PARTICIPANT OF SEROTONIN TURNOVER RATE IN THE BRAIN ON BARBITAL WITHDRAWAL CONVULSION

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Abstract—The correlation between the development of barbital (B)-withdrawal signs and alterations in the metabolism of brain 5-hydroxytryptamine (5-HT) was studied. Barbital (B)-dependent rats were prepared by the B-Admixed Food method (DAF method). The B-dependent rats were grouped according to the following 5 states: G-I, B-dependent state; G-II, B-withdrawal state; G-III, cross-administration of nitrazepam (NZP) following B-withdrawal; G-IV, cross-administration of chlorpromazine (CPZ) following B-withdrawal; and G-V, cross-administration of phenytoin following B-withdrawal. The controls were comprised of naive rats (G-VI) and naive rats dosed in the same manner as the dependent rats. The brain 5-HT synthesis rate and the brain 5-hydroxyindole acetic acid (5-HIAA) elimination were measured at 44 to 48 hr after B-withdrawal in all groups (when withdrawal convulsion was still persisting in the B-withdrawn rats of the G-II group). The brain 5-HT synthesis rate was elevated significantly (P<0.001) in the rats with persistent B-withdrawal convulsion (G-II) as compared with that in the B-dependent rats (G-I). Cross-administration of nitrazepam caused the elevation of 5-HT synthesis rate with B-withdrawal to be inhibited to the dependent level in parallel with the inhibition of B-withdrawal signs. On the other hand, CPZ and phenytoin, which inhibit B-withdrawal convulsion slightly, failed to recover completely the 5-HT turnover rate during B-withdrawal. From these results, it is obvious that the elevation of the brain 5-HT synthesis rate with B-withdrawal plays an important role in eliciting B-withdrawal convulsion.

There have been studies on the relationships between the threshold value for acute convulsion induced by electric shock or with pentetrazol (PTZ) and the brain monoamines (1-5). Kilian et al. (6) suggested the probable involvement of 5-HT in PTZ convulsion from the following findings: the reduction in the brain norepinephrine (NE) or 5-hydroxytryptamine (5-HT) contents lowered the threshold for electro-shock convulsion; pretreatment by L-DOPA or 5-hydroxytryptophan (5-HTP; a precursor of 5-HT) to cause the elevation of dopamine (DA). NE or 5-HT raised the threshold for the convulsion; and in the rats with the brain 5-HT content reduced by the administration of p-chlorophenylalanine (PCPA; a 5-HT synthesis inhibitor), the threshold for PTZ convulsion was low (6). There is also a report (1) indicating that DA plays a principal role in the convulsion. However, there are varying opinions on what causes the con-
vulsion, depending on the convulsion model, animal species or animal strain used. There are a number of papers about the variations in brain monoamines in acute or chronic application (7) or after withdrawal of alcohol (8–15), but only few papers on barbiturate dependence (16). By observing B-withdrawal convulsion and changes in brain catecholamines with application of α-methyl-p-tyrosine (α-MT) during B-withdrawal, Morgan et al. (17) noted that changes in these parameters were in no way correlated with each other. From the viewpoint of drug dependence, no studies have been made on the reversibility of withdrawal signs, i.e., whether the neuron activities that have changed with B-withdrawal will recover to the pretreatment level on recovery of barbital withdrawal signs or in parallel with the inhibition of B-withdrawal signs with the cross-application of a drug of the same type.

In an investigation of the relationships of drug dependence to changes in the brain monoamines, it is essential to characterize the changes in monoamines in the non-withdrawn dependent, the withdrawn, and the cross-administered state with other drugs. Tagashira et al. (18, 19) had been successful in providing a drug dependence model in rats exposed to barbiturates by the DAF (Drug Admixed Food) method. This model is similar to the drug dependence in man or large animals such as dogs and monkeys with respect to changes in withdrawal signs with the passage of time, their severities and durations. The DAF method, as a model for sedative-hypnotic dependence with high reproducibility, but with less individual difference and variations, can be applied not only for screening of drug dependence liability, but also for studying the mechanism of drug dependence formation and of studying the mechanism of drug dependence formation and of elimination of withdrawal signs. This study was made to investigate the relationship between changes in brain serotonin metabolism and the evolution of withdrawal signs at the stage during which convulsion persisted during B withdrawal.

MATERIALS AND METHODS

Male Sprague-Dawley rats (supplied from Tokyo Laboratory Animals Co., Tokyo) were used which weighed 100 to 120 g at the start of the study. There rats were given free access to food and drinking water, housed in individual cages in a room with the light turned on at 8:00 and turned off at 20:00, and air-conditioned to maintain room temperature (22±2°C) and a relative humidity of 55±5%. Barbital (B) and nitrazepam (NZP) were administered as mixtures with a powder feed (CA-1; Japan Clea, Tokyo). Tranylcypromine (Tcp) was dissolved in saline and chlorpromazine (CPZ) and phenytoin (PHT) dissolved in distilled water.

Procurement of B-dependent rats: Following the method of Tagashira et al. (18), foods containing B at 2 different concentrations were simultaneously provided in each cage. Foods containing 0.5 and 1 mg of B per g of food were provided during the first 4 days; those containing 1 and 2 mg/g, on days 5–10, those containing 2 and 4 mg/g, on days 11 to 16, those containing 4 and 6 mg/g, on days 17 to 26, and those containing 6 and 8 mg/g, on days 27 to 36, so as to gradual increase the amounts of B ingested by the rats. On this dosage schedule, severely B-dependent rats were produced.

Effects of cross-application of test drugs on B-withdrawal signs: When B was withdrawn from the B-dependent rats at 9:00 a.m. (by means of replacing the drug-admixed foods with a normal food), withdrawal signs such as hyperirritability, muscle fasciculation, tremor, muscle twitching, hyperkinesia and clonic-tonic convulsion developed and persisted between 17 and 48 hr after with-
drawal. At 48 hr after withdrawal, the rats were examined for magnitudes of weight loss, and severities, frequencies and durations of convulsion to evaluate the severities of withdrawal signs.

The B-dependent rats were divided into the G-I (B-dependent) group, G-II (natural withdrawal) group, and G-III to V (cross-application) groups. NZP (G-III) was given to completely inhibit B-withdrawal signs, and CPZ (G-IV) and PHT (G-V) were cross-administered as the drug to partially suppress B-withdrawal signs including convulsion. NZP was given as a mixture with food containing 1.5 mg of NZP per g (20) from the 0 hr of withdrawal and onward. CPZ at a dosage of 30 mg/kg was cross-applied orally, 4 times in total, at 5- to 6-hr intervals between 31 and 48 hr of withdrawal. PHT at a dosage of 40 mg/kg was cross-applied orally 5 times at 6-hr intervals between 9 and 48 hr of withdrawal. For the measurement of 5-HT synthesis rate, 20 mg/kg of Tcp (21), a monoamine oxidase inhibitor, was applied at 44 to 48 hr of withdrawal when the B-withdrawal signs still persisted. The rats were decapitated before or 1 hr after the dosing, and their brains were assayed for 5-HT and its metabolite, 5-HIAA. The rats on natural withdrawal (G-II), naive rats (G-VI) or naive rats cross-dosed with the drugs on the same schedule as for the cross-physical dependence liability test were used as controls.

Assay methods: A slight modification of the procedure for 5-HT and 5-HIAA determination by Perez-Cruet et al. (22) was used.

The rat brains were removed quickly and each was homogenized with 0.1 N hydrochloric acid containing 0.5% ascorbic acid. The homogenate was neutralized with 1 N sodium hydroxide and deproteinized by the addition of 10% zinc sulfate. The supernatant was transferred into another test tube, saturated with sodium chloride, neutralized with 1 N hydrochloric acid, and washed with 10 ml of benzene to remove indoleacetic acid. Ten ml of n-butylacetate was added to the product, the mixture was shaken, and centrifuged. The n-butylacetate layer was used for the determination of 5-HIAA and the water layer for the determination of 5-HT. The 5-HT extracted with butanol in this process was re-extracted with phosphate buffer (pH 7.0). 5-HT was determined after condensation with ninhydrin (Wako Pure Chemical Industries, Osaka) by the method of Snyder et al. (23) to increase its fluorescence sensitivity and specificity.

RESULTS

Withdrawal signs during B-withdrawal and on cross-application of test drugs: In the group of rats dependent on low dosages of B (2 and 4 mg B/g food) (MD group), moderate withdrawal signs, i.e., muscle twitching, vocalization, systemic tremor, hyperreflexia and hyperirritability were noted from about 24 hr of withdrawal and onward. The B-withdrawal was also associated with marked weight loss with a

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<th>Table 1. Grades of barbital withdrawal signs in rats</th>
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<tr>
<td>Mild: Anorexia, restlessness, weight loss (approximately 5%)</td>
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<tr>
<td>Moderate: Hyporeflexia, hypopnea, aggressiveness, vocalization on touching, hyperirritability, mild temors, rearing, abnormal posture (kangaroo style_), ear-twitching, muscle rigidity, impaired motor activities, ataxia, weight loss (approximately 10%)</td>
</tr>
<tr>
<td>Severe: Aggravated temors (including head temors), fascicular twitching (nuchal twitching), hyperthermia (1.5-2.0°), clonic-tonic convulsion, hyperkinesia, grand mal type convulsion, wild running &quot;run fits&quot;, weakness after convulsion, death, weight loss (more than 15%)</td>
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maximum loss of 7.5% at 36 hr after withdrawal. In the group treated gradually to the highest dosage (6 and 8 mg B/g food) (SD group), weight loss persisted until about 48 hr of withdrawal as noted in the MD group, with the maximum loss of about 11.0% at 48 hr after withdrawal. Mild to moderate withdrawal signs appeared from about 17 hr of withdrawal and onward, and severe withdrawal signs such as clonic-tonic convulsion (C-TC) were frequent in all rats between 24 and 48 hr of withdrawal (Fig. 1).

The dosing of naive rats with NZP (1.5 mg/g food) resulted in the appearance of generalized muscle relaxation, staggering gait, ptosis and head shaking from about 12 hr after the dosing and onward. The oral dosing with CPZ and PHT also caused the manifestations of CNS depression, muscle relaxation, ptosis, etc. which were similar to those observed with NZP. Hypothermia and hypokinesia were especially striking with CPZ.

The cross-application of NZP during B withdrawal was followed by no rapid weight losses which were seen in the control group on B-withdrawal, and the rats treated with NZP showed the same circadian rhythm of body weight as naive rats (Fig. 1). This cross-dosing also inhibited the other withdrawal signs almost completely. The cross-dosing with CPZ, unlike that with NZP, did not inhibit the weight loss, and the CPZ-treated rats exhibited a weight loss pattern similar to that of the controls on B-withdrawal (Fig. 2). This cross-dosing further inhibited the frequency of withdrawal convulsion, but failed to inhibit vocalization, hyperirritability and tremor. The cross-dosing with PHT inhibited the weight loss to about 7% as compared with the maximum loss of 11% in the controls on B-withdrawal (Fig. 2). It also partially inhibited the withdrawal signs, with hyperreflexia, tremor and clonic convulsion still persisting.

Brain 5-HT synthesis rate and 5-HIAA elimination during B-withdrawal and on cross-application of test drugs: Figures 3 and 4 illustrate the brain 5-HT and 5-HIAA levels, 5-HT synthesis rate and 5-HIAA elimination in the B-dependent groups (MD and SD.

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**Fig. 1.** Time course of changes in body weight by barbital-withdrawal in rats made mildly (——) and severely (—) dependent on barbital and the inhibitory action of nitrazepam on barbital withdrawal signs. Nitrazepam-admixed food (1 mg/g food) was substituted for barbital-admixed food from onset of barbital-withdrawal to 44 hr after withdrawal.
Fig. 2. Effects of treatment with chlorpromazine and phenytoin on the decrease in body weight by barbital-withdrawal in rats made severely dependent on barbital (6- and 8 mg barbital/g food). Chlorpromazine and phenytoin were cross-administered 4 times at 5-6 hr intervals and 5 times at 6 hr intervals, respectively.

Fig. 3. Biosynthesis rates of 5-hydroxytryptamine (5-HT) in brain of a rat mildly dependent on barbital (maintenance level of 2-and-4 mg barbital/g food). Biosynthesis rates of 5-HT and elimination rate of 5-hydroxyindole acetic acid (5-HIAA) was determined from the initial rate of 5-HT accumulation and elimination if 5-HIAA after administration of the monoamine oxidase inhibitor, tranylcypromine (20 mg/kg, i.p.). The 0 min data points in Fig. denotes the steady level of 5-HT and 5-HIAA.
groups) and the group on natural withdrawal as compared with these parameters in the untreated group. No difference was noted in brain 5-HT and 5-HIAA levels or 5-HT synthesis rate and 5-HIAA elimination between the naive rats in a steady state and the non-withdrawn, dependent rats. The natural withdrawal of B, however, was followed by statistically significant (P<0.05) elevations of both 5-HIAA levels and 5-HT synthesis rate in the MD and SD groups. Also, the brain 5-HIAA elimination decreased

![Graphs showing biosynthesis rates of 5-HT in brain of a rat severely dependent on barbital.](image)

**Fig. 4.** Biosynthesis rates of 5-HT in brain of a rat severely dependent on barbital (maintenance level of 6-and-8 mg barbital/g food). Procedures for determination of 5-HT and 5-HIAA were the same as those in the legend of Fig. 3.

**Table 2.** Effects of cross-administration of nitrazepam, chlorpromazine and phenytoin during barbital withdrawal on 5-HT metabolism in brain

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<tr>
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<th>5-HT (µg/g brain)</th>
<th>5-HT synthesis rate (µg/g brain/hr)</th>
<th>5-HIAA</th>
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<tr>
<td>Dependent control</td>
<td>100.0 ± 5.3</td>
<td>100.0 ± 0.215</td>
<td>100.0 ± 10.8</td>
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<tr>
<td>Withdrawal control</td>
<td>104.5 ± 2.9</td>
<td>153.5 ± 0.330</td>
<td>154.1 ± 14.1</td>
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<tr>
<td>Withdrawal + nitrazepam</td>
<td>95.7 ± 7.6</td>
<td>93.0 ± 0.200</td>
<td>81.9 ± 10.9</td>
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<tr>
<td>Withdrawal + chlorpromazine</td>
<td>109.5 ± 10.6</td>
<td>115.5 ± 0.248</td>
<td>109.8 ± 6.6</td>
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<tr>
<td>Withdrawal + phenytoin</td>
<td>117.2 ± 4.8</td>
<td>119.6 ± 0.257</td>
<td>126.2 ± 9.6</td>
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Steady state level of 5-HT, 5-HIAA and synthesis rate of 5-HT are shown with those of the dependent control of barbital taken as 100. Significantly different from barbital-dependent control, *P<0.05, **P<0.01.
Fig. 5. Inhibitory action of nitrazepam on elevation of 5-HT synthesis rate and enhancement of suppressed elimination of 5-HIAA caused by barbital withdrawal. Nitrazepam was substituted for barbital from onset of barbital withdrawal to 44 hr after withdrawal.

strikingly in the SD group. The cross-application of NZP which inhibits B-withdrawal signs completely and of CPZ and PHT which apparently inhibit the withdrawal signs caused changes in the brain 5-HT synthesis rate corresponding to the inhibition of the withdrawal signs (Table 2). In other words, the cross-dosing with NZP inhibited and recovered the elevated 5-HT synthesis rate and the depressed brain 5-HIAA elimination during B-withdrawal to almost the same level as those in naive or B-dependent rats (Fig. 5). In comparison, the cross-dosing with CPZ and PHT gave rise to a tendency for the inhibition of the 5-HT synthesis rate which was slight compared with that induced with NZP.

DISCUSSION

In a previous paper (24), Tagashira et al. described the effects on B-withdrawal signs of cross-dosing with B, NZP, and 5-HT metabolism-related substances, i.e., 5-hydroxytryptophan (5-HTP, a 5-HT precursor), p-chlorophenylalanine (PCPA, a 5-HT synthesis inhibitor) and tranylcyromine (monoamine oxidase inhibitor). They further studied changes in 5-HT and 5-HIAA levels in a steady state with the cross-dosing, and found that the application of tranylcypromine during B-withdrawal both in mildly and severely dependent rats (25) was followed within 10 min by the appearance of clonic-tonic convulsion in all rats. This lead to the presumption that brain monoamines play an important role in the development of withdrawal convulsion. The present study was designed to observe changes in brain 5-HT synthesis rate for the purpose of investigating the mechanism of Tcp-induced B-withdrawal convulsion from the aspect of 5-HT metabolism.

It is possible to study whether changes in 5-HT metabolism are specific for physical dependence on B by means of comparing the following states: a) the B-dependent state (G-I) and the naive state (G-IV), b) B-withdrawn state (G-II) and a state in
which B-withdrawal signs are inhibited by cross-dosing with NZP (G-III), or c) states in which B-withdrawal signs including convulsion are partially inhibited with CPZ and PHT, respectively, G-IV and G-V. The 5-HT level and 5-HT synthesis rate in the steady state in B-dependence did differ significantly from those in the naive state, and 5-HT metabolism was not enhanced until B was withdrawn. Therefore, the elevation of 5-HT synthesis rate was proven to play an important role in the elicitation of B-withdrawal signs.

Moreover, the elevation of 5-HT synthesis rate showed a reversible response pattern which recovered to a dependent or naive state in parallel with the inhibition of B-withdrawal signs with the cross-application of NZP. Considering these responses during natural withdrawal and on cross-dosing, elicitation of B-withdrawal convulsion resulted in elevation of 5-HT metabolism. In mildly dependent and withdrawn rats, a significant elevation of 5-HT synthesis rate was also observed upon appearance of convulsion with the tranylcypromine challenge. Thus, the elevation of 5-HT synthesis rate may probably be related to the elicitation of B-withdrawal convulsion.

In a previous study (24), we found that when 5-HT metabolism was enhanced by the cross-application of 5-HTP (a precursor of 5-HT) during B-withdrawal, the rats on B-withdrawal fell into a sedative, associated with reduced severities of withdrawal convulsion. The reduction in 5-HT level with reserpine or PCPA was followed by a tendency for B-withdrawal signs including convulsion to intensify. Because PCPA is known to induce insomnia (26, 27), this tendency may have resulted from the intensification of insomnia, one of the B-withdrawal signs, with this agent.

Morgan et al. (17) reported that reduction in catecholamine content with α-MT resulted in inhibition of B-withdrawal convulsion, and dl-propranolol, especially 1-propranolol, a β-adrenoceptor blocker, also inhibited the withdrawal convulsion (28). These findings indicate that not only the activity of serotonergic neurons but also that of adrenergic neurons is elevated in the B-withdrawn state.

Considering these findings together, it is not clear only from the results of this study whether the enhancement of 5-HT metabolism as observed here acts as a trigger for B-withdrawal convulsion or if it is a mere biological compensatory reaction at the onset of the withdrawal convulsion. We may speculate on the possibility that the adrenergic neurons act to excite the convulsion and the serotonergic neurons act to inhibit it, and 5-HT regulates the excitability of the adrenergic neurons.

In convulsion, it has been found that the elevation of the brain 5-HT synthesis rate during B-withdrawal is related to the evolution of B-withdrawal convulsion.

REFERENCES


