FORMATION OF PHYSICAL DEPENDENCE ON BARBITURATES AND CEREBRAL MONOAMINES

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Abstract—The effects of cerebral monoamine-related substances during the course of formation of barbital (B) dependence were studied in male S.D. rats by the B-admixed food method. The monoamine-related substances, p-chlorophenylalanine (PCPA), tranylcypromine (Tcp), a-methyl-p-tyrosine (a-MT), methamphetamine (MA) and reserpine were administered during each B treatment period, except in the control group that was fed a food admixed with B only. All groups were examined for B withdrawal signs during each of 2 B withdrawal periods. Compared with the control group, B withdrawal signs tended to be mild in the PCPA-treated group, remained similar in the Tcp-treated group, were severe in the a-MT-treated group, very mild in the MA-treated group, and very severe in the reserpine-treated group. These findings suggested B withdrawal signs may tend to be reduced in severity if the activity of catecholaminergic neurons is kept elevated during the course of formation of physical dependence on B, and they suggested that acquirement of tolerance to and formation of dependence on barbiturates tend to be reduced under a condition of reduced cerebral 5-HT activity.

Morgan et al. (1, 2) reported that barbital (B) withdrawal convulsions were related to cerebral monoamines in such a manner that cerebral dopamine (DA) increased during B withdrawal, and they further showed that administration of a-methyl-p-tyrosine (a-MT), a catecholamine (CA) synthesis inhibitor, inhibited the convulsions. On the other hand, Tagashira et al. (3, 4) reported that the turnover rate of cerebral 5-hydroxytryptamine (5-HT) was elevated during B withdrawal, and the inhibition of B withdrawal signs with a sedative-hypnotic on which the experimental animals showed cross-dependence resulted in the reversible restoration of the 5-HT turnover rate to the level before the withdrawal. In a previous paper (5), we described the results of a study in which the animal models for tranylcypromine (Tcp)-precipitated convulsions during B dependence and withdrawal (6) were treated with a few agonists or antagonists of CA- or 5-HT-mediated nerves, and these results implied that cerebral monoamines might play some important roles in the onset of B withdrawal convulsions. These studies were made to investigate the developmental mechanism of B withdrawal signs.

On the other hand, there has been virtually no report on the relation of the formation of B dependence to cerebral monoamines. In another previous paper (7), we reported that concurrent administration of propranolol, a β-adrenoceptor blocker, and B resulted in a significantly inhibited formation of B dependence and suggested the probable involvement of cerebral monoamines in the formation of B dependence. For this reason, we administered agonists and antagonists of the 5-HT- and CA-mediated nerves in the
course of B dependence formation to investigate the relation of the formation of B dependence to cerebral monoamines.

Materials and Methods

Five-week-old male Sprague-Dawley rats, each weighing about 120 g (supplied from Tokyo Laboratory Animals Co., Tokyo), were reared on a commercial (normal) diet (CA-1; Japan Clea, Tokyo) for acclimatization for one week, and those which weighed 160 to 170 g and were healthy were divided into groups of 6 to 7 each before they were used in the study. All rats were reared on the same commercial diet in an animal room air-conditioned to 22±2°C and maintained at 55±5% relative humidity throughout the study.

1. Effects of cerebral monoamine-related substances on the formation of barbital (B) dependence

The rats which were able to stay on a rotarod for 300 sec under the condition described in section 1–2 were divided into 6 groups of 6 rats each and made physically dependent on B by the DAF method already established by Tagashira et al. (8) under the dose schedule as described for group 1 below. The agonists and antagonists of the 5-HT and CA-mediated nerves were administered to these rats on the following dose schedules during each B treatment period:

Group 1 (control group): treated with B mixed with the normal food at the initial dose levels of 0.5 and 1 mg/g food for 4 days, then at 1 and 2 mg/g food for 6 days, and at 2 and 4 mg/g food for 6 days; this was followed by B withdrawal for 2 days (fed on B-free normal food); then rats were treated with B at 4 and 6 mg/g food for 10 days and at the final dose levels of 6 and 8 mg/g food for 10 days; this was followed by B withdrawal (again fed on B-free normal food).

Group 2: treated with B in the same manner as group 1 and additionally with 200 mg/kg of p-chlorophenylalanine (PCPA), twice weekly, i.p. Treatment with PCPA was ceased 7 days prior to B withdrawal at each stage of 2 and 4 mg B/g food and the final doses of 6 and 8 mg B/g food. B-admixed food (4 and 6 mg/g food) and the same dose of PCPA as pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food.

Group 3: treated with B in the same manner as group 1 and additionally with 5 mg/kg of tranylcypromine (Tcp), twice weekly, p.o. Treatment with Tcp was ceased 7 days prior to B withdrawal at each stage of 2 and 4 mg B/g food and the final doses of 6 and 8 mg B/g food. B-admixed food (4 and 6 mg/g food) and the same dose of Tcp as pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food.

Group 4: treated with B in the same manner as group 1 and additionally with 100 mg/kg of α-methyl-p-tyrosine (α-MT), twice daily (10:00 A.M. and 5:00 P.M.), i.p. Treatment with α-MT was continued until on the day of B withdrawal (24 hr after the last α-MT administration) at each stage of 2 and 4 mg B/g food and the final doses of 6 and 8 mg B/g food. B-admixed food (4 and 6 mg/g food) and the same dose of α-MT as pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food.

Group 5: treated with B in the same manner as group 1 and additionally with 5 mg/kg of methamphetamine (MA), twice daily (10:00 A.M. and 5:00 P.M.), i.p. Treatment with MA was continued until on the day of B withdrawal (24 hr after the last MA administration) at each stage of 2 and 4 mg B/g food and the final doses of 6 and 8 mg B/g food. B-admixed food (4 and 6 mg B/g food) and the same dose of MA as pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of
2 and 4 mg B/g food.

Group 6: treated with B in the same manner as group 1 and additionally with 2 mg/kg of reserpine, at 4-day intervals, i.p. Treatment with reserpine was ceased 3–4 days prior to B withdrawal at each stage of 2 and 4 mg B/g food and the final doses of 6 and 8 mg B/g food. B-admixed food (4 and 6 mg B/g food) and the same dose of reserpine as pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food.

1-1. Changes in body weight and food consumption: The rats were weighed (as a marker for B dependence formation) at a given time (between 10:00 and 11:00 A.M.) every day from the beginning of B treatment until the end of the 7-day withdrawal period. Their food consumption was measured at the same time every day during each B treatment period to calculate daily and mean B intake at each dose level of B.

1-2. Rotarod test: Each time body weight and food consumption were measured during each B treatment period, the rats were also tested for rotarod performance (with the rod rotating at 5.3 revolutions per minute) to measure the grade of motor incoordination.

1-3. Formation of physical dependence on B: The rats were observed for withdrawal signs and formation of physical dependence on B during each B withdrawal period.

1-4. Changes in cerebral 5-HT and 5-HIAA levels: Groups 7 to 12, each comprised of 6 rats, were treated with B and the cerebral monoamine-related substances in the same manner as groups 1 to 6 (with group 7 as the control), and they were assayed for cerebral 5-HT and 5-HIAA at 48 hr of B withdrawal (9).

2. Effects of cerebral monoamine-related substances on the duration of B-induced sleep

Prior to single-dose administration of 250 mg/kg of B (i.p.), 6 groups of 7 naive rats were treated with cerebral monoamine-related substances on the following schedules: Group 13: control group, no pre-treatment. Group 14: treated with 200 mg/kg of PCPA, i.p., 24 hr before B. Group 15: treated with 10 mg/kg of Tcp, i.p., 24 hr before B. Group 16: treated with 250 and 150 mg/kg of α-MT, i.p., 24 hr and 1 hr before B, respectively. Group 17: treated with 5 mg/kg of MA, i.p., 30 min before B. Group 18: treated with 2 mg/kg of reserpine, i.p., 24 hr before B.

The time until onset of B-induced sleep (onset time) and the duration of the sleep (sleeping time) were measured with the disappearance and reappearance of the righting reflex as markers.

3. Statistical analysis

Analysis of variance was applied to the analysis of the data obtained for statistically significant differences on the grade of motor incoordination and the weight loss during B withdrawal between the control and the groups treated additionally with the monoamine-related substances. The Student's t-test was applied to the analysis of the data for statistically significant differences in the cerebral 5-HT, cerebral 5-HIAA, and the onset time between the groups.

Results

1. Effects of cerebral monoamine-related substances on the formation of B dependence

1-1. Changes in body weight and food consumption: Weight gain compared with group 1 was retarded in all but group 3 (Figs. 1 and 2). Daily food consumption and B intake in all groups were similar to the values in group 1, except in group 6 which showed low food consumption and B intake (Figs. 1 and 2).

1-2. Rotarod test: Groups 2 to 6 showed no significant difference in rotarod performance compared with group 1 (Fig. 2).

1-3. Formation of physical dependence on B: During the first 48 hr of each B withdrawal
Fig. 1. Alterations in body weight, food consumption during consecutive administration of barbital (B) alone and B plus monoamine-related substances. B was administered under a gradually increasing dosage schedule. The treatments with each of the monoamine-related substances and withdrawal of these drugs were as shown in Materials and Methods. B-admixed food (4 and 6 mg/g food) and the same dose of monoamine-related substances were re-administered as done during pre-withdrawal of B from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food. The data are expressed as the mean ± S.E. of 6 rats.

Fig. 2. Comparison between inhibition of rotarod performance (upper figure) and daily B intake during consecutive administration of B alone and B plus monoamine-related substances. B-admixed food (4 and 6 mg/g food) and the same doses of each drug as during pre-withdrawal were re-administered from the day on 2 mg B/g food after B withdrawal at the stage of 2 and 4 mg B/g food. The data are expressed in as the mean ± S.E. of 6 rats.
period, group 2 showed weight losses similar to those in group 1, but tended to recover the losses rather quickly thereafter. Group 3 showed weight losses similar to those in group 1. Groups 4 and 6 exhibited greater weight losses than group 1, while group 5 showed no weight loss (Fig. 3).

In group 1, the B withdrawal signs other than weight loss were vocalization, muscle twitch, exaggerated pinna reflex, and clonic-tonic convulsion. The severity of these signs correlated with the magnitude of weight loss. In other words, only mild straub tail was noted in group 2 and 5, which also showed a slight or marked inhibition of weight loss, while group 3 showed B withdrawal signs similar in grade to those of group 1. Groups 4 and 6, where the weight losses were greater than in group 1, showed obviously more exaggerated pinna reflex, more severe hyper-irritability, more severe hyperreflexia, and more frequent and longer-lasting clonic-tonic convulsions than group 1.

1-4. Changes in cerebral 5-HT and 5-HIAA levels: The cerebral 5-HT level was significantly increased (P<0.01) in groups 2 and 3 as compared with the level in group 1. The 5-HIAA level was significantly increased (P<0.01) in group 6, but significantly decreased (P<0.01) in groups 2 and 5 (Table 1).

2. Effects of cerebral monoamine-related substances on the duration of B-induced sleep

Figure 4 illustrates the effects of the cerebral monoamine-related substances on the sleep-inducing action of B. Group 11 showed prolongation of onset time and reduction in sleep time compared with control group 7. In groups 9 and 12, B-induced sleep was prolonged, especially for group 9 in which 6 out of the 7 rats died from augmented sleep. Group 4 showed no effect of α-MT on the B-induced sleep.

Discussion

In a previous study (5, 7), we administered agonists or antagonists of the CA- or 5-HT-mediated nerves during B withdrawal following formation of B dependence and revealed

| Table 1. Changes in cerebral 5-HT and 5-HIAA levels of a rat at 48 hr of barbital withdrawal (maintenance level of 6 and 8 mg barbital/g food) |
|-----------------|-----------------|-----------------|
|                 | 5-HT (μg/g brain) | 5-HIAA (μg/g brain) |
| Control         | 0.733±0.059      | 0.264±0.013      |
| PCPA 200 mg/kg  | 1.076±0.048 (P<0.01) | 0.185±0.012 (P<0.05) |
| Tranylcypromine 5 | 0.986±0.034 (P<0.05) | 0.246±0.012 |
| α-MT 100        | 0.875±0.055      | 0.239±0.009      |
| Methamphetamine 5 | 0.854±0.049      | 0.181±0.016 (P<0.01) |
| Reserpine 2     | 0.717±0.049      | 0.336±0.015 (P<0.01) |

Each cerebral monoamine-related substance was administered in combination with barbital. Barbital was chronically administered with barbital admixed food from the initial concentration, 0.5 and 1 mg B/g food, to the final concentration, 6 and 8 mg B/g food, over 36 days (refer to Materials and Methods).
that cerebral monoamines played an important role in the occurrence of B withdrawal convulsions. Beyond this, there have been but a few papers published on the relation between the course of B dependence formation and these cerebral monoamines. The present study showed that cerebral 5-HT and CA were largely responsible for the remission or intensification of B dependence formation as well for elicitation of B withdrawal convulsions (5). In the MA-treated group, the severity of the withdrawal signs was markedly reduced compared with the signs in the control group. In other words, it was revealed that in the course of B dependence formation, the formation of physical dependence on B was reduced when the CA-mediated nerves were kept stimulated. From the findings with the administration of α-MT and reserpine, it was further revealed that when cerebral CA was reduced, the withdrawal signs of B intensified.

Blum et al. (10) observed that when α-MT was administered in combination with continuous administration of ethanol (EtOH), it significantly potentiated EtOH withdrawal convulsions without affecting EtOH metabolism. This finding and the findings in the present study suggest that the activity of cerebral CA is the factor responsible for the grade of physical dependence formation, which is common for the barbiturate-alcohol type drugs.

In a previous paper (5, 7), we reported that administration of α-MT, disulfiram, and dl-propranolol during B withdrawal inhibited B withdrawal convulsions and other withdrawal signs. This finding, together with the findings in our present study, implies that the activities of the CA-mediated nerves may be inhibited in the course of B dependence formation and that abrupt withdrawal of B in such a condition may result in elevation of the activities of the CA-mediated nerves in a rebound manner.

In both the MA- and the PCPA-treated groups, B withdrawal signs were alleviated. On the other hand, it is known that MA is anorexigenic and that this action is accompanied by the development of tolerance. In this study, although there were practically no differences in food consumption and B intake between the group treated with B plus MA and the groups treated with the other combinations, B withdrawal signs were strikingly inhibited in the former. The antagonism of MA against the CNS depressant action of B, as represented by the reduction in the duration of B-induced sleep (Fig. 4), may be one of the probable reasons for this phenomenon. It is further known that MA promotes the release of CA from the catecholaminergic neurons and that it inhibits the uptake of CA by such neurons to increase CA in the nerve terminals and consequently to excite the CNS (11). The rotarod test, however, during continuous administration of B revealed that the administration of MA twice daily failed to reduce B-induced motor
incoordination or to influence the acquirement of tolerance by the rats. From these findings, we consider that MA exerts its effect on the formation of B dependence or consequently reduces B withdrawal signs through changing the sensitivity of α- or β-adrenoceptors by its continuous administration, rather than through changing CA content. Thomas and Handly (11) reported that continuous administration of MA elevated the sensitivity of α-adrenoceptors.

In another study, we found that combined administration d/-propranolol, a β-adrenoceptor blocking agent, with B during the formation of B dependence resulted in obvious reduction in the formation of physical dependence on B (7). In our present study, the combined administration of α-MT with B resulted in the development of strikingly severe B withdrawal signs compared with those in the control group. These findings suggest that B withdrawal signs may tend to be reduced in severity if the activity of catecholaminergic neurons —especially the sensitivity of α-adrenoceptors— is kept elevated during the course of formation of physical dependence on B.

It has been reported in connection with the activities of 5-HT-mediated nerves that prolonged administration of MA decreased 5-HT and 5-HIAA in all parts of the brain (12). From the significant decrease in 5-HIAA in the MA-treated group as shown in Table 1, the reduced formation of B dependence by continuous administration of MA may be thought to result from the inhibition of 5-HT metabolism. On the other hand, the B dependence formation-reducing mechanism of PCPA may be thought to be such that PCPA inhibits the formation of cerebral 5-HT (13) and at the same time potentiates the activity of tyrosine hydroxylase, a CA synthesizing enzyme (14), to indirectly elevate the activities of the CA-mediated nerves.

Frankel et al. (15) reported that PCPA reduced tolerance to the motor incoordination induced by EtOH and pentobarbital, and from the finding that combined administration of PCPA with these drugs caused no changes in the metabolisms of EtOH and pentobarbital, they suggested the probable involvement of cerebral 5-HT in the acquirement of tolerance to these 2 agents. In our present study, neither significant inhibition of motor incoordination nor reduced acquirement of tolerance was observed in the PCPA-treated group. This discrepancy may in part be attributed to the procedure used by Frankel et al. (15) of administering only PCPA every day from 5 days before the administration of EtOH and pentobarbital and thereafter in combination with them, which placed the animals in a condition in which cerebral 5-HT was reduced severely (95%). In any event, these findings indicate that the acquirement of tolerance to and the formation of dependence on barbiturates tend to be reduced under a condition of reduced cerebral 5-HT activity.

Tcp is a drug which has been used clinically as an anti-depressant (16). The principal action of Tcp is the inhibition of monoamine oxidase. The increase in contents of both CA and 5-HT (Table 1) showed no significant effect on the B dependence formation. The present study suggests that decrease in cerebral monoamines has a large effect on B dependence formation.

In our present study, it was shown that changes in the metabolisms of cerebral monoamines as well as in the sensitivity of the respective receptors were influential on the course of B dependence formation. This phenomenon is of particular interest because in view of our previous findings (5–7) that reduction in the neural activity of CA during B withdrawal inhibited B withdrawal signs and reduction in the neural activity of 5-HT intensified the signs, the aforementioned relationship of cerebral monoamines with the
formation of B dependence is in inverse
correlation to their relationship with elicitation
of B withdrawal signs.

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