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Effects of Moclobemide on Forced-Swimming Stress and Brain Monoamine Levels in Mice

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MIURA, H., M. NAOI, D. NAKAHARA, T. OHTA AND T. NAGATSU. *Effects of moclobemide on forced-swimming stress and brain monoamine levels in mice.* PHARMACOL BIOCHEM BEHAV 53(2) 469–475, 1996.—Moclobemide [Ro 11-1163, *p*-chloro-N-(2-morpholinoethyl)benzamide, AURORIX] is known as an antidepressant and a reversible inhibitor of type A monoamine oxidase. In the present study, a forced swimming test was applied to mice to evaluate behavioral and neurochemical effects of this drug. During forced swimming posture of immobility, a typical behavioral change, was observed, and biochemical analysis of the brain revealed significant changes in the monoamine levels. The norepinephrine concentration was reduced, while that of its product was increased, indicating increase in norepinephrine turnover. The stress increased the levels of dopamine, serotonin, and their metabolites. Moclobemide significantly improved the immobility elicited by the test, and it could prevent the changes in the turnover of norepinephrine, dopamine, and serotonin induced by the stress. These results suggest that moclobemide may improve the behavioral changes induced by the forced swimming through its effects on monoamine metabolism.

Moclobemide Forced swimming Stress model Brain monoamines Type A monoamine oxidase inhibitor

THE FORCED swimming test has been considered to be a model of the depressive state (14–16). In this test, animals such as mice are put into water, and initially they show vigorous activity trying to escape from it. After a while, however, they show a typical posture of immobility in the water, which can be prevented by many antidepressant drugs. Even though numerous pharmacological data have been accumulated, the biochemical process in the brain underlying this immobility remains to be clarified (5). In addition, the effects of antidepressants and other drugs on the biochemical changes caused by the forced swimming have been scarcely reported (5). On the other hand, forced swimming may be regarded as acute inescapable stress, and we may use this animal model as that of an acute stress. Therefore, we may compare neurochemical findings based on this model with those on other stress models reported previously.

Recently we reported the relation between certain behavioral changes and neurochemical changes in the brain, and also the effects of RS-8359, (±)-4-(4-cyanophenyl)amino-6,7-dihydro-7-hydroxy-5H-cyclopenta[d]-pyrimidine, a new reversible MAO-A inhibitor, on these changes (12).

Moclobemide [Ro 11-1163, *p*-chloro-N-(2-morpholinoethyl)-benzamide, AURORIX], is another reversible inhibitor of MAO, preferentially of type A, and it has been shown to have antidepressant effects on humans. The neurochemical and pharmacological characteristics of moclobemide have been studied (2,3,6,7,10), and the drug was reported to affect monoamine levels in the brain of nonstressed animal (6,7,10), and also to improve behavior changes induced by forced swimming (2).

In the present study, employing the forced swimming test as a stress model, we studied acute effects of moclobemide on the behavioral and neurochemical changes induced by the stress in mice. To evaluate the biochemical changes, we measured the levels of norepinephrine (NE), 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), dopamine (DA), homovanilic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in five brain regions: cerebral

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cortex, cerebellum, striatum, thalamus-hypothalamus, and brainstem.

METHOD

Animals

Experimental animals used were male BALB/cA (SPF) mice weighing 20 to 25 g. Five or six mice were housed together per cage with free access to food and drinking water. A 12 L : 12 D cycle was maintained, and the room temperature was kept at 21–23°C. The protocols of the animal experiments were approved by the Ethics Committee of Nagoya University School of Medicine.

Materials

Moclobemide was synthesized at Sankyo (Tokyo, Japan). The following drugs or chemicals were purchased from commercial sources: NE, DA, citric acid, sodium acetate, and EDTA 2Na from Nacalai tesque (Kyoto, Japan); DOPAC, HVA, MHPG, 5-HIAA, and isoproterenol (ISO) used as an internal standard for analysis of monoamines, from Sigma (St. Louis, MO); 5-HT from Merck (Darmstadt, Germany); and sodium octanesulfonate (SOS) from Aldrich (Milwaukee, WI). Moclobemide was suspended in 0.2% sodium carboxymethylcellulose (CMC) solution, and given to mice by intraperitoneal (IP) injection.

Forced Swimming Test

This test was performed on male mice according to a modified version (2) of the original method (14–16). We used a resin bucket instead of the glass cylinder used in previous studies (2,14–16), because in the glass cylinder scoring of immobility of BALB/cA mice was difficult (11). With the resin bucket, as immobility appeared with prolonged latency, the scoring time was lengthened in this study compared with that used in the original method to stabilize the immobility score (12). One hour after the test drug or vehicle had been administered IP, each mouse was exposed separately to water in the bucket (height, 28 cm; diameter, 26.5 cm). The container was filled with tap water to a depth of 15 cm, and the temperature was kept at 21–23°C. On the initial exposure, the mice typically made vigorous efforts to escape from the water. Then the animals gradually lapsed into a state of behavioral immobility. Each mouse were subjected to a single 40 min trial of forced swimming. For behavioral scoring, the presence (+) or absence (−) of immobility lasting for 10 to 15 s was scored at 30-s intervals over 20 subsequent observations for each 10-min session. The total (+) score for each mouse was counted, with the theoretically maximum value of the score being 20. The mean immobility score of the experimental groups (n = 8) was calculated. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test.

Sample Preparation

The mice were divided into two groups. One was the nonstress control that received neither swimming test nor medication, and 1 h after the vehicle had been injected IP, these mice were sacrificed by cervical dislocation. The other was the stress group, which was exposed to forced swimming. One hour after the Ip injection of drug or vehicle, these mice were dropped into water, and 40 min later they were killed by the same means as the former group. The brains were removed immediately, and dissected into five regions, i.e., the cerebral cortex, cerebellum, striatum, thalamus-hypothalamus, and brain stem, by a slight modification of the reported method (4,13).

The samples were weighed and then added to ice-cold 0.1 M perchloric acid (PCA) solution (10 vol/wet weight) containing 0.2 mM sodium pyrosulfite, 0.04 mM EDTA 2Na, and 1 μM ISO as an internal standard. Next, they were sonicated and centrifuged at 22,000 × g for 10 min at 4°C. The supernatant was filtered through a Millipore HV filter (0.45 μm pore size), and then applied to high-performance liquid chromatography (HPLC) with electrochemical detection (ECD).

HPLC-ECD Determination of the Brain Levels of Biogenic Monoamines and Their Major Metabolites

The amounts of monoamines and their metabolites in the brain extracts were quantitatively measured by HPLC with ECD; the systems used were as follows: System 1, for determination of NE, DA, HVA, 5-HT, and 5-HIAA, was composed of an EP-10 pump (Eicom, Kyoto, Japan), an ECD-100 ECD (Eicom), an Eicompac MA-5ODS reverse-phased column (4.6 × 150 mm, Eicom), a WE-3G working electrode (Eicom) set at 650 mV against an Ag/Cl reference electrode, and a Chromatocorder 12 recorder (GL Sciences, Tokyo, Japan). The mobile phase consisted of 90 mM sodium acetate, 35 mM citric acid, 130 μM EDTA, 230 μM SOS, and 11% methanol; and the flow rate was 0.8 ml/min. System 2, for analysis of MHPG, comprised an LC-9A pump (Shimadzu, Kyoto, Japan), a Coulochem 5100A ECD (ESA, Bedford, MA), and an Inertosil reverse-phased ODS-2 column (4.6 × 250 mm, GL Sciences). A model 5021 conditioning ECD cell was set at +50 mV, the first and the second electrodes of a model 5011 analytical cell were set at 0 and +400 mV, and the output of the second electrode was monitored with a C-R6A chrmatopac (Shimadzu). The mobile phase was 90 mM sodium acetate-35 mM citric acid buffer, pH 4.35, containing 130 μM EDTA, 2 mM SOS, and 11% methanol; and the flow rate was 0.6 ml/min.

The concentration of each compound measured by system 1 was calculated by comparison with the internal and external

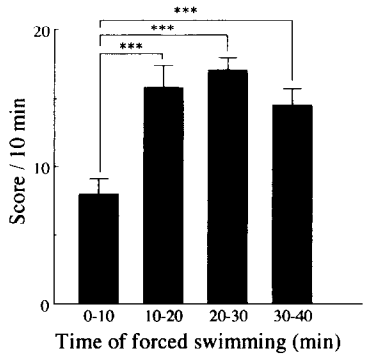

FIG. 1. Time course of immobility scores during forced swimming. The presence (+) or absence (−) of immobility lasting for a minimum of 5 s within 30-s intervals was measured for each subject. The total number of + scores was counted in each 10-min session. Mean and SEM (n = 8) were calculated for each experimental group. Statistical analyses were performed by one-way repeated measure ANOVA followed by Fisher's PLSD test, $F(3, 21) = 17.23$, $p < 0.001$. ***$p < 0.001$.

standards; and that of MHPG assayed by system 2, with the external standard. Statistical analyses were performed by the unpaired two-tailed *t*-test and ANOVA, followed by Fisher's PLSD test.

RESULTS

The validity of forced swimming as a stress model to mice was examined from the time course study of the immobility score. The immobility score was determined at 30-s intervals for 40 min, and the sum of the score was calculated for every 10 min. As shown in Fig. 1, the immobility score gradually increased with the test time, reaching to a plateau in 20 min, and then remained stable for the following 20 min. Thus, in the present study, we set the duration of forced swimming for 40 min, and determined the immobility score during the last 10 min (30–40 min).

Based on the behavioral data, we examined changes in monoamine levels in mice having been subjected to forced swimming for 40 min. The contents of the monoamines and their metabolites were measured in five brain regions, and the results are shown in Table 1A and B. Forced swimming affected the monoamine levels markedly. The NE level decreased significantly, whereas that of MHPG increased significantly in all the brain regions examined (Table 1A); and the ratio of MHPG/NE increased except in the case of the cerebellum (Table 1B). The sum of NE and MHPG did not change significantly except for the decrease ($p < 0.05$) in the brain

TABLE 1

CHANGES IN MONOAMINE LEVELS BY FORCED SWIMMING

1A

Region	Stress	Monoamine Level nmol/g NE	MHPG	DA	HVA	5-HT	5-HIAA
Cerebral cortex	(−)	2.28 ± 0.07	0.43 ± 0.08	0.22 ± 0.02	0.20 ± 0.03	0.53 ± 0.02	0.49 ± 0.01
	(+)	1.85 ± 0.03*	1.34 ± 0.24†	0.31 ± 0.03	0.29 ± 0.02†	0.86 ± 0.04‡	0.71 ± 0.03‡
Cerebellum	(−)	2.10 ± 0.05	0.40 ± 0.05	n.d.	n.d.	0.13 ± 0.01	0.24 ± 0.01
	(+)	1.83 ± 0.06†	0.72 ± 0.09†	n.d.	n.d.	0.23 ± 0.03†	0.39 ± 0.03*
Striatum	(−)	2.39 ± 0.06	0.33 ± 0.04	12.71 ± 0.14	4.35 ± 0.23	0.84 ± 0.06	0.58 ± 0.02
	(+)	1.98 ± 0.10†	0.87 ± 0.13*	14.66 ± 0.21†	3.78 ± 0.24	1.43 ± 0.08‡	0.93 ± 0.06*
Thalamus-hypothalamus	(−)	4.86 ± 0.11	0.57 ± 0.01	0.77 ± 0.02	0.63 ± 0.03	1.06 ± 0.02	1.40 ± 0.04
	(+)	3.84 ± 0.11‡	1.30 ± 0.24†	1.06 ± 0.09†	0.96 ± 0.07*	2.03 ± 0.06‡	1.94 ± 0.08‡
Brain stem	(−)	4.24 ± 0.03	0.84 ± 0.12	0.36 ± 0.02	0.31 ± 0.01	1.51 ± 0.03	1.44 ± 0.04
	(+)	3.19 ± 0.04‡	1.33 ± 0.11†	0.44 ± 0.03	0.42 ± 0.06	2.37 ± 0.03‡	2.04 ± 0.11*

Monoamine levels were measured as described in the text. (−), nonstress control group; (+), stress group, which suffered forced swimming for 40 min. Number of animals used for each group was 4. n.d., not detectable. Statistical analyses were performed by unpaired two-tailed *t*-test between nonstress and stress groups.

*$p < 0.01$.
†$p < 0.05$.
‡$p < 0.001$.

1B

Region	Stress	MHPG/NE	HVA/DA	5-HIAA/5-HT	Monoamine Level nmol/g NE + MHPG	DA + HVA	5-HT + 5-HIAA
Cerebral cortex	(−)	0.193	0.880	0.932	2.71	0.41	1.02
	(+)	0.713*	0.952	0.831	3.16	0.59*	1.56†
Cerebellum	(−)	0.188	n.d.	1.839	2.50	n.d.	0.37
	(+)	0.360	n.d.	1.767	2.48	n.d.	0.61‡
Striatum	(−)	0.139	0.343	0.694	2.72	17.06	1.41
	(+)	0.408‡	0.258*	0.650	2.80	18.44‡	2.36
Thalamus-hypothalamus	(−)	0.117	0.820	1.325	5.42	1.40	2.46
	(+)	0.326*	0.922	0.958†	5.25	2.02‡	3.97†
Brainstem	(−)	0.199	0.867	0.953	5.08	0.67	2.95
	(+)	0.400‡	0.942	0.861	4.46*	0.86	4.41†

Monoamine levels were measured as described in the text. (−), nonstress control group. (+), stress group, which suffered forced swimming for 40 min. Number of animals used for each group was 4. n.d., not detectable. Statistical analyses were performed by unpaired two-tailed *t*-test between nonstress and stress groups.

*$p < 0.05$.
†$p < 0.001$.
‡$p < 0.01$.

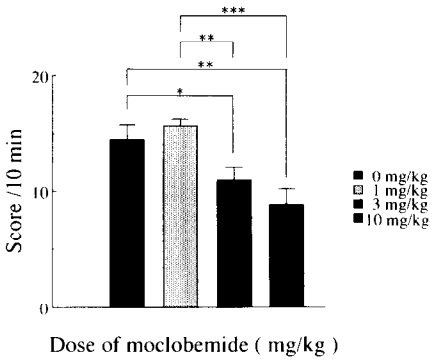

FIG. 2. Effect of moclobemide on immobility score during the last 10-min period (from 30 to 40 min) of forced swimming. Mean and SEM (n = 8) were calculated for groups treated with each dose of moclobemide. Statistical analyses were performed by one-way factorial ANOVA followed by Fisher's PLSD test, $F(3, 28) = 7.70$, $p < 0.001$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

stem (Table 1B). The DA level significantly increased in the striatum and thalamus-hypothalamus, and the HVA level increased in the cerebral cortex and thalamus-hypothalamus (Table 1A). The ratio of HVA/DA did not significantly change, except in the case of the striatum, where it decreased (Table 1B). A significant increase in the sum of DA and HVA amounts was noted in the cerebral cortex ($p < 0.05$), striatum, and thalamus-hypothalamus ($p < 0.01$, Table 1B). Both 5-HT and 5-HIAA levels significantly increased in all the five regions (Table 1A), whereas a decrease in the 5-HIAA/5-HT ratio was statistically significant only in the thalamus-hypothalamus (Table 1B). The sum of 5-HT and 5-HIAA also significantly increased in all the five regions (Table 1B: cerebellum, $p < 0.01$; other regions, $p < 0.001$).

Moclobemide was administered to mice 1 h before forced swimming at doses of 1, 3, and 10 mg/kg, and the effect of moclobemide on the behavioral change caused by forced swimming was then examined. As shown in Fig. 2, the pretreatment with moclobemide decreased the immobility score significantly in a dose-dependent manner at the dose of 3 mg/kg or higher.

The effects of moclobemide on the biochemical changes in the brain were examined at the same doses found to improve the behavioral changes, i.e., 3 mg/kg and 10 mg/kg. The pretreatment of mice with moclobemide changed the levels of monoamines and their metabolites significantly (Tables 2, 3, and 4). The ratio of monoamine metabolite level/monoamine level, and the sum of the amounts of a given monoamine and its metabolites are summarized in Tables 2, 3, and 4. Moclobemide significantly increased the concentration of NE, whereas it significantly decreased that of MHPG in all the five brain regions (Table 2). The ratio of MHPG/NE decreased significantly in all the brain regions except in the thalamus-hypothalamus, whereas the sum of NE and MHPG amounts increased in all the brain regions (Table 2). A significant in-

TABLE 2

EFFECTS OF MOCLOBEMIDE ON CHANGES IN NE AND MHPG LEVELS ELICITED BY FORCED SWIMMING

Region	Dose	Number	Monoamine Level nmol/g			
			NE	MHPG	NE + MHPG	MHPG/NE
Cerebral cortex	0 mg/kg	4	1.85 ± 0.03	1.34 ± 0.24	3.17	0.71
	3 mg/kg	5	5.91 ± 0.14*	0.57 ± 0.06†	6.48*	0.10*
	10 mg/kg	5	4.55 ± 0.25*§	0.53 ± 0.03*	5.07*§	0.12*
Cerebellum	0 mg/kg	4	1.83 ± 0.06	0.72 ± 0.09	2.49	0.36
	3 mg/kg	5	5.81 ± 0.08*	0.40 ± 0.06†	6.21*	0.07*
	10 mg/kg	5	4.35 ± 0.32*§	0.27 ± 0.02*	4.62*§	0.07*
Striatum	0 mg/kg	4	1.98 ± 0.10	0.87 ± 0.13	2.80	0.41
	3 mg/kg	5	4.94 ± 0.11*	0.35 ± 0.04*	5.29*	0.07*
	10 mg/kg	5	3.88 ± 0.12*§	0.29 ± 0.02*	4.17*§	0.08*
Thalamus-hypothalamus	0 mg/kg	4	3.84 ± 0.11	1.30 ± 0.24	5.25	0.33
	3 mg/kg	5	6.79 ± 0.09*	0.72 ± 0.06‡	7.51*	0.11
	10 mg/kg	5	5.77 ± 0.17*§	0.63 ± 0.06†	6.40†¶	0.11
Brain stem	0 mg/kg	4	3.19 ± 0.04	1.33 ± 0.11	4.47	0.40
	3 mg/kg	5	5.60 ± 0.08*	0.74 ± 0.03*	6.33*	0.13*
	10 mg/kg	5	5.06 ± 0.11*§	0.62 ± 0.04*	5.68*#	0.12*

Monoamine levels were measured as described in the text. Statistical analyses were performed by one-way factorial ANOVA followed by Fisher's PLSD test. In the analysis of NE: cerebral cortex, $F(2,11) = 126.5$, $p = 0.0001$; *cerebellum*, $F(2,11) = 90.60$, $p = 0.0001$; striatum, $F(2,11) = 165.02$, $p = 0.0001$; thalamus-hypothalamus, $F(2,11) = 124.60$, $p = 0.0001$; brain stem, $F(2,11) = 186.83$, $p = 0.0001$.

In the analyses of MHPG: cerebral cortex, $F(2,11) = 13.15$, $p < 0.01$; cerebellum, $F(2,11) = 14.22$, $p < 0.001$; striatum, $F(2,11) = 18.64$, $p < 0.001$; thalamus-hypothalamus, $F(2,11) = 7.06$, $p < 0.05$; brain stem, $F(2,11) = 33.92$, $p = 0.0001$.

In the analyses of NE + MHPG: cerebral cortex, $F(2,11) = 58.30$, $p < 0.0001$; cerebellum, $F(2,11) = 88.30$, $p < 0.0001$; striatum, $F(2,11) = 49.95$, $p < 0.0001$; thalamus-hypothalamus, $F(2,11) = 25.18$, $p < 0.0001$; brain stem, $F(2,11) = 54.78$, $p = 0.0001$.

In the analyses of MHPG/NE: cerebral cortex, $F(2,11) = 22.26$, $p < 0.001$; cerebellum, $F(2,11) = 16.23$, $p < 0.001$; striatum, $F(2,11) = 33.20$, $p < 0.0001$; thalamus-hypothalamus, $F(2,11) = 2.28$, $p < 0.05$; brain stem, $F(2,11) = 52.45$, $p = 0.0001$.

Between 0 mg/kg and 3 mg/kg, 0 mg/kg and 10 mg/kg: *$p < 0.001$, †$p < 0.01$, ‡$p < 0.05$.

Between 3 mg/kg and 10 mg/kg: §$p < 0.001$, ¶$p < 0.05$, #$p < 0.01$.

TABLE 3

EFFECTS OF MOCLOBEMIDE ON CHANGES IN DA AND HVA LEVELS INDUCED BY FORCED SWIMMING

Region	Dose	Number	Monoamine Level nmol/g DA	HVA	DA + HVA	HVA/DA
Cerebral cortex	0 mg/kg	4	0.31 ± 0.03	0.29 ± 0.02	0.59	0.95
	3 mg/kg	5	0.34 ± 0.06	0.25 ± 0.03	0.60	0.83
	10 mg/kg	5	0.42 ± 0.02	0.21 ± 0.01	0.63	0.49*§
Cerebellum	0 mg/kg	4	n.d.	n.d.	n.d.	n.d.
	3 mg/kg	5	n.d.	n.d.	n.d.	n.d.
	10 mg/kg	5	n.d.	n.d.	n.d.	n.d.
Striatum	0 mg/kg	4	14.66 ± 0.21	3.78 ± 0.24	18.44	0.26
	3 mg/kg	5	13.68 ± 0.41	2.47 ± 0.05†	16.15	0.18‡
	10 mg/kg	5	19.04 ± 0.88‡¶	3.26 ± 0.29§	22.30†¶	0.17‡
Thalamus-hypothalamus	0 mg/kg	4	1.06 ± 0.09	0.96 ± 0.07	2.02	0.93
	3 mg/kg	5	1.25 ± 0.03	0.75 ± 0.03†	2.00	0.60‡
	10 mg/kg	5	1.36 ± 0.07*	0.63 ± 0.02‡§	1.98	0.46‡§
Brain stem	0 mg/kg	4	0.44 ± 0.03	0.42 ± 0.06	0.86	0.94
	3 mg/kg	5	0.53 ± 0.03	0.34 ± 0.02	0.87	0.64†
	10 mg/kg	5	0.62 ± 0.05*	0.28 ± 0.02*	0.90	0.45‡

Monoamine levels were measured as described in the text. Statistical analyses were performed by one-way factorial ANOVA followed by Fisher's PLSD test.

In the analyses of DA; cerebral cortex, $F(2,11) = 2.11$, $p < 0.05$; striatum, $F(2,11) = 22.66$, $p = 0.0001$; thalamus-hypothalamus, $F(2,11) = 5.63$, $p < 0.05$; brain stem, $F(2,11) = 4.53$, $p < 0.05$.

In the analyses of HVA:cerebral cortex, $F(2,11) = 3.34$, $p < 0.05$; striatum, $F(2,11) = 8.85$, $p = 0.01$; thalamus-hypothalamus, $F(2,11) = 17.87$, $p < 0.001$; brain stem, $F(2,11) = 4.06$, $p < 0.05$.

In the analyses of DA + HVA: cerebral cortex, $F(2,11) = 0.154$, $p < 0.05$; striatum, $F(2,11) = 18.71$, $p = 0.001$; thalamus-hypothalamus, $F(2,11) = 0.03$, $p < 0.05$; brain stem, $F(2,11) = 0.08$, $p < 0.05$.

In the analyses of HVA/DA: cerebral cortex, $F(2,11) = 4.99$, $p < 0.05$; striatum, $F(2,11) = 14.61$, $p = 0.001$; thalamus-hypothalamus, $F(2,11) = 42.64$, $p < 0.0001$; brain stem, $F(2,11) = 21.71$, $p < 0.001$.

Between 0 mg/kg and 3 mg/kg, 0 mg/kg and 10 mg/kg: $*p < 0.05$, $\dagger p < 0.01$, $\ddagger p < 0.001$.

Between 3 mg/kg and 10 mg/kg: $\S p < 0.05$, $\| p < 0.001$, $\# p < 0.01$.

crease in DA was detected in the striatum, thalamus-hypothalamus, and brain stem, and a decrease in HVA was confirmed in the same regions (Table 3). The ratio of HVA/DA was decreased significantly in the cerebral cortex, striatum, thalamus-hypothalamus, and brain stem, but the sum of DA and HVA amounts was not affected except for an increase in the striatum (Table 3). Although 5-HT increased in all the regions examined, 5-HIAA decreased significantly in all of them (Table 4). The ratio of 5-HIAA/5-HT significantly decreased in the five regions, whereas the sum of their amounts was not altered except for an increase in the cerebral cortex (Table 4).

DISCUSSION

In the present study, the effects of forced swimming on levels of monoamines and their metabolites in the brain were examined. We applied forced swimming as a model of acute inescapable stress, and the present results may be compared with those employing other stress models. In other stress models, such as acute foot shock stress (8) and chronic environmental stress (1), the metabolic rates of monoamines were reported to increase in the brain. MAO is the major catabolic enzyme of monoamines, and type A MAO oxidizes monoamine neurotransmitters such as 5-HT and NE, whereas DA is a substrate of both type A and B MAO. Because monoamines are sequestered in synaptic vesicles and MAO is localized in the outer membrane of mitochondria, monoamines are oxidized after their release into the extracellular space and reuptake in the terminals or in glia or other cells; thus, the enhanced metabolic rate of monoamines may correspond to their increased release from the nerve terminals.

The present data document that forced swimming affected markedly the monoamine levels in the brain. A decrease in the NE and an increase in the MHPG level were confirmed in all the five brain regions (Table 1A), indicating enhanced NE turnover. However, the sum of their amounts was not changed, except in the brain stem (Table 1B), suggesting no remarkable change in NE biosynthesis. These results support the view that under an acute uncontrollable stress the enhanced release of NE may increase its catabolism more than its synthesis, which results in a reduction in NE concentration (21).

In the striatum and thalamus-hypothalamus, the DA level increased, but HVA increased in the cerebral cortex and thalamus-hypothalamus (Table 1A). The HVA/DA ratio, reflecting DA turnover, did not change except in the striatum, while the sum of their amounts, an index of DA synthesis, was enhanced in the cerebral cortex, striatum, and thalamus-hypothalamus (Table 1B). These data suggest that in the DA system forced swimming increased the synthesis rather than the turnover. Synthesis of DA increased by stress is considered to be due to an increase in the activity of the rate-limiting enzyme, tyrosine hydroxylase [L-tyrosine, tetrahydropteridine: oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2, TH]. In the mouse brain, TH activity was reported to be enhanced in vivo and in vitro by acute immobilization stress (13).

Our present data clearly demonstrate that 5-HT and 5-HIAA levels as well as their sum increased in all the five brain

TABLE 4

EFFECTS OF MOCLOBEMIDE ON CHANGES IN 5-HT AND 5-HIAA LEVELS CAUSED BY FORCED SWIMMING

Region	Dose	Number	Monoamine Level nmol/g			
			5-HT	5-HIAA	5-HT + 5-HIAA	5-HIAA/5-HT
Cerebral cortex	0 mg/kg	4	0.86 ± 0.04	0.71 ± 0.03	1.57	0.83
	3 mg/kg	5	1.15 ± 0.02*	0.51 ± 0.03	1.67	0.45*
	10 mg/kg	5	1,48 ± 0.06*§	0.36 ± 0.03*¶	1.84†	0.24*¶
Cerebellum	0 mg/kg	4	0.23 ± 0.03	0.39 ± 0.03	0.61	1.78
	3 mg/kg	5	0.30 ± 0.01†	0.23 ± 0.02*	0.53	0.75*
	10 mg/kg	5	1.89 ± 0.14†	0.43 ± 0.03*¶	2.32	0.23*§
Thalamus-hypothalamus	0 mg/kg	4	2.03 ± 0.06	1.94 ± 0.08	3.97	0.96
	3 mg/kg	5	3.18 ± 0.03*	1.35 ± 0.05*	4.53	0.43*
	10 mg/kg	5	3.51 ± 0.16*#	0.92 ± 0.09*¶	4.43	0.26*§
Brain stem	0 mg/kg	4	2.37 ± 0.03	2.04 ± 0.11	4.41	0.86
	3 mg/kg	5	3.29 ± 0.10*	1.60 ± 0.06‡	4.89	0.49*
	10 mg/kg	5	3.93 ± 0.13*§	1.21 ± 0.15*#	5.13	0.30*§

Monoamine levels were measured as described in the text. Statistical analyses were performed by one-way factorial ANOVA followed by Fisher's PLSD test.

In the analyses of 5-HT: cerebral cortex, $F(2,11) = 52.35$, $p = 0.0001$; cerebellum, $F(2,11) = 28.814$, $p < 0.0001$; striatum, $F(2,11) = 4.47$, $p < 0.05$; thalamus-hypothalamus, $F(2,11) = 51.41$, $p = 0.0001$; brain stem, $F(2,11) = 54.49$, $p = 0.0001$.

In the analyses of 5-HIAA: cerebral cortex, $F(2,11) = 38.71$, $p = 0.0001$; cerebellum, $F(2,11) = 33.36$, $p < 0.0001$; striatum, $F(2,11) = 45.10$, $p < 0.0001$; thalamus-hypothalamus, $F(2,11) = 42.35$, $p = 0.0001$; brain stem, $F(2,11) = 12.878$, $p = 0.01$.

In the analyses of 5-HT + 5-HIAA: cerebral cortex, $F(2,11) = 5.40$, $p = 0.05$; cerebellum, $F(2,11) = 1.91$, $p < 0.05$; striatum, $F(2,11) = 0.15$, $p < 0.05$; thalamus-hypothalamus, $F(2,11) = 3.18$, $p = 0.05$; brain stem, $F(2,11) = 3.33$, $p = 0.05$.

In the analyses of 5-HIAA/5-HT: cerebral cortex, $F(2,11) = 69.13$, $p = 0.0001$; cerebellum, $F(2,11) = 128.90$, $p < 0.0001$; striatum, $F(2,11) = 203.33$, $p < 0.0001$; thalamus-hypothalamus, $F(2,11) = 258.48$, $p = 0.0001$; brain stem, $F(2,11) = 86.56$, $p = 0.0001$.

Between 0 mg/kg and 3 mg/kg, 0 mg/kg and 10 mg/kg: *$p < 0.001$, †$p < 0.05$, ‡$p < 0.01$.

Between 3 mg/kg and 10 mg/kg: §$p < 0.001$, ¶$p < 0.01$, #$p < 0.05$.

regions (Table 1A and B). In the thalamus-hypothalamus, the 5-HIAA/5-HT ratio decreased significantly (Table 1B). These results suggest that the stress enhanced synthesis of 5-HT rather than its turnover. In previous studies, acute immobilization stress increased 5-HT release in the rat hippocampus, as shown by in vivo voltammetry; and this increase was shown to be ascribable to an increased availability of tryptophan (8,11). In vivo microdialysis also demonstrated 5-HT release in the rat hypothalamus by immobilization stress (17). These previous reports seem to support our present observation. The changes in the brain monoamine levels by different types of stress have been already reported, but the intensity of changes, the type of monoamines affected, and the brain regions, where the monoamine levels were changes, are different by the type of stress. The difference in the changes of monoamine levels may be due to the intensity (9) and the type (18,20) of exercise or to training to the exercise (19). These results suggest that the changes in the monoamine metabolism after stress should be carefully elucidated, because we cannot exclude the possible reflects of the movements.

In this article, it was found that moclobemide significantly reduced the immobility score (Fig. 2). Moclobemide was reported to inhibit the activity of type A MAO (3,6,7,10). Moclobemide was reported to increases the levels of NE, DA, and 5-HT in the brains of rats without stress and to decrease those of their metabolites, indicating it to be an inhibitor of MAO (3,6,7,10). Our data demonstrate that in the NE system moclobemide could prevent the reduction in NE and increase in MHPG level by forced swimming (Tables 1A and 2), and that it reduced the enhanced turnover ratio. NE levels increased by about two or three times from those of the nonstress control, and MHPG levels were almost the same to those before stress (Tables 1A and 2). Thus, the drug increased the sum of amounts of NE and MHPG. Previous experiments showed that moclobemide does not modify the activity of various enzymes involved in the synthesis of monoamines, i.e., tyrosine hydroxylase, aromatic L-amino acid decarboxylase, dopamine-β-hydroxylase, and phenylethanolamine-N-methyltransferase (6). But our data suggests that moclobemide may accelerate the catecholamine synthesis in vivo. In the DA system the DA level was increased and the HVA level was decreased by moclobemide (Table 3). It also reduced the turnover ratio without changing the synthesis rate enhanced by the stress. Because DA is a substrate of both type A and type B MAO, and the chages in DA metabolism may be due the inhibiton of type A MAO by moclobemide itself, or that of type B by a metabolite of moclobemide, Ro16-6491[N-(2-aminoethyl)-*p*-chlorobenzamide] (6). In the 5-HT system, the compound further increased the 5-HT level enhanced by forced swimming but decreased the 5-HIAA level enhanced by the stress even to a level lower than that of nonstress control (Tables 1A and 4), so it also reduced the turnover ratio. But it did not change the enhanced synthesis rate. As a conclusion, the effects of moclobemide on DA and 5-HT metabolism may probably be ascribed to the inhibition of MAO, where dose-dependent effects were seen. On the other hand, the effects of moclobemide on NE metabolism were not dose dependent. This may be due to the different effects of moclobemide on the biosynthesis and catabolism of these monoamines. These results suggest that the effect of moclobemide on behavioral changes elicited by forced swimming may be related to that on monoamine metabolism.

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