Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 775–783, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00024-5

Effect of Aversive Stimulation on 5-Hydroxytryptamine and Dopamine Metabolism in the Rat Brain

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Received 26 April 1996; Revised 18 December 1996; Accepted 12 February 1997

GE, J., N. M. BARNES, B. COSTALL AND R. J. NAYLOR. *Effect of aversive stimulation on 5-hydroxytryptamine and dopamine metabolism in the rat brain.* PHARMACOL BIOCHEM BEHAV **58**(3) 775–783, 1997.—The neurochemical consequences of aversive behavior based on novelty, rat social interaction, have been assessed in various rat brain regions utilizing high-performance liquid chromatography coupled with an electrochemical detector (HPLC-ECD) technique. The present studies indicated that compared to animals from the home cage, those exposed to the high-light aversive unfamiliar test condition had significantly increased levels of 5-hydroxyindoleacetic acid (5-HIAA), the metabolite of 5-hydroxytryptamine (5-HT), in the tested brain regions including amygdala, entorhinal cortex, frontal cortex, temporal cortex, tuberculum olfactorium, hippocampus, nucleus accumbens, and striatum. The levels of 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the metabolites of dopamine (DA), were increased in tuberculum olfactorium, nucleus accumbens, and striatum. When compared to the low-light familiar test condition (LF), the levels, following exposure to the high-light unfamiliar situation, of 5-HIAA were significantly increased in the amygdala, entorhinal cortex, tuberculum olfactorium, hippocampus, and nucleus accumbens, while the 5-HIAA levels remained unchanged in the frontal cortex, temporal cortex, and striatum. The DOPAC and HVA levels were also increased by the HU situation in the amygdala, tuberculum olfactorium, and nucleus accumbens. An increase was also found for the levels of DA in the amygdala. Such effects were prevented by diazepam or the 5-HT_3 receptor antagonist ondansetron. It is concluded that the aversive test condition of the social interaction test (HU) increases 5-HT and DA turnover throughout the rat brain. Such effects might be related to the sensitivity to novel anxiolytic drug of the social interaction test. © 1997 Elsevier Science Inc.

Social interaction Locomotor activity Neurochemical consequence Turnover 5-Hydroxytryptamine Dopamine Ondansetron Diazepam Rat brain

ALTHOUGH the psychological and physiological manifestations of anxiety have been studied extensively for many years, the biochemical mechanisms underlying the pathology are less clearly understood. In the past decade, investigations have demonstrated that many central neurotransmitters systems such as 5-HT, DA, GABA, ACh, and NA are probably involved in behavioral responses to aversive situations and anxiety in humans (8,34–36). For instance, several studies have shown that central mesocortical dopamine neurons (9,15) and the noradrenergic system (11,41) may be activated by a variety of stress paradigms, such as electric foot shock, exposure to novelty, and conditioned fear, and the stress-induced increases in the mesocortical dopamine neuronal function were blocked by classic anxiolytic agents such as benzodiazepine receptor agonists (9,30,45). This evidence appears to suggest that central dopaminergic and noradrenergic systems may be involved in some forms of anxiety or fear (34).

During the last decade, in the search for alternatives to the benzodiazepines, 5-hydroxytrypaminergic agents have received the most attention (8,26,27,32,36,43). The direct evidence for

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the involvement of 5-HT in the control of anxiety comes from previous findings that electrical stimulation of midbrain raphe nuclei, which contain 5-HT cell bodies, or systemic administration of the precursor of 5-HT, 5-hydroxytryptophan (5-HTP), or injection of 5-HT directly into the dorsal raphe nuclei, results in aversive behaviors in the animal models, increasing suppressed behavior by punishment in conflict tests, increasing exploratory behavior in the black compartment in the mouse white–black box tests (12,13,48); such responses were antagonized by administration of benzodiazepine receptor agonists, e.g., diazepam, and 5-HT_{1A} receptor agonists such as buspirone, or the 5-HT_3 receptor antagonists such as ondansetron (12,13,48). Lesions produced by administration of *p*-chlorophenylalanine (*p*-CPA), which reduces 5-HT neurotransmissions by inhibition of tryptophan hydroxylase, produced anxiolytic-like effects in the rat social interaction tests (23) and conflict procedures (19). The potent anxiolytic agents, benzodiazepine receptor agonists, have been shown to possess the ability to reduce 5-HT neurotransmission by inhibiting 5-HT synthesis and metabolism (8,10,36,48). Taken together, the pharmacological studies suggest that a reduction in the function of 5-HT, NA, or DA systems leads to anxiolytic effects, and an increase in 5-HT, NA, or DA transmission results in anxiogenic effects (8,34,36).

Therefore, it would seem that the central neurotransmitters play an important role in the control of anxiety. The pharmacological methodology used for testing anxiolytic agents such as conflict procedure and nonpainful stressors (social interaction test, elevated plus-maze, white–black box test) are based on the aversive behaviors in the animal models. The question arose as to whether these models also produce changes of central neurotransmitter levels? The present study was designed to investigate the neurochemical consequences of exposure to an aversive situation, i.e., the high-light, an unfamiliar test condition of the social interaction test. Furthermore, we investigated whether the changes in neurotransmitter contents could be prevented by prior administration of diazepam or the 5-HT_3 receptor antagonist ondansetron.

METHOD

Experimental Animals

Male hooded-Lister rats (Bradford bred) weighing 250–300 g, 11 to 15 weeks old were housed in groups of five at a constant temperature (21.0 ± 1.0°C), 40–50% humidity, 12-h light–dark cycle with lights on between 0700 h and 2000 h. Rats were allowed access to food (CRM diet; Labsure) and water ad lib.

Behavioral Studies

All observations were performed in a wooden test box 62 × 62 × 33 cm (L × W × H) with nine black lines drawn on each side of the floor. The box was placed on the floor in the center of the experiment room. This was a small, quiet room adjacent to the animal holding room and the rats were observed via a Hitachi video system equipped with a low light-sensitive lens. Experiments requiring strong lighting (H) were performed using a 100-W white light source 75 cm above the floor of the box giving 380 lx, and experiments requiring dim light (L) were performed under low lighting conditions in the behavioral laboratory giving 3.5 lx.

All animals were transferred to the experimental holding room at least 2 h before testing. Testing involved placing each member of a pair of rats in opposite corners of the box and then leaving them undisturbed for 10 min while recording their behavior remotely on videotape. The behavioral assessments were subsequently made from the recordings. The time spent in social interaction was measured and expressed as a cumulative total for a 10-min session. When familiar conditions were used, rats were exposed to the arena in the same pair for 10 min, and then put back into the home cages in groups of five, for 3 consecutive days under low-light conditions. The biochemical experiments were carried out on the third day. The behaviors that comprised social interaction were: following with contact, sniffing of partner, crawling over and climbing under partner, genital investigation of partner, tumbling, boxing, and grooming. Locomotor activity, the number of line crossings on the floor of the arena, were also measured over a 10-min test period.

Experimental Design

Experiment 1. In this experiment, both groups of the rats were transferred to the experimental holding room at least 2 h before testing, the normal animals were taken from home cages and killed by cervical dislocation, and the brain areas were dissected. In the HU group, a pair of rats was taken from different animal cages and put in the social interaction testing box, illuminated by high light, for 10 min, the animals were killed by cervical dislocation immediately after one social interaction testing, and the brains were removed and dissected.

Experiment 2. All animals were transferred to the experimental holding room at least 2 h before testing. In the familiar low-light group, animals were taken from different cages, and the same pair of rats were subject to the social interaction paradigm for 10 min under low level of illumination, and then put back into the home cages in groups of five, on 3 consecutive days. In unfamiliar high light, the animals were taken from different cages and placed in the social interaction paradigm for 10 min under high levels of illumination. All animals were killed by cervical dislocation immediately after the third social interaction testing, and the brains were removed for measurement of neurotransmitters and their metabolites.

Experiment 3. In the present experiment, all animals were transferred to the experimental holding room at least 2 h before testing. In the LF condition, animals were taken from different cages, and the same pair of rats was subject to the social interaction paradigm for 10 min under low levels of illumination, and then put back into the home cages in groups of five, on 3 consecutive days prior to assessment of drug effect in the social interaction test. On the third day, rats received a 45-min pretreatment of either vehicle (10% v/v of PEG in saline, 1.0 ml/kg, IP), diazepam (10% v/v of PEG in saline, 2.5 mg/kg, IP), or ondansetron (10% v/v of PEG in saline, 10 μg/kg, IP) and were then subject to the social interaction paradigm for 10 min under low or high illumination between 'familiar' or 'unfamiliar' pairs. After the 10-min social interaction testing, all animals were killed by cervical dislocation and brains were immediately removed for measurement of neurotransmitter levels.

Biochemical Studies

The drug- and vehicle-treated rats were killed by cervical dislocation immediately after the 10-min interaction test. The brains were rapidly removed and nuclei dissected before they were immediately frozen in liquid nitrogen. Brain tissues were homogenized (ultra-Sonic homogeniser; Soniprep 150 MSE) in 200 μl 0.2 M perchloric acid (AnalaR, BDH) containing 0.4 mM sodium metabisulphite (BDH), DHBA (3,4-Dihydroxy-

benzylamine hydrobromide, 100 μM, catecholamine internal standard) and *N*-methyl-5-HT (100 μM, indoleamine internal standard) and stored in liquid nitrogen prior to assay. To assay indoleamines and catecholamines, tissue homogenates were centrifuged (15,600 × *g*, 15 min, 4°C) and 60 μl of the supernatant was added to 60 μl of indoleamine/homovanillic acid mobile phase and applied to the indoleamine/homovanillic acid high-performance liquid chromatography with electrochemical detection (HPLC-ECD) system optimized to detect the indoleamines (5-HTP, 5-HT, 5-HIAA, and homovanillic acid). Catecholamines were isolated from a further 60 μl of supernatant using an alumina extraction technique (3). The extracted catecholamines were immediately applied to the HPLC-ECD optimized for the separation and detection of catecholamines.

The HPLC-ECD systems consisted of either Waters 510 or 6000 A pumps (Waters Associates) connected to a Hypersil-ODS (250 × 4.6 mm, 5 μm particle size) analytical column (HPLC Technology) via an automatic injector (Wisp 710b, Waters Associates). The eluate from the analytical column was passed into a ECD (BAS LC-4A or LC-4B amperometric detector with TL5 glassy carbon working electrodes and silver:silver chloride reference electrodes). The working electrode was maintained at a potential of +700 mV relative to the reference electrode for the analysis of catecholamines and +800 mV relative to the reference electrode for the analysis of indoleamines and homovanillic acid (Bioanalytical Systems Inc.). Peaks due to the oxidation of compounds in the column eluates were recorded on a printing integrator (3392A Hewlett-Packard). Each HPLC-ECD system, with the exception of the integrator, was maintained at 4°C in a temperature-controlled cabinet.

The optimized mobile phase for the separation of indoleamines and homovanillic acid consisted of a mixture of 0.2 M disodium hydrogen orthophosphate (AnalaR grade, BDH) and 0.1 M citric acid (HPLC grade, BDH), pH 6.3, with 11% v/v methanol (HPLC grade, May and Baker) and 2.0 mM tetraethylammonium chloride (Eastman Kodak). The mobile phase was filtered through a 0.2 μm filter (Rainin Instrument Co. Inc.) and degassed by sonication under vacuum. The mobile phase was pumped through the analytical column at a rate of 1.3 ml/min.

The optimized mobile phase for the separation of catecholamines consisted of a mixture of 0.2 M disodium hydrogen orthophosphate and 0.1 M citric acid with 18.0 ml/l of 0.1 M octanesulphonic acid (HPLC grade, Fisons plc), made up in glacial acetic acid (Analytical reagent grade, May and Baker Ltd.), pH 4.0 with 11% v/v methanol. The mobile phase was filtered and degassed as for the indoleamine/homovanillic acid mobile phase and was pumped through the analytical column at 1.3 ml/min.

Drugs

Ondansetron (hydrochloride dihydrate, Glaxo Laboratories, Ware, Herts, UK) and diazepam (Sigma) were suspended in 10% v/v of polyethylene glycol (PEG) in saline. All drugs were administered in a volume of 1 ml/kg IP.

TABLE 1

THE EFFECT OF HIGH LEVELS OF ILLUMINATION BETWEEN UNFAMILIAR PAIRS OF RATS (HU) ON THE LEVELS O F 5-HT, 5-HIAA, DA, DOPAC, AND HVA IN THE RAT BRAIN AREAS

	5-HT	5-HIAA	DA	DOPAC	HVA
Amygdala					
CON	934 ± 29	245 ± 8	490 ± 35	34 ± 2	—
HU	877 ± 21	299 ± 13*	805 ± 73†	49 ± 6	—
Entorhinal cortex					
CON	842 ± 22	164 ± 6	66 ± 4	—	—
HU	846 ± 16	236 ± 14†	57 ± 3	—	—
Frontal cortex					
CON	610 ± 25	86 ± 4	26 ± 3	5 ± 1	—
HU	674 ± 25	120 ± 7†	30 ± 2	9 ± 1	—
Temporal cortex					
CON	536 ± 18	122 ± 3	—	—	—
HU	532 ± 14	164 ± 9†	—	—	—
Tuberculum olfactorium					
CON	725 ± 35	193 ± 11	1614 ± 126	151 ± 12	94 ± 9
HU	696 ± 49	256 ± 16†	1683 ± 62	252 ± 9†	126 ± 10*
Hippocampus					
CON	300 ± 14	166 ± 11	—	—	—
HU	288 ± 10	243 ± 14†	—	—	—
Nucleus accumbens					
CON	575 ± 30	288 ± 12	7512 ± 657	664 ± 50	344 ± 24
HU	671 ± 51	385 ± 12†	7422 ± 506	870 ± 48†	449 ± 23†
Striatum					
CON	390 ± 21	212 ± 10	7167 ± 233	592 ± 22	322 ± 19
HU	617 ± 51*	326 ± 17†	6809 ± 143	679 ± 22*	600 ± 61†

All the levels are expressed as the absolute amount (pg/mg wet brain tissue). Values represent the mean ± SEMs of 8–10 determinations. Significant increases or decreases in responding compared to control values (rats taken from home cages (CON) are indicated as *$p < 0.05$, and †$p < 0.001$ (one-way ANOVA followed by Dunnett's *t*-test). (—): below the detectable limits.

Data Analysis

To analyze the behavior and neurochemical data, one-way ANOVA followed by Dunnett's *t*-test was used for all three experiments. A *p*-value of 0.05 or less was required for significance.

RESULTS

Brain Catecholamine and Indoleamine Levels of Unfamiliar Paired Rats Under High Illumination (HU) and of Control Rats

Compared to the normal rats taken from their home cages, it was found that rats exposed to the high-light unfamiliar test condition had significantly altered levels of 5-HIAA, DOPAC, and HVA. In the amygdala, HU had increased the 5-HIAA levels by 20%, $F(1, 18) = 3.741$, $p < 0.01$, the DA levels were elevated by 65%, $F(1, 18) = 4.268$, $p < 0.001$, by the HU situation, while the 5-HT and DOPAC levels remained unchanged in this area. In the entorhinal cortex, frontal cortex, and temporal cortex, the HU had increased levels of 5-HIAA by 40%, $F(1, 16) = 1.426$, $p < 0.01$, 28%, $F(1, 13) = 1.010$, $p < 0.01$, and 34%, $F(1, 15) = 1.613$, $p < 0.01$, respectively, over the home cage control values.

In the tuberculum olfactorium, the 5-HIAA, DOPAC, and HVA levels were increased by the HU situation by 33%, $F(1, 16) = 2.493$, $p < 0.01$, 70%, $F(1, 17) = 1.533$, $p < 0.01$, and 35% $F(1, 17) = 6.106$, $p < 0.01$, respectively, while the 5-HT and DA levels were not significantly affected. In the hippocampus, the HU situation increased the levels of 5-HIAA by 47%, $F(1, 18) = 4.863$, $p < 0.001$, while the 5-HT levels remained unchanged. In the nucleus accumbens, the levels of 5-HIAA, DOPAC, and HVA were increased by the HU conditions by 34%, $F(1, 14) = 1.605$, $p < 0.01$, 30%, $F(1, 16) = 2.440$, $p < 0.01$, and 30%, $F(1, 15) = 1.86$, $p < 0.01$, respectively, over the control levels.

Finally, in the striatum, the HU situation caused increases in the levels of 5-HT, 5-HIAA, DOPAC, and HVA by 58%, $F(1, 18) = 2.455$, $p < 0.01$, 54%, $F(1, 18) = 3.643$, $p < 0.01$, 10%, $F(1, 18) = 4.665$, $p < 0.05$, and 86%, $F(1, 18) = 2.454$, $p < 0.001$, respectively, over the control values (Table 1).

Brain Catecholamine and Indoleamine Levels of Unfamiliar Paired Rats Under High Illumination (HU) and Familiar Paired Rats Under Low Illumination (LF)

Rats exposed to the LF situation showed a time-dependent increase in the social interaction levels, whereas the locomotor activity was found to have a time-dependent decrease over 3 consecutive days. In contrast, the HU situation showed a marked decrease in the levels of social interaction while the locomotor activity was significantly increased by the HU situation when compared to the FL levels on the third day (Fig. 1).

In the amygdala, the HU situation caused a 90% increase in the DA levels over the LF control values, $F(1, 16) = 3.319$, $p < 0.001$. The DOPAC and 5-HIAA levels were also increased by the HU situation. In the entorhinal cortex, the HU condition caused significant increases in the 5-HIAA levels.

The 5-HIAA, DOPAC, and HVA levels in the tuberculum olfactorium were significantly increased by the HU situation compared to the LF control levels. In the hippocampus, the 5-HIAA levels were increased by the HU situation over the LF control values.

In the nucleus accumbens, the HU situation caused increases in the levels of 5-HIAA, DOPAC, and HVA. In the striatum, the HU situation significantly increased the DOPAC levels compared to the FL control values (Table 2).

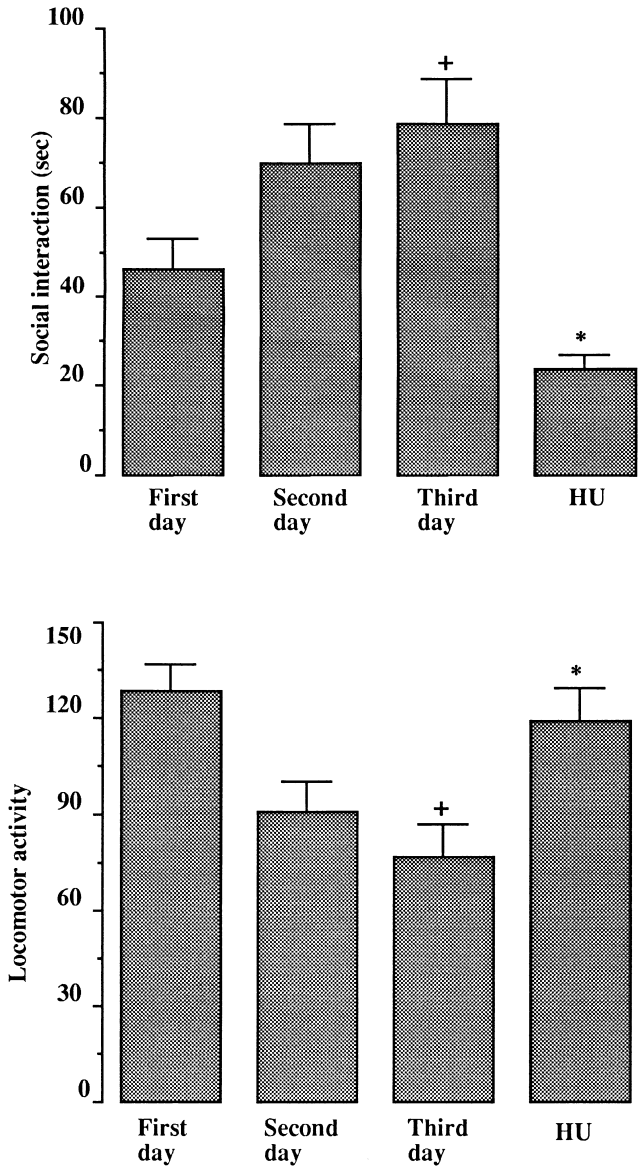

FIG. 1. The effect of low levels of illumination between same pairs of rats in three consecutive days and high levels of illumination between different pairs of rats (HU) on the levels of social interaction and locomotor activity. Values represent the mean ± SEMs of 8–10 determinations. Significant increases or decreases in responding are indicated as $*p < 0.05$ (compared to the value from the third day under low light) and $+p < 0.01$ (compared to the value from the first day under low light) (one-way ANOVA followed by Dunnett's *t*-test).

Effect of Diazepam and Ondansetron on Social Interaction, Locomotor Activity, and Monoamine Levels in Rat Brain Under HU and LF Conditions

The HU situation decreased the social interaction levels. A 45-min pretreatment of diazepam (2.5 mg/kg, IP) and ondansetron (10 μg/kg, IP) prevented such effect of HU on social interaction levels (Fig. 2). Under low light and familiar

TABLE 2

THE EFFECT OF HIGH LEVELS OF ILLUMINATION BETWEEN UNFAMILIAR PAIRS OF RATS (HU) AND LOW LEVELS OF ILLUMINATION BETWEEN FAMILIAR PAIRS OF RATS (LF) ON THE LEVELS OF 5-HT, 5-HIAA, DA, DOPAC, AND HVA IN THE RAT BRAIN AREAS

	5-HT	5-HIAA	DA	DOPAC	HVA
Amygdala					
LF	769 ± 23	253 ± 10	379 ± 27	40 ± 4	—
HU	742 ± 25	356 ± 20‡	711 ± 77†	92 ± 11‡	—
Entorhinal cortex					
LF	569 ± 26	116 ± 6	53 ± 4	—	—
HU	580 ± 121	146 ± 4†	54 ± 3	—	—
Frontal cortex					
LF	1033 ± 58	86 ± 2	—	—	—
HU	951 ± 72	98 ± 3	—	—	—
Temporal cortex					
LF	467 ± 24	125 ± 5	—	—	—
HU	422 ± 15*	129 ± 5	—	—	—
Tuberculum olfactorium					
LF	1285 ± 32	195 ± 9	1753 ± 93	251 ± 11	31 ± 2
HU	1529 ± 62*	262 ± 12‡	2091 ± 127	369 ± 25‡	44 ± 3†
Hippocampus					
LF	396 ± 10	267 ± 7	—	—	—
HU	403 ± 17	325 ± 18†	—	—	—
Nucleus accumbens					
LF	707 ± 24	349 ± 12	6226 ± 268	764 ± 13	78 ± 2
HU	725 ± 34	457 ± 32†	6377 ± 273	920 ± 50*	101 ± 6†
Striatum					
LF	641 ± 22	255 ± 10	4713 ± 266	418 ± 27	70 ± 6
HU	564 ± 21*	287 ± 14	4956 ± 200	533 ± 33*	92 ± 10

All the levels are expressed as the absolute amount (pg/mg wet brain tissue). Values represent the mean ± SEMs of 8–10 determinations. Significant increases or decreases in responding compared to the FL control values are indicated as *$p < 0.05$, †$p < 0.01$ and ‡$p < 0.001$ (one-way ANOVA followed by Dunnett's *t*-test). (—): below the detectable limits.

conditions neither diazepam nor ondansetron pretreatment had any significant effects on rat social interaction levels or locomotor activities (Fig. 2).

The neurochemical studies showed that the catecholamine and indoleamine levels were, to different extents, affected by diazepam or ondansetron. In the amygdala, the HU situation caused increases in the levels of 5-HIAA, DA, and DOPAC. Diazepam (2.5 mg/kg, IP) significantly reversed the HU-induced increases in the 5-HIAA, DA, and DOPAC levels (Fig. 3). Under the LF condition, however, diazepam increased the DA and DOPAC levels and had no effect on the 5-HIAA levels. Ondansetron (10 μg/kg, IP) significantly antagonized the HU-induced increases of the DA levels, but had no effects on the 5-HIAA and DOPAC levels in this area. Ondansetron also caused decreases of the 5-HT levels by approximately 40%, $F(2, 26) = 186.5$, $p < 0.001$, over the vehicle-treated HU control values (Fig. 3).

In the hippocampus, the HU situation caused significant increases in the 5-HIAA levels. Pretreatment with diazepam or ondansetron completely prevented such increases (Fig. 3).

In the nucleus accumbens, the HU situation increased the DOPAC and HVA levels over the vehicle-treated LF control values. Administration of diazepam or ondansetron significantly prevented the HU-induced increases in these levels (Fig. 3). Diazepam and ondansetron also caused some increases in the DOPAC levels under the LF conditions. In other tested brain areas, the HU situation caused, to different extents, increases of the 5-HIAA, DOPAC, and HVA levels; however, pretreatment of neither diazepam nor ondansetron altered such levels.

DISCUSSION

The present studies have investigated the neurochemical consequences of aversive behavior of social interaction in various rat brain areas using a high-performance liquid chromatography technique coupled with an electrochemical detector (HPLC-ECD). The present biochemical results provide evidence that the aversive situation induced by the novelty of the environment and partner causes marked increases in the levels of 5-HIAA, DOPAC, and HVA without effect on basal DA and 5-HT levels (i.e., increased 5-HT and DA turnover) throughout the rat brain, especially the mesolimbic areas. The dopamine levels were also markedly elevated by such aversive stimulation in the rat amygdala. A pretreatment of diazepam or the 5-HT_3 receptor antagonist ondansetron prevented the increases of 5-HT and DA turnover and DA levels in the amygdala induced by the aversive stimulation.

Behavior in the social interaction test is modulated by varying illumination and novelty (22). The high-light, unfamiliar test condition, in which both rats are unfamiliar with each other and the arena, is the most aversive situation, whereas the low-light familiar situation is the lowest aversive. In the first experiment, we compared the rats from home cage to those exposed to the high-light aversive unfamiliar test condition. The present neurochemical results showed that the high-light aversive unfamiliar test condition increased 5-HT and

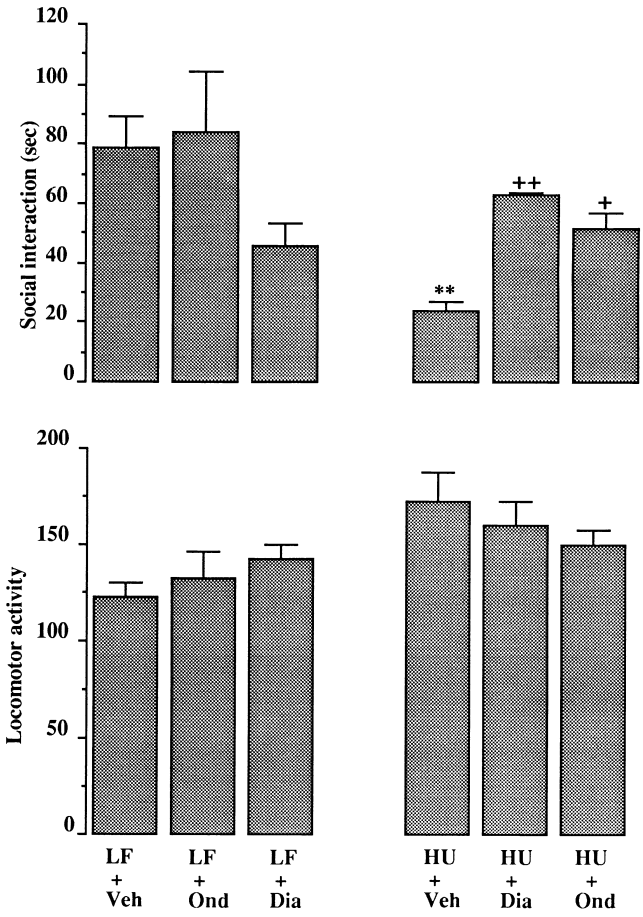

FIG. 2. The effect of pretreatment of vehicle (1.0 ml/kg, IP, 10% v/v of PEG in saline, Veh), diazepam (2.5 mg/kg, IP, 10% v/v of PEG in saline, Dia) or ondansetron (10 μg/kg, IP, 10% v/v of PEG in saline, Ond) on the levels of social interaction and locomotor activity under LF. Values represent the mean ± SEMs of 8–10 determinations. Significant increases or decreases in responding are indicated as $^{**}p < 0.05$ (compared to the value from the third day under low light condition) and $+p < 0.05$ and $++p < 0.01$ (compared to the value from HU control group) (one-way ANOVA followed by Dunnett's *t*-test).

DA metabolism by increasing the levels of 5-HIAA, DOPAC, and HVA without effect on basal 5-HT and DA levels in all the tested brain areas, except that the basal DA levels in amygdala were elevated by approximately twofold by such aversive stimulation. Given the ratio of 5-HIAA to 5-HT as turnover of 5-HT and DOPAC and HVA to DA as turnover of DA, the present studies provided evidence that under high level of aversive stimulation induced by the high-light and unfamiliar test condition, the 5-HT and DA systems were highly activated and, therefore, the 5-HT and DA turnovers were increased throughout the brain areas, especially the mesolimbic system.

In the second experiment, the same pair of animals were placed in the social interaction test paradigm for 10 min each day for 3 consecutive days under low levels of illumination (LF). This procedure allowed the animals to become familiar with the environment and the partner, thereby avoiding the novelty of such factors and hence reducing the aversive levels. Over 3 consecutive days, the levels of social interaction were increased while the locomotor activity was decreased in a time-dependent manner. The high levels of illumination between unfamiliar pairs of rats (UH) caused marked reductions in the level of social interaction and increases in locomotor activity, suggesting that the decreases in social interaction reflect an increased level of anxiety, and increases in the social interaction levels reflect a decreased levels of anxiety (21,22,25). Consistent with the behavioral results, the basal levels of 5-HT and DA and their metabolites measured under LF situation were comparable to the levels from normal animals from their home cages, indicating that the aversive levels were minimized under LF condition. Paralleling with reduction of social interaction levels between two unfamiliar rats under high illumination (HU), the 5-HT and DA turnover were significantly elevated by the HU stimulation only in rat amygdala, entorhinal cortex, tuberculum olfactorium, hippocampus, and nucleus accumbens, which have been shown to be mainly associated with anxiety disorders (8,35,36), whereas the 5-HT turnover in frontal cortex, temporal cortex, and striatum remain unchanged. Such finding may suggest that: the first, the 5-HT and/or DA system are more sensitive to the HU aversive stimulation in the limbic system but less sensitive in the other areas; and the second, the LF situation still causes some extent of aversive stimulation, although such stimulation was minimized (21,22). However, the present studies were unable to determine whether the changes in the 5-HT and DA turnover induced by LF situation is time dependent, and which factor among levels of illumination, novelty to environment, and partner plays a more important role in such neurochemical changes. Further investigations are needed to clarify these test conditions. No matter which factor contributes more to the aversive stimulation-induced neurochemical changes, the high-illumination unfamiliar aversive stimulation elevated the 5-HT and DA turnover in the rat mesolimbic regions.

Pretreatment with diazepam or the selective 5-HT_3 receptor antagonist, ondansetron, significantly prevented the reduction of the social interaction levels induced by HU situation but failed to modify such levels under the LF condition. Consistently, diazepam and ondansetron also prevented the HU stimulation-induced increases in the 5-HT and DA turnover and elevated DA levels in the rat amygdala, confirming that the increased 5-HT and DA turnover were the result of aversive stimulation and sensitive to the antianxiety drugs.

The involvement of the 5-hydroxytryptaminergic system in the control of aversive behavior and anxiety in humans has been demonstrated in previous studies (see the introductory paragraphs). The present studies provide neurochemical evidence to show that the aversive situation induced by novelty of a partner and environment elevated 5-hydroxytryptaminergic neuronal activity and further increased 5-HT metabolism in the rat brain, especially the limbic areas. The increased 5-HT neuronal activity was also found in the previous studies using other animal models of anxiety. For example, it has been demonstrated that stressors, startle, and some anxiogenic agents increase the 5-HT turnover, 5-HT and 5-HIAA levels, and 5-HT release in the rat brain (1,14,17,18,28,42). Using the intercerebral microdialysis technique, we have previously reported that chemical stimulation of yohimbine and FG7142 elevated the extracellular levels of 5-HT and 5-HIAA in the rat frontal cortex. Furthermore, pretreatment with anxiolytic and putative anxiolytic agents prevented such responses (29), while systemic administration of the benzodiazepine agonists flurazepam and diazepam reduced the 5-HT release in the rat hippocampus (29,44). The increase of 5-HT release in rat hippocampus has also been reported during behavior on the elevated plus-maze (49) and during the anxiogenic response as-

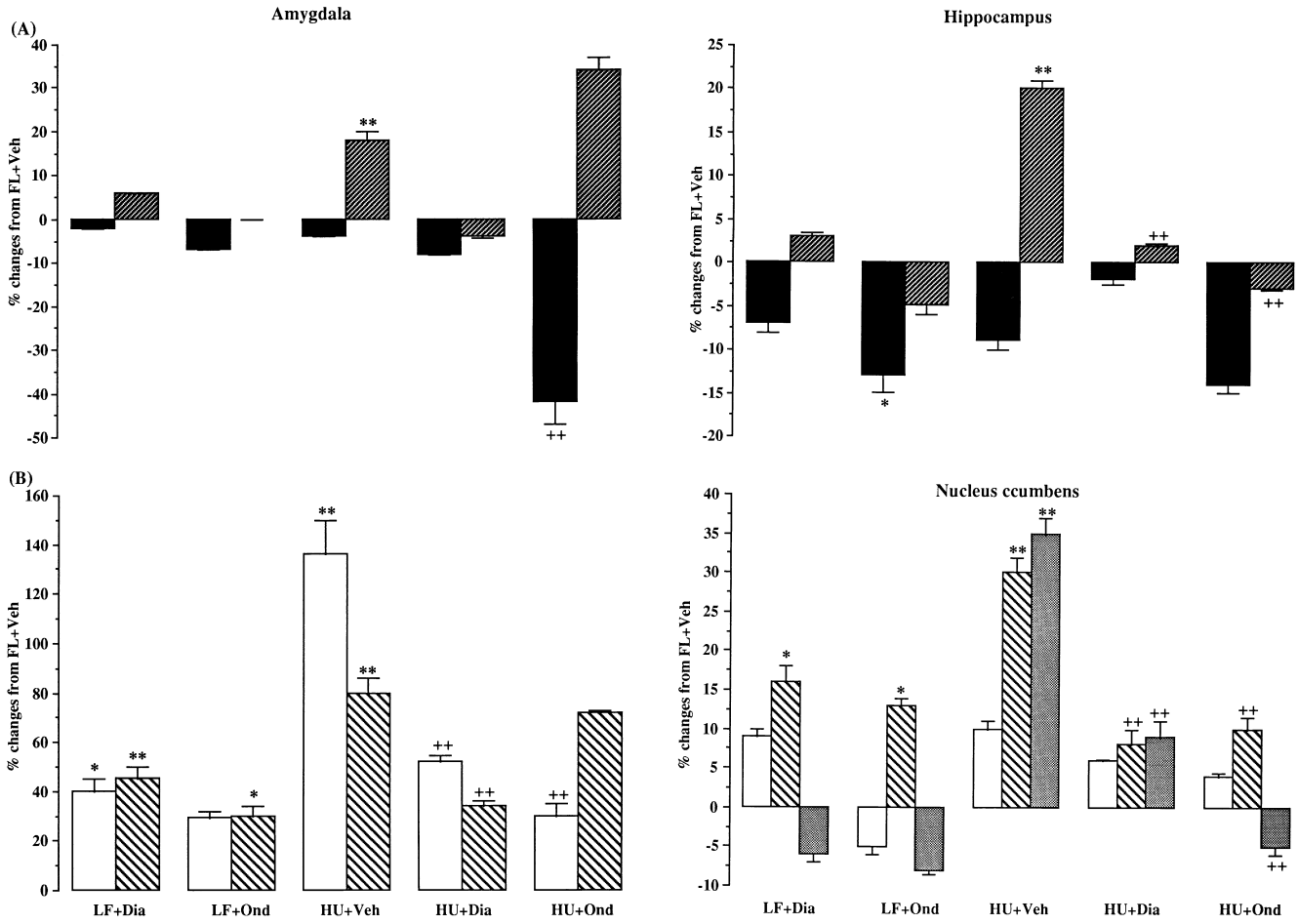

FIG. 3. The effect of pretreatment of vehicle (Veh, 1.0 ml/kg, IP, 10% v/v of PEG in saline), diazepam (Dia, 2.5 mg/kg, IP, 10% v/v of PEG in saline) or ondansetron (Ond, 10 μg/kg, IP, 10% v/v of PEG in saline) on the levels of 5-HT (solid columns), 5-HIAA (closed-striped diagonal columns), DA (open columns), DOPAC (loose-striped diagonal columns) and HVA (dotted columns) in the rat amygdala, hippocampus, and nucleus accumbens under LF and HU condition. All the levels are expressed as percentage changes from vehicle control values (FL + Veh). Values represent the mean ± SEMs of 8–10 determinations. Significant increases or decreases in responding compared to the FL control values are indicated as $*p < 0.05$ and $**p < 0.01$ and compared to the HU control values are indicated as $++p < 0.01$ (one-way ANOVA followed by Dunnett's *t*-test).

sociated with benzodiazepine withdrawal (2). Most recently, File et al. (24) reported that both a 5-min plus-maze trial and exposure to the high-light familiar condition of the social interaction test caused significant increases in evoked release of [^{3}H]5-HT from slices of hippocampus, but far more greater changes were found in the uptake of 5-HT in such behavior tests. Taken together, it could be assumed that aversive stimulation increases central 5-hydroxytryptaminergic activity (especially in the mesolimbic areas) via either increase in 5-HT release or decrease in 5-HT uptake, and therefore, increases 5-HT metabolism. Such effects may contribute to the neurochemical mechanism of anxiolytic action of benzodiazepines and 5-HT type (5-HT_{1A} agonists and antagonists, 5-HT_2 and 5-HT_3 antagonists) anxiolytics.

The involvement of dopaminergic systems in anxiety have also been described before. Previous studies have shown that the mesocortical dopaminergic neurons were activated by a variety of stress paradigms such as electric foot shock, exposure to novelty, and conditioned fear (9,15,17,20,38,46,47). Such stressors increased catecholamine turnover in rat frontal cortex (47), nucleus accumbens (20), and ventral tegmental area (VTA) (15). Consistent with these findings, the present results indicate that an aversive situation enhanced dopamine turnover in the rat tuberculum olfactorium, nucleus accumbens, and striatum, suggesting that dopaminergic neuronal activity is also increased by aversive stimulation.

The most important finding of the present study was that the basal dopamine levels were increased by two- to three-fold by the HU situation in the rat amygdala, in comparison with either the vehicle control or LF situation. The amygdala is involved in the central limbic system, which plays an important role in the control of anxiety, and several studies have suggested that the amygdala is of crucial importance in aversive classical conditioning and probably anxiety in animals and humans (5,31,33,39,40). For instance, amygdalectomy has been indicated to reduce the response to threatening stimuli in monkeys, increase punished responding in rats, and block the conditioned fear induced by the potentiated startle response (5,16,33,37). Electrical stimulation of the central nucleus of the amygdala produces a cessation of ongoing behavior (4),

which is the critical measure of fear or anxiety in several animal models such as the operant conflict test, the conditioned emotional response, social interaction, and freezing itself. Clinical studies have also demonstrated that electrical stimulation of the amygdala elicits feelings of fear or anxiety as well as autonomic reactions indicative of fear in humans (7,31). The precise mechanisms of involvement of dopamine in amygdala underlying the pathology of anxiety is not clearly understood. Investigators have also indicated that the projections from the central nucleus of the amygdala to the ventral tegmental area (VTA), which also parallel the 5-HT ascending projections from dorsal raphe nucleus to amygdala and frontal cortex, may mediate stress-induced changes in dopamine turnover (47). Nevertheless, the increased dopaminergic neuronal activity in amygdala may be one of the most important factors in the control of anxiety, and the interaction between the 5-HT and DA systems may also exist in such mechanisms.

In summary, the present studies demonstrated that the aversive stimulation of the social interaction test (HU) increases 5-HT and DA turnover throughout the rat brain, especially the mesolimbic ares. The DA levels were also increased by aversive situations in the rat amygdala. Such responses were prevented by diazepam or ondansetron. It could be concluded that the increases in 5-hydroxytryptaminergic and/or dopaminergic activities leads to an anxiogenic response, and that reductions of this response may be associated with anxiolytic activities, and the increase of DA levels in amygdala may be an important factor in the control of anxiety.

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