ± 0.85‡	17.85 ± 0.94*
ATII	200.0 μg/kg	SC	27.17 ± 1.53‡	18.33 ± 0.80*
14-day administration of ATII				
Control vehicle		SC	42.30 ± 0.85	27.15 ± 1.25
ATII	2.0 μg/kg	SC	34.83 ± 5.07	12.83 ± 2.30
ATII	200.0 μg/kg	SC	30.50 ± 1.68	12.67 ± 0.67*

Each group contained six mice. The latency (in minutes) to the first hypoxic seizures was measured.
*$p \leq 0.01$ vs. controls.
†$p \leq 0.05$ vs. controls.
‡$p \leq 0.001$ vs. controls.

the latency to the first hypoxia-induced convulsive seizures. ATII administered ICV at doses of 0.5, 1.0, and 2.0 μg per mouse significantly decreased the latency to seizure occurence both in asphyctic and hemic hypoxia (except for the dose of 2.0 μg per mouse in hemic hypoxia, where latency tended to increase). ATII administered SC at single doses of 2 and 200 μg/kg body wt. also significantly decreased the latency to seizures both in asphyctic and hemic hypoxia [$F(2, 83) = 25.17$, $p \leq 0.05$].

On chronic SC administration (2 and 200 μg/kg body wt.), ATII tended to decrease the latency to seizures (at a dose of 200 μg/kg body wt. in hemic hypoxia, the difference vs. controls was significant). ANOVA did not reveal any significant difference in the latency to seizures between ICV and SC (acute and chronic) routes of administration of ATII [$F(1, 83) = 2.62$, $p \leq 0.05$].

The DA level in the forebrain significantly decreased by ATII at a dose 2.0 μg per mouse ICV (Fig. 1—group 3) as compared to control nonhypoxic (normoxic) mice (Fig. 1—group 1) and unsignificantly decreased as compared to hypoxic (asphyctic and hemic) mice (Fig. 1—group 2). The DA level was significantly decreased only in hemic hypoxia (Fig. 1—group 2). The effect of hypoxia on the background of ATII did not differ from the independent effect of ATII: $F(2, 65) = 13.04$, $p \leq 0.05$. ANOVA revealed no significant difference in the DA level between ICV and SC (acute and chronic) routes of administration of ATII.

ANOVA revealed both hypoxia and ATII treatment as significant variables, determining a decrease of brain NE level [hypoxia: $F(2, 63) = 4.77$, $p \leq 0.05$; ATII: $F(1, 63) = 14.79$, $p \leq 0.05$]. The forebrain level of NE was significantly reduced in the two models of hypoxia without ATII (Fig. 2, group 2) and with ATII at a dose of 2.0 μg per mouse ICV (Fig. 2, group 4) vs. nonhypoxic controls (Fig. 2, group 1). The NE level after ATII at a dose of 2.0 μg per mouse ICV (Fig. 2, group 3) did not differ compared with control nonhypoxic (normoxic) mice (Fig. 2, group 1) or with hypoxic (both types) mice (Fig. 2, group 2). ANOVA revealed no significant differences in the NE level between the ICV and SC (acute and chronic) routes of administration of ATII.

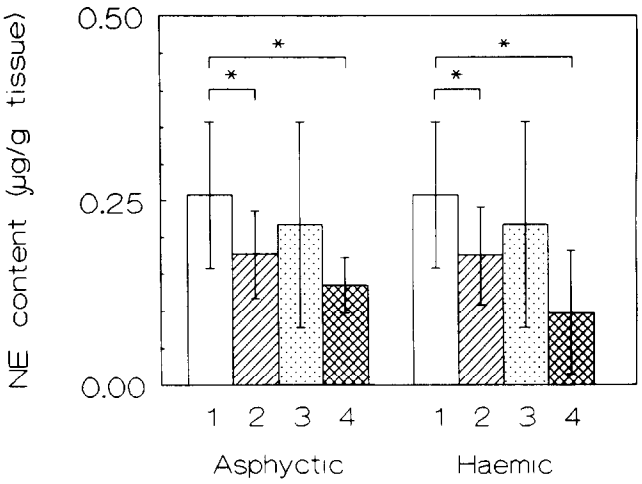

FIG. 2. Effects of ATII (ICV) and hypoxia on the NE level (μg/g tissue ± SD) in the forebrain of mice. 1, control group (normoxia); 2, hypoxia; 3, ATII (2 μg per mouse, ICV) in normoxia; 4, hypoxia on the background of ATII. *$p \leq 0.05$

The forebrain level of 5-HT was significantly reduced by ATII at a dose of 2.0 μg per mouse ICV only in nonhypoxic (normoxic) mice (Fig. 3, group 3) compared with nonhypoxic controls (Fig. 3, group 1). The 5-HT level tended to decrease after hemic hypoxia. ANOVA revealed no significant differences in the forebrain 5-HT level between the ICV and SC (acute and chronic) routes of administration of ATII.

DISCUSSION

Our study showed that the octapeptide ATII administered acutely and chronically in mice shortened the latency to hypoxia-induced convulsions (both in asphyctic and hemic hypoxia). ATII significantly decreased the NE level in the forebrain of hypoxic mice in comparison with control animals and

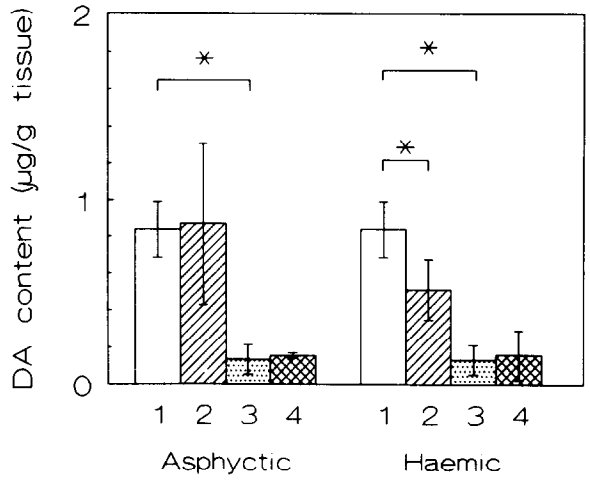

FIG. 1. Effects of ATII (ICV) and hypoxia on the DA level (μg/g tissue ± SD) in the forebrain of mice. 1, control group (normoxia); 2, hypoxia; 3, ATII (2 μg per mouse, ICV) in normoxia; 4, hypoxia on the background of ATII. *$p \leq 0.05$

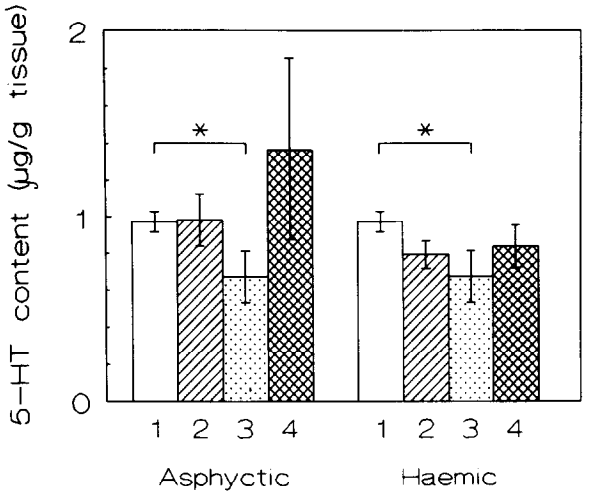

FIG. 3. Effects of ATII (ICV) and hypoxia on the 5-HT level (μg/g tissue ± SD) in the forebrain of mice. 1, control group (normoxia); 2, hypoxia; 3, ATII (2 μg per mouse, ICV) in normoxia; 4, hypoxia on the background of ATII. *$p \leq 0.05$

did not significantly reduce the DA level in hypoxic animals. The 5-HT level in hemic hypoxic mice was slightly reduced. ATII significantly decreased the DA and 5-HT levels in the forebrain of nonhypoxic (normoxic) mice. Our findings on the neurochemical effects of hypoxia are consistent with some data in the literature. Thus, a decrease in the brain levels of DA and NE has been reported in hemic hypoxia (1). During hypoxia a significant decline in tyrosine hydroxylase activity was observed in different brain areas (hemispheres, striatum, and midbrain) (10). Hypoxia reduces the NE level mainly in the hypothalamus and cortex, which is associated with a reduced NE turnover (11). Thus, the hypoxia-induced reduction of DA and NE reflects the decline in central DAergic and noradrenergic activity. The brain level of 5-HT and the brain tryptophane hydroxylase activity are slightly affected by hypoxia (10), but severe hypoxia reduces the brain 5-HT concentration (13). The lack of a significant difference in the effects of ATII on the monoamine levels between both routes of administration might be due to the satisfactorial transfer of ATII through the blood-brain barrier.

The decreased brain concentrations of DA and NE after ATII in normoxic conditions could be explained by the increased DA and NE use, turnover, and release. It is not clear, however, whether these effects result from direct action of ATII on DA- and NE-containing nerve terminals or from an indirect action on DAergic and noradrenergic neurons. Our findings are in agreement with the general opinion that brain catecholamines have an integral role in mediating the central effects of ATII (5-9).

It is possible that ATII performs as a neuromodulator in the brain by modifying synaptic transmission in normoxic and hypoxic conditions. This role of ATII has been supported by studies that indicate that ATII alters the synthesis, release, and neuronal uptake of catecholamines by interacting with specific ATII receptors on catecholaminergic nerve terminals (18,19). ATII increases the release of sympathetic transmitter by activating prejunctional ATII receptors (24). On single ICV administration, ATII increases NE in the midbrain and cerebral cortex under normal conditions (9). In conscious rats, ATII, administered ICV, significantly increases NE release in the paraventricular nucleus of the hypothalamus, as shown by microdialysis coupled to high performance liquid chromatography with electrochemical detection. This effect is abolished by an AT_1 receptor nonpeptide antagonist, DuP 753, demonstrating that it is mediated by an AT_1 receptor (17).

Many findings suggest the participation of transmitter mechanisms (DAergic, noradrenergic, GABAergic, and cholinergic) in the behavioral effects of ATII (5-8,23). ATII influences NE release in brain regions as hypothalamus, thalamus, and amygdala of stressed rats (9), which may determine the suppressive effects of ATII on the behavioral responses upon defensive burying paradigms in rats (20).

Hypoxia may change the ability of ATII receptors to influence NE release at the presynaptic level, where the presynaptic α_2-receptors (autoreceptors) usually inhibit NE release (17). This in turn would change the interactions between ATII receptors (both subtypes) and α_2-autoreceptors—that is, the ability of ATII presynaptically to control NE release.

In conclusion, ATII decreases the latency to acute hypoxia (asphyctic and hemic)-induced convulsions. This effect of ATII is associated with nonsignificantly reduced levels of DA and NE in the forebrain of mice. No significant changes in the brain concentration of 5-HT were evaluated both in hypoxia and in hypoxia with ATII. Taken together, ATII-induced changes in hypoxia (prohypoxic effect) are accompanied by slight alterations in the monoamine metabolism.

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