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Effect of 1-Amino-5-Bromouracil on Brain Monoamine Metabolism in Rats

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MIYAZAKI, S., M. IMAIZUMI AND H. MACHIDA *Effect of 1-amino-5-bromouracil on brain monoamine metabolism in rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 471–475, 1994.—The effect of 1-amino-5-bromouracil (ABU), a novel central-acting agent, on monoaminergic neurotransmitter levels of rat brain was investigated. Under the nonstressed condition, ABU (20 and 30 mg/kg intraperitoneally [IP]) did not affect monoamine metabolism, whereas diazepam (5 mg/kg IP) increased the 3-methoxy-4-hydroxyphenylethylene glycol (MHPG)/noradrenaline (NA) ratio. One-hour immobilization stress increased the MHPG/NA ratio in various brain regions of drug-naive rats, but did not increase the homovanilic acid (HVA) plus 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) ratio or the 5-hydroxyindole acetic acid (5-HIAA)/serotonin (5-HT) ratio. Pretreatment with ABU or diazepam suppressed the activation of noradrenergic neurons induced by immobilization stress. By contrast, electric foot shock stress increased the MHPG/NA and HVA + DOPAC/DA ratios. Pretreatment with ABU or diazepam suppressed the activation of noradrenergic and dopaminergic cortical neurons by electric foot shock stress. These results indicate that these two physiologic stresses affected monoaminergic neurons differently and that their effects were suppressed by ABU and diazepam.

1-Amino-5-bromouracil (ABU) Anxiety Immobilization stress Electric foot shock stress Monoamine metabolism

1-AMINO-5-BROMOURACIL (ABU), a novel central-acting agent, shows anxiolytic effects in Geller-type conflict tests in rats and in Vogel-type conflict tests in mice (10,12). It also suppresses spontaneous locomotor activity, potentiates drug-induced anesthesia, and causes myorelaxation and anesthetic activity in mice. The pharmacologic profile of ABU is similar to that of diazepam, but ABU is more selective in its anti-conflict effect than is diazepam. ABU has not shown an affinity for the benzodiazepine receptor, and is a candidate for a new type of anxiolytic that has a unique action. In a previous drug discrimination study, we found that the discriminative stimulus properties of ABU were similar, but not identical, to those of diazepam (11).

Many studies have reported that different kinds of stress can induce activation of different types of monoamine metabolism and the release of monoaminergic neurotransmitters in rodent brain. Immobilization stress has been shown to increase noradrenaline (NA) turnover (8,20), NA release (23), and serotonin (5-HT) turnover (17); electric foot shock stress has been shown to increase dopamine (DA) turnover (6,7) and 5-HT turnover (14). Psychological stress also increases NA release (22) and 5-HT turnover (14). These stress-induced monoaminergic neuronal activations are reportedly suppressed by anxiolytic benzodiazepines, such as diazepam (3,8,9,16,22), suggesting an anti-stress activity. Because the activation of turnover of monoaminergic neurotransmitters is related to fear and anxiety, the anxiolytic effect of diazepam may involve this activity (8,16).

In the present study, we investigated the antistress activity of ABU and its effect on turnover of brain monoaminergic neurotransmitter, compared with that of diazepam, to help elucidate the mechanism of the central action of ABU.

METHOD

Subjects

Male Wistar rats (Clea Japan, Inc., Tokyo, Japan) were used at 10 weeks of age. The animals were housed under standard conditions (23 ± 1°C, 55 ± 5% humidity, light-dark cycle with the light on between 6000 and 1800 h), with free access to water and food. We cared for these animals accord-

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ing to "Guidelines for Animal Experimentation" (by the Japanese Association for Laboratory Animal Science, 1987). The present experimental protocol was planned according to these guidelines and permitted by the Animal Committee in our company.

Drugs

Diazepam was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). ABU was synthesized by Yamasa Corporation (Choshi, Japan). The drugs were suspended in physiologic saline containing 0.5% carboxymethyl cellulose and were injected intraperitoneally (IP) in a fixed volume of 0.1 ml/100 g body wt.

Stress Procedure

Immobilization stress was applied by enclosing rats in flexible wire mesh for 60 min, as previously described (8). The procedure for electric foot shock stress was as follows: Rats were placed in the shock chamber with a grid floor (23 cm in width, 27 cm in length, and 26 cm in height), and exposed to inescapable electric foot shocks (100 V, 15 s), with a fixed 20-s resting interval, for a total of 30 min.

Experimental Procedure

The effects of diazepam (5 mg/kg) and ABU (20 and 30 mg/kg) on the turnover of neurotransmitters in brain regions were examined in nonstressed and stressed rats. The rats in the nonstress groups were injected with vehicle or drugs, 30 min before being killed, and were then placed in their home cages. The rats in the stress groups received the same injections and were exposed to immobilization stress or electric foot shock stress 5 or 15 min after the injections, respectively. The nonstressed controls for the immobilization stress were injected with vehicle and placed in their home cages for 65 min. As a control for electric foot shock stress, the nonstressed rats were injected with vehicle and placed in the shock chamber without electric foot shock for 15 min.

Tissue Preparation and Biochemical Determination

Immediately after release from the stress, the rats were killed by microwave irradiation (5 kW, 1.2 s) using a microwave applicator (TMW-6402; Toshiba, Tokyo, Japan), and decapitated. The brains were removed and dissected into the following discrete regions, as previously described (5), with some modifications: cerebral cortex, amygdala containing pyriform cortex and temporal cortex, striatum, hippocampus, midbrain, thalamus plus hypothalamus, and pons plus medulla oblongata. Immediately after the dissection, the tissues were frozen in dry ice, weighed, and stored at −80°C until assayed. The brain tissues were homogenized with an ultrasonic cell disrupter (Model US-150T; Nihonseiki Co. Ltd., Tokyo, Japan) in ice-cold 0.2 M perchloric acid containing 0.1 mM Na_2-EDTA, 0.3 mM Na_2SO_3, and 100 ng of isoproterenol as an internal standard. The homogenates were adjusted to pH 3.0 by adding 1 M CH_3COONa and centrifuged at 18,000 × *g* for 10 min. The supernatants were filtered through a 0.45-μm membrane filter and applied to the HPLC system to detect the presence of NA, 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), DA, homovanilic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, and 5-hydroxyindole acetic acid (5-HIAA). The details of the procedure for HPLC analysis have been described previously (13).

Statistical Analysis

The 5-HT, DA, and NA turnover rates were obtained from the ratio of concentrations of 5-HIAA and 5-HT, HVA plus DOPAC and DA, and MHPG and NA, respectively. Each value was converted to a percent of controls and expressed as the mean ± SE. Statistical analysis was performed using the two-tailed Student's *t*-test.

RESULTS

Table 1 shows the basal levels of monoamines and their metabolites in brain regions of the vehicle-treated nonstressed rats. In these nonstressed rats, the 5-HIAA/5-HT ratio and the HVA + DOPAC/DA ratio were not affected by treatment with either dose of ABU or diazepam (Fig. 1a and b). The MHPG/NA ratio was significantly increased by treatment with diazepam at a dose of 5 mg/kg in all brain regions, but not by ABU (Fig. 1c).

The 5-HIAA/5-HT ratio and the HVA + DOPAC/DA ratio were not significantly changed by the immobilization stress (Fig. 2a and b). No significant effects of ABU and diazepam were seen for the 5-HIAA/5-HT and HVA + DOPAC/DA ratios. In contrast, the MHPG/NA ratio significantly increased in all brain regions of rats compared with those in nonstressed controls (Fig. 2c). The stress-induced increase in

TABLE 1

REGIONAL CONCENTRATIONS OF MONOAMINES AND THEIR METABOLITES IN NONSTRESSED RAT BRAIN

	MHPG	NA	HVA	DOPAC	DA	5-HIAA	5-HT
CTX	108.3 (6.1)	274.6 (9.8)	155.1 (19.8)	104.0 (10.4)	471.2 (35.7)	195.4 (4.7)	376.4 (15.4)
AMY	126.3 (8.6)	248.7 (12.9)	68.3 (5.4)	58.7 (8.0)	296.3 (20.5)	171.9 (13.0)	337.6 (8.3)
STR	94.2 (9.2)	192.4 (24.8)	1069.8 (91.0)	930.0 (76.7)	6885.1 (675.4)	337.8 (14.2)	371.1 (19.9)
HIC	136.2 (18.1)	335.6 (16.0)	64.6 (9.4)	64.2 (11.1)	289.1 (22.5)	306.0 (10.5)	321.3 (28.5)
THA	370.6 (20.3)	1197.2 (36.8)	80.8 (13.8)	165.6 (27.2)	571.7 (31.4)	509.0 (62.7)	1011.9 (55.0)
MID	252.7 (11.5)	532.6 (58.3)	46.7 (4.0)	96.2 (6.2)	252.9 (35.4)	648.9 (63.4)	1012.9 (87.5)
OBL	169.9 (5.0)	409.1 (12.2)	48.8 (3.3)	32.6 (2.1)	45.5 (2.4)	267.4 (11.6)	339.7 (11.5)

The rats were pretreated with vehicle 30 min before death and were not exposed to stress. They were killed by microwave irradiation, and the concentrations of the monoamines and their metabolites were evaluated. All values were expressed as the mean (nanograms per gram of tissue, wet weight) of six animals. Numbers in parenthesis represent SE. CTX, cerebral cortex; AMY, amygdala containing pyriform cortex and temporal cortex; STR, striatum; HIC, hippocampus; THA, thalamus plus hypothalamus; MID, midbrain; OBL, pons plus medulla oblongata.

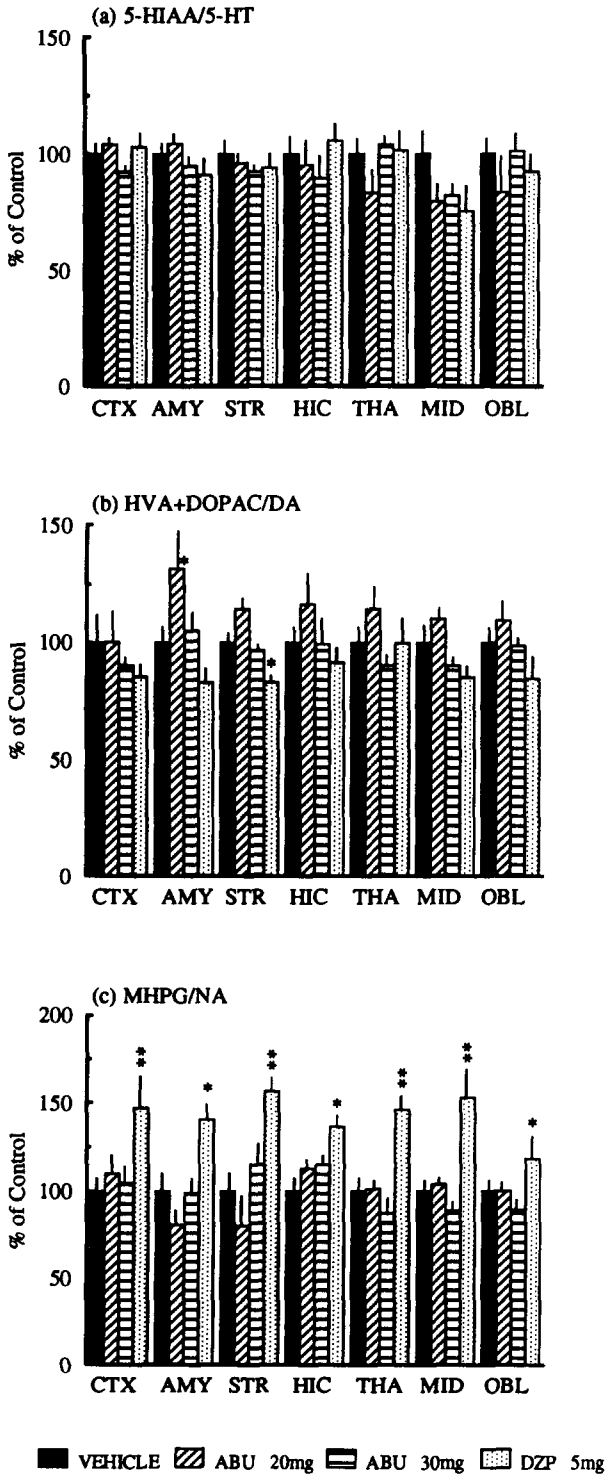

FIG. 1. The effect of ABU and diazepam on turnover rates of monoaminergic neurotransmitters in brain regions in nonstressed rats: (a) the 5-HIAA/5-HT ratio, (b) the HVA+DOPAC/DA ratio, and (c) the MHPG/NA ratio. Each value indicates the mean ± SE of six animals expressed as a percent of the control value. CTX, cerebral cortex; AMY, amygdala containing pyriform cortex and temporal cortex; STR, striatum; HIC, hippocampus; THA, thalamus plus hypothalamus; MID, midbrain; OBL, pons plus medulla oblongata. Statistical significance as compared with rats pretreated with vehicle: $*p < 0.05$, $**p < 0.01$.

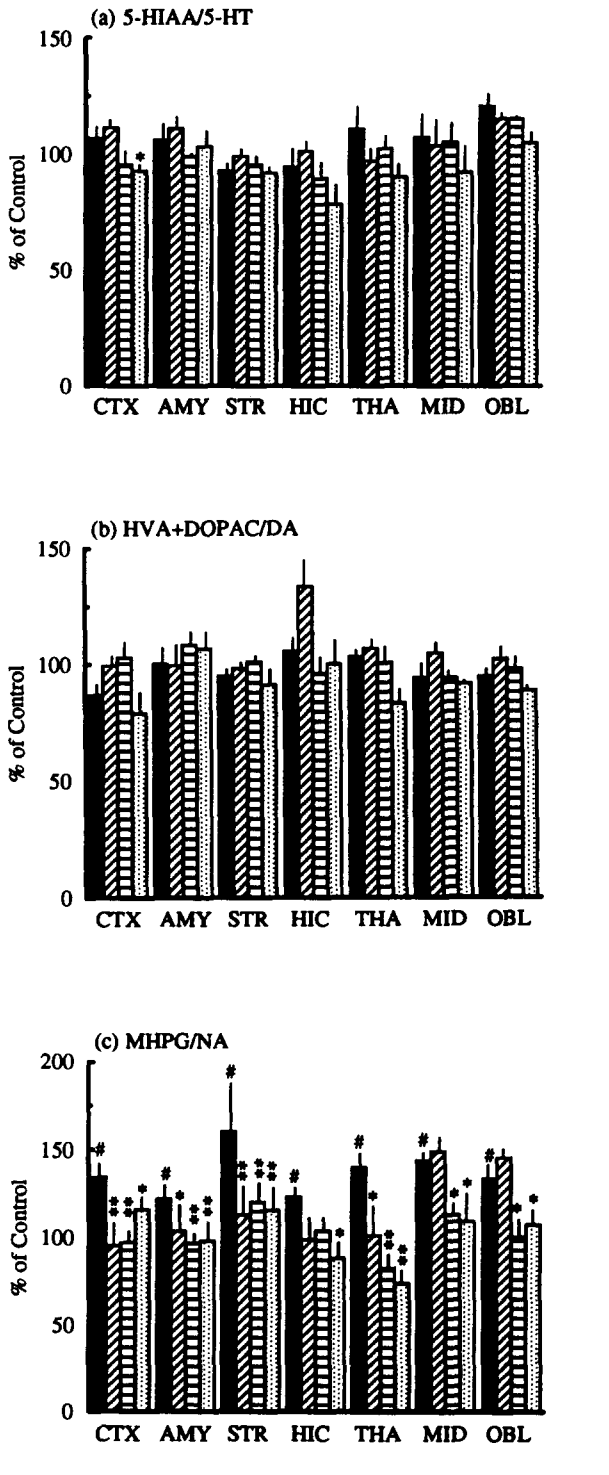

FIG. 2. The effect of ABU and diazepam on turnover rates of monoaminergic neurotransmitters in brain regions in immobilization stressed rats: (a) the 5-HIAA/5-HT ratio, (b) the HVA+DOPAC/DA ratio, and (c) the MHPG/NA ratio. Each value indicates the mean ± SE of 6 animals expressed as a percent of the control value. Statistical significance, $\#p < 0.05$ as compared with nonstressed rats pretreated with vehicle; $*p < 0.05$, $**p < 0.01$ as compared with stressed rats pretreated with vehicle.

the MHPG/NA ratio was significantly attenuated by pretreatment with ABU at doses of 20 and 30 mg/kg, and by diazepam at a dose of 5 mg/kg, in almost all brain regions.

The 5-HIAA/5-HT ratio was significantly increased in the cortex and hippocampus by electric foot shock stress, as compared with nonstressed controls (Fig. 3a). The stress-induced increase in the 5-HIAA/5-HT ratio was not affected by the pretreatments with either dose of ABU and diazepam. The HVA+DOPAC/DA ratio and the MHPG/NA ratio were also increased by the foot shock stress in almost all brain regions (Fig. 3b and c). The stress-induced increase in the HVA+DOPAC/DA ratio was attenuated by ABU at a dose of 30 mg/kg in the cortex and amygdala, and by diazepam at a dose of 5 mg/kg in amygdala. The stress-induced increase in the MHPG/NA ratio was attenuated by ABU at a dose of 30 mg/kg in the cortex, amygdala, and pons plus medulla oblongata. The increase was also attenuated by diazepam at a dose of 5 mg/kg in amygdala and pons plus medulla oblongata. However, these observed increases were not attenuated by ABU at a dose of 20 mg/kg.

DISCUSSION

It has been reported that NA turnover and NA release in rat brain regions are increased by immobilization stress (4,20,21,23), and that the increases in NA turnover and the release induced by immobilization stress were attenuated by diazepam (8). As was shown in the present study, NA turnover was increased by the immobilization stress in all brain regions. Pretreatment with ABU, like diazepam, attenuated the increase in NA turnover in various brain regions. Although 5-HT turnover in rat brain was reported to be increased by immobilization stress using a different stress procedure (17), our results showed that 5-HT turnover in rat brain did not change. This is consistent with a previous report (18).

Electric foot shock stress reportedly increases DA turnover (2,6,7) and 5-HT turnover (14) in rat brain regions. We showed increases not only in 5-HT and DA turnover, but also in NA turnover. There are few reports on changes in NA turnover. The present results may be supported by previous reports, in which the mesocortical DA system was suggested to increase the sensitivity of the NA system (18). The mesocortical DA system, which innervates the frontal cortex, and mesolimbic DA system are activated by the locus coeruleus (LC)-NA system during stress exposure (15). From these reports and the present results, the NA and DA systems may be activated by each other during stress exposure. The increase in NA turnover by electric foot shock stress was attenuated by ABU and diazepam in pons plus medulla oblongata containing the LC and amygdala and/or cortex. The increase in DA turnover was also attenuated by these drugs in amygdala and/or cortex. Cortical DA pathways are reported to play a critical role in the regulation of emotional states, whereas the striatum DA pathways are strictly involved in the control of motor activity (3). Our results support the proposition that cortical DA pathways play a critical role in the regulation of emotional states.

At a dose of 30 mg/kg ABU suppressed an increase in NA turnover by electric foot shock stress in a manner similar to diazepam at a dose of 5 mg/kg, but ABU at a dose of 20 mg/kg showed no effect. This is in contrast to the response to immobilization stress, in which pretreatment with ABU at a dose of 20 mg/kg attenuated the stress-induced increase in NA turnover. It is possible that the electric foot shock stress is more stressful than immobilization stress. The present results

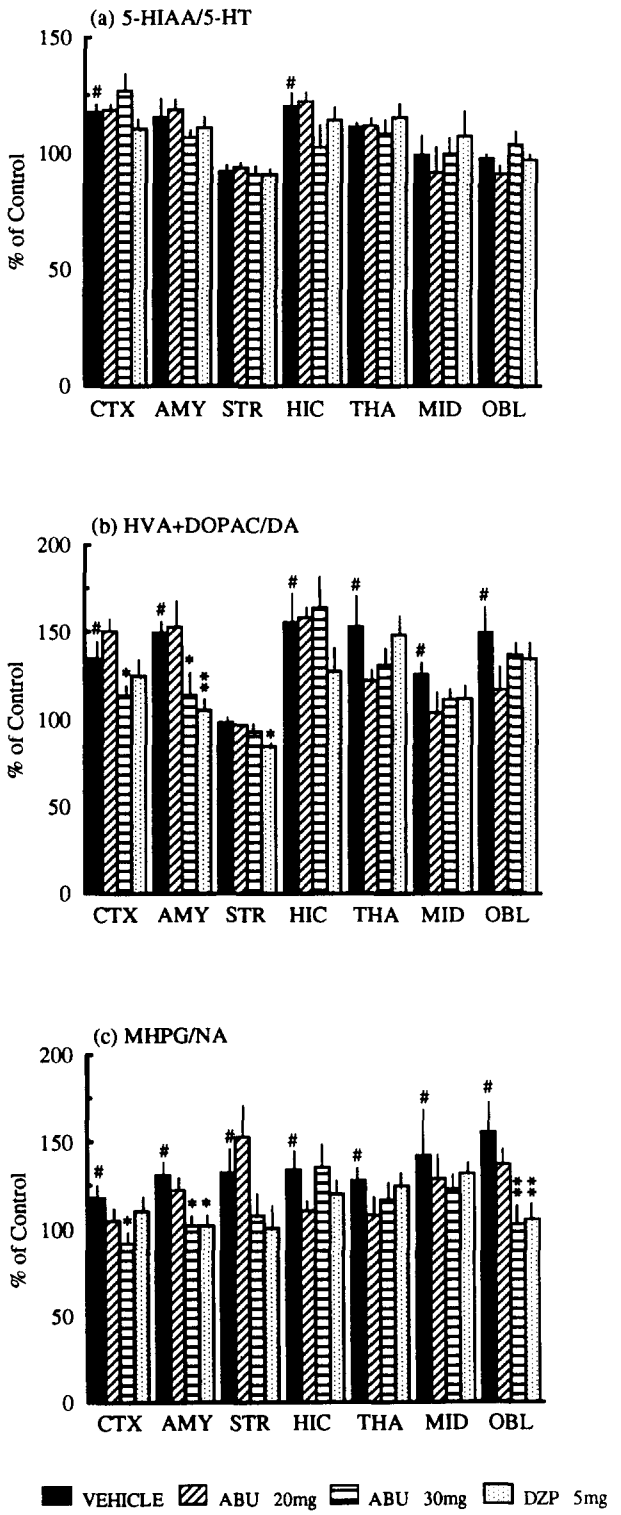

FIG. 3. The effect of ABU and diazepam on turnover rates of monoaminergic neurotransmitters in brain regions in electric foot shock stressed rats: (a) the 5-HIAA/5-HT ratio, (b) the HVA+DOPAC/DA ratio, and (c) the MHPG/NA ratio. Each value indicates the mean ± SE of six animals expressed as a percent of the control value. Statistical significance: #$p < 0.05$ as compared with nonstressed rats pretreated with vehicle; *$p < 0.05$, **$p < 0.01$ as compared with stressed rats pretreated with vehicle.

suggest that the modification of monoamine metabolism depends on the type and intensity of stress.

Our results showed that NA turnover increased in both immobilization and electric foot shock stress. Furthermore, ABU and diazepam attenuated activation of NA turnover found by the two different stress conditions in amygdala and pons plus medulla oblongata, which contains the LC, innervating noradrenergic neurons in many brain regions. Electric stimulation of the LC has been shown to cause anxiety- or fear-related behavioral changes in monkeys (19). Similarly, a drug that increases the neuronal activity of the LC and releases NA in terminal sites, such as piperoxan, an α_2-antagonist, has been shown to induce anxiety-like responses in monkeys (19). Reportedly, hyperemotionality in stressed animals can be modulated by regulation of brain noradrenergic systems in the LC, amygdala, and hypothalamus (1,6). These reports and the present data suggest that the noradrenergic system is important in the expression of stress and anxiety.

1-Amino-5-bromouracil did not affect the turnover of brain monoaminergic neurotransmitters in nonstressed rats, whereas pretreatment with diazepam increased NA turnover in various brain regions. Stress is known to increase the turnover of brain monoaminergic neurotransmitters. ABU, like diazepam, blocked the increase in turnover of neurotransmitters in various brain regions in stressed rats. These results suggest that ABU behaves as an antistress agent. Because the antistress activity of ABU was observed at doses that showed an anticonflict activity in rats and mice, it is possible that the attenuating effect of ABU on the hyperactivation of noradrenergic and dopaminergic systems may be one of the mechanisms of its central action.

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