PHYSICAL DEPENDENCE ON MEPROBAMATE AFTER REPEATED ORAL ADMINISTRATION IN RATS

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Abstract—The physical dependence potential of meprobamate (MPB) was compared with that of phenobarbital (PHB) and codeine (COD) to ascertain whether MPB produces definite physical dependence in the rat. Rats were treated orally with MPB (maintenance dosage=800 mg/kg x 2/day), PHB (100x2) or COD (50x2) twice a day (10:00 a.m. and 5:00 p.m.) for a total of 21 days; the treatment was ceased for 3 days after administration for 7 days, and the last dosing was performed on day 27. During intoxication and after the withdrawal, the MPB treated rats exhibited behaviour and withdrawal signs similar to those seen in the PHB treated rats, but not the COD treated rats. After withdrawal of drugs, definite weight loss was observed in all the rats given drugs, and the recovery of the MPB and PHB treated rats was clearly later than that of the COD treated rats. A long-lasting increase in rectal temperature was observed after the withdrawal in the MPB and PHB treated rats; a decrease was seen in the COD treated rats. From these results, it is concluded that definite physical dependence on MPB, similar to that on PHB but different from that on COD, was developed after repeated oral administration for a total of 21 days in the rat.

Dogs and monkeys are suitable species to evaluate sedative-hypnotics physical dependence liability (1-7). However, it is inadequate to use these animals as a test animal for the primary screening of drugs because they are expensive animals and their bleeding and experimental procedures are complicated. In view of this, studies with rats would be valuable for the primary testing. Sedative-hypnotics such as barbiturates and benzodiazepine analogues produce physical dependence in rats when given orally or subcutaneously once or twice daily (8-11) and when given as a solution in drinking water (12-15) or as a drug-admixed food (16, 17). Meprobamate, however, has produced only uncertain physical dependence in rats so far (15, 18-21), although it produces comparatively severe physical dependence upon the repeated administration in dogs (22), monkeys (7) and man (23-25). If the rat is as useful as the dog and monkey for barbiturate-type physical dependence producing test, meprobamate, like other sedative-hypnotics, should produce definite physical dependence upon the repeated administration in the rat. Thus, we designed an experiment to compare the physical dependence potential of meprobamate with phenobarbital in rats.

Materials and Methods

Animals and drugs: Male Wistar rats, weighing 150 to 160 g, were divided into 4 groups of 9 to 12 animals each and housed in individual cages at 24-25°C. Rats were allowed access to laboratory chow (CE-2, Clea Japan, Inc.) and tap water ad libitum throughout the experiment. Meprobamate (MPB), phenobarbital (PHB) and codeine phosphate (COD) were used. Drugs were
dissolved or suspended in a constant volume of 5 ml/kg in a 0.5% gum tragacanth aqueous solution (VEH).

**Drug treatments:** Each drug was administered orally to the rat twice a day (10:00 a.m. and 5:00 p.m.) for a total of 21 days. The experiment was divided into three periods as shown in Fig. 1. In the first and second periods, drug administration was performed for 7 days and thereafter ceased for 3 days. Drugs were withdrawn after administration for 7 days in the third period. Dosages of MPB were 200, 400 and 800 mg/kg x 2/day in the first, second and third periods, respectively; and they were 50, 50 and 100 mg/kg x 2/day, respectively, for PHB. The dosage (50 mg/kg x 2/day) of COD was maintained at the same level in all the periods.

**Equipment and experimental procedure:** Body weight was measured using a digital meter (Model PS 1200, Mettler) with an accuracy of 0.1 g, and body (rectal) temperature was measured using a thermistor probe (Natsume, Japan). Electroconvulsion was evoked by an electrical stimulus delivered at 20 or 15 mA for 0.2 sec by means of surface electrodes applied to both the ears of each rat. Pressure pain threshold was determined by the modified Haffner's method using an apparatus designed by Nakamura and Shimizu (26). Mechanical pain was induced by pressing the tail of rats, and the bite response was measured in mm (1 mm=12.5 g pressure) with an arbitrary cutoff pressure of 45 mm. Forty-six hr after withdrawal of the drugs, the rats were put singly into a transparent cage (32×21×13 cm), and the manifestation of withdrawal signs was observed for 20 min (27).

**Statistical method:** Student's t-test was used in statistical analyses.

**Results**

**Changes in body weight:** Body weight changes during administration of each drug are shown in Fig. 1. In the second period, MPB and PHB produced weight gain more than VEH during drug administration. Dosages of MPB and PHB were increased to 800 and 100 mg/kg x 2/day, respectively, in the third period, since marked weight loss did not occur during the first and second cessations in the rats receiving MPB and PHB. Consequently, the body weight decreased temporally after 4 days in the PHB treated rats, though the increase became more intensive in the MPB treated rats. This decrease might be due to the dosages which was much higher than the tolerance level developed already to PHB; the PHB treated rats exhibited staggering gait at the morning dosing and were in anaesthesia at the evening dosing. On the other hand, the body weight decreased markedly during the first cessation in the rats receiving COD, which was used as a control drug producing a physical dependence different from barbiturates, and thus, the dosage was maintained throughout the experiment.

After withdrawal of each drug, severe
Fig. 2. Time-course of withdrawal signs after withdrawal of meprobamate, phenobarbital and codeine. (A) Body weight: The ordinate shows the change rate in body weight based on that at 5:00 p.m. on day 27. Open and closed marks show the change rate at 10:00 a.m. and 5:00 p.m., respectively. (B) Body temperature: Rectal temperature was measured 7 and 3 hr before and 17, 21, 24 and 41 hr after the last dosing (5:00 p.m. on day 27) and then further twice a day (10:00 a.m. and 5:00 p.m.) for 6 days. Each point and vertical bar represent the mean and S.E.M. □, meprobamate; ○, phenobarbital; △, codeine; ●, vehicle. *0.01<P<0.05 and **P<0.01, significantly different from the vehicle group.

weight loss (Fig. 2A) and other withdrawal signs were observed in all the rats given drugs. The maximum decrease rate of the MPB, PHB and COD treated rats were 7.08%, 11.1% and 13.8% (P<0.01, based on the weight at 5:00 p.m. on day 27), respectively, and the recovery of the COD treated rats was clearly faster than those of the MPB and PHB treated rats.

Changes in body temperature: In the rats treated with MPB and PHB, a marked increase in rectal temperature was observed 17 hr after the last dosing, the significant increase lasted until 72 hr, and thereafter, the slight increase lasted until 144 hr (Fig. 2B); the decrease was seen in the COD treated rats. When a single dose of MPB (800 mg/kg), PHB (100 mg/kg), COD (50 mg/kg) or VEH (5 ml/kg) was administered orally to the naive rats (N=10, each), a slight increase in rectal temperature was noted in only the rats given MPB and PHB 24 hr after dosing, and the increase recovered after 48 hr (data not shown). Consequently, the changes in body temperature caused by the withdrawal were apparently different from those by a single administration of these drugs.

Other withdrawal signs: After 24 hr of the withdrawal, the manifestation of hyperirritability on handling became more intensive in the rats treated with drugs. The MPB treated rats moved continuously during the observation period of 20 min and manifested frequent rearings and tail elevation. The PHB treated rats exhibited behaviour similar to the MPB treated rats, but somewhat weaker than that of MPB. On the other hand, the COD treated rats exhibited wet dog shakes, diarrhea and writhing syndrome with a decrease in spontaneous locomotor activity.

The appearance of electroconvulsion was found in 10 out of 12 rats and 9 out of 9 rats treated with MPB and PHB, respectively, but no electroconvulsion was noted in the COD treated rats. The normal rats, approx. the same body weight as the COD treated rats, were...
Table 1. Behavioural changes after the withdrawal in the rats receiving repeated administration of meprobamate, phenobarbital or codeine

| Drug               | Dose mg/kg/day p.o. | n  | Body wt. mean(g) ± S.E.M. | Electroconvulsion number of rats convulsed / tested | Electroconvulsion number of rats with tonic convulsion | Pressure pain threshold mean(mm) ± S.E.M. |
|--------------------|---------------------|----|--------------------------|-----------------------------------------------------|-------------------------------------------------------|----------------------------------------|
| Vehicle            |                     | 11 | 227.5 ± 3.5              | 3/11                                                | (0)†                                                  | 42.2 ± 2.5                             |
| Meprobamate        | 1600                | 12 | 232.0 ± 5.3              | 10/12*                                               | (1)†                                                  | 35.5 ± 1.1                             |
| Phenobarbital      | 200                 | 9  | 213.7 ± 4.3              | 9/ 9**                                               | (5)†                                                  | 35.8 ± 4.6                             |
| Codeine phosphate  | 100                 | 9  | 171.2 ± 5.5              | 0/ 9                                                 | (0)‡                                                  | 23.0** ± 1.5                           |
| Untreated control  |                     | 8  | 167.3 ± 2.7              | 1/ 8                                                 | (0)‡                                                  | 48.8 ± 1.3                             |

*: time (hr) after the last administration of each drug. †: Electroconvulsion was evoked by a single electrical stimulation with 20 mA for 0.2 sec. ‡: 15 mA for 0.2 sec. *: 0.01<P<0.05 and **P<0.01, significantly different from the vehicle group.

Discussion

The dosage of MPB was increased to 1600 mg/kg/day in the third period, but the body weight continued to increase much more. In the other experiment, 2 out of 10 naive rats died after a single oral administration of 800 mg/kg of MPB. Thus, the present results demonstrate that definite physical dependence on and tolerance to MPB was developed after repeated oral administration of MPB only for a total of 3 weeks, though its maintenance dosage was as high as 1600 mg/kg/day, 8 times that of PHB. It has been reported that the dosage needed to produce physical dependence in monkeys was 100 mg/kg p.o. once a day and 400 mg/kg p.o. twice a day for PHB and MPB, respectively (7), and that oral dosages of PHB and MPB which substitute for 100 mg/kg sodium barbital in barbital-physically dependent dogs were 30 mg/kg twice a day and 150 mg/kg four times a day, respectively (5). Therefore, the relative potency of MPB to PHB in rats was almost in accord with that in monkeys and dogs. It has been reported that a single oral dose of 200 mg/kg of MPB produces severe motor impairment and inattentiveness to the activities of other monkeys, and 400 mg/kg produces light anaesthesia in normal monkeys (7). In the present study, similar pharmacological actions were produced after a single oral administration of 400 and 800 mg/kg of MPB in normal rats. Thus, the dosage in the rat was about two times higher than that in the monkey. These results suppose that physical dependence on these drugs is developed when the dosage which produces a definite action on the central nervous system is given for an adequate period and also that the difference in the dosage between rats and monkeys is due to the species difference in the dosage to produce the action on the
Weight loss is widely used as one of reliable withdrawal signs of sedative-hypnotics, but this sign is also noted after morphine withdrawal. Weight loss seen after withdrawal of MPB and PHB lasted longer than that of COD in the rats (Fig. 2A). However, it is difficult to distinguish the sedative-hypnotics withdrawal from the narcotics withdrawal only by the observation of weight loss. Convulsion is one of the characteristic withdrawal signs of sedative-hypnotics, but the spontaneous convulsion is graded into the severe signs in dogs, monkeys and rats (7, 28, 29). Hyperthermia is also used as a withdrawal sign of sedative-hypnotics, but there is not any detailed observation on changes in body temperature before and after withdrawal of sedative-hypnotics in rats. The present results, measured in detail with the time-course, demonstrate that changes in body temperature are available as a characteristic withdrawal sign that is quantitatively measurable in rats.

There were many differences in withdrawal signs between MPB and PHB (sedative-hypnotics) and COD (narcotics). However, it was common to both types of the drugs that behaviour opposite to the withdrawal sign was noted during intoxication of these drugs: e.g., MPB produces an increase of weight gain (appetite), hypothermia, decrease in electroconvulsion threshold (anti-convulsive), sedation and hypomotility; and COD produces hyperthermia, decrease in pain threshold (analgesic), hypermotility and constipation. This phenomenon is interesting with respect to the mechanism of producing physical dependence.

From these results, it is concluded that definite physical dependence on MPB, similar to that on PHB, but different from that on COD, was developed after repeated oral administration for a total of 21 days in the rat. This result supports that the rat is one of the suitable species that can be used for the physical dependence producing test of sedative-hypnotics.

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