FURTHER STUDIES ON THE DETERMINANT ROLE OF BRAIN LEVEL OF PENTOBARBITAL FOR THE DEVELOPMENT OF ACUTE HYPNOTIC TOLERANCE

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Controlling the brain level of pentobarbital and the duration of hypnosis at the initial treatment in relation to development of acute hypnotic tolerance was studied. Simultaneous treatment of bemegride or TRH with pentobarbital attenuated the hypnotic effect of pentobarbital in a dose dependent manner, but neither the brain level of pentobarbital nor the development of tolerance was modified by this treatment. The effect of TRH was further demonstrated in rats by concomitant intracarotid infusion with pentobarbital maintaining the brain concentration of pentobarbital and also the duration of exposure of the brain to pentobarbital under a constant condition. On the other hand, THC significantly prolonged the hypnosis induced by pentobarbital but did not potentiate the effect of pentobarbital to develop acute tolerance. Thus, the brain level of pentobarbital at the initial treatment is the primary determinant for the development of acute tolerance and the duration of hypnosis is not the essential factor in this mechanism.

Keywords—pentobarbital; bemegride; TRH; THC; acute hypnotic tolerance; brain level of pentobarbital

INTRODUCTION

In the previous paper we reported that a single i.p. injection of pentobarbital developed hypnotic tolerance 24 h after the injection and that the primary determinant for the development of this type of acute tolerance was the brain level of the drug at the initial treatment. Namely, bemegride attenuated the hypnotic effect of pentobarbital, but neither the brain level of pentobarbital nor the development of tolerance was modified and full duration of hypnosis was not necessary in this mechanism.

In the present paper we describe further evidences to confirm our previous results controlling the brain level of pentobarbital and also the duration of hypnosis.

MATERIALS AND METHODS

General—Male mice of dd strain weighing 20 to 25 g and male rats of Wistar strain weighing 200 to 250 g were used and divided into groups each consisting 10 animals. The following drugs were used: pentobarbital-Na (PB, Tanabe), thyrotropin-releasing hormone (TRH, Takeda), bemegride (BG, Bayer) and Δ⁹-tetrahydrocannabinol (THC, Prof. Nishioka, Kyushu University).

Drug Administration—Drugs were dissolved in saline solution and administered intraperitoneally so as to contain the dosage in a volume of 0.1 mg/10 g of body weight of mice. In the case of THC, because of its insolubility in saline solution, it was dissolved in 1% Tween 80 solution.

For intracarotid infusion, rats were used instead of mice mainly due to the technical difficulties. Under light ether anesthesia a cannula was inserted to the unilateral carotid artery, and after recovery from anesthesia, namely, the animals regained their response against corneal or tail pinch stimulation, infusion of the drug solu-
Acute Hypnotic Tolerance to Pentobarbital

...tion was started. Twenty mg of pentobarbital and/or 5 mg of TRH in one ml saline solution was infused for 10 min at a constant rate of 40 μl/min using continuous infusion pump. Saline solution alone was given to the control animals.

Evaluation of the Degree of Developed Tolerance — Twenty-four hours after the initial treatment the hypnotic effect of i.p. test dose of pentobarbital, 45 mg/kg in mice and 30 mg/kg in rats, was estimated. The hypnotic effect was evaluated by measuring the sleeping time, i.e. the time elapsed from loss to regain of the righting reflex, and to minimize the diurnal variations of the effect, experiments were performed during 13:00 to 16:00 in the afternoon. In the infusion experiments, the effect was compared with that in non-treated animals.

RESULTS

Dose of Initial Treatment and the Degree of Tolerance

Twenty-four hours after the initial injection of pentobarbital, tolerance developed against the hypnotic effect and the degree of tolerance was dependent on the dose at the initial treatment (Fig. 1). As recognized from Fig. 1, for the development of this type of acute tolerance the dose required at the initial treatment must exceed the challenging dose on the 2nd day.

Effect of Bemegride on the Development of Tolerance

In proportion to the dose of bemegride the sleeping time induced by 45 mg/kg pentobarbital was shortened and some animals failed to sleep by the combination of more than 40 mg/kg of bemegride (7 and 9 out of 10 animals by 40 and 80 mg/kg of bemegride, respectively). On the 2nd day, the same degree of tolerance was formed regardless of the duration of hypnosis at the initial treatment (Fig. 2). Thus, combined treatment with bemegride did not affect the development of tolerance to pentobarbital.

Effect of TRH on the Development of Tolerance

Combined i.p. treatment with TRH also attenuated the hypnotic effect of pentobarbital in a dose dependent manner without affecting the development of tolerance to the test dose of pen-

![Fig. 1. Relationship between Initial Dose and Degree of Tolerance to the Hypnotic Effect of Pentobarbital in Mice](image-url)

Each group of animals was treated with varying i.p. doses of PB on the 1st day and 24 h after the treatment the sleeping time induced by test dose, 45 mg/kg, of PB was determined. Significantly different from the sleeping time induced by 45 mg/kg i.p. PB on the 1st day (* p < 0.05, ** p < 0.01).
tobarbital on the 2nd day (Fig. 3).

When pentobarbital was intracarotidally infused to rats, 20 mg/ml solution for 10 min at a rate of 40 μl/min, animals became tolerant to the hypnotic effect of 30 mg/kg of pentobarbital 24 h after the termination of infusion. Simultaneous

FIG. 2.  Effect of Bemegride on the Development of Hypnotic Tolerance to Pentobarbital in Mice
Each group of animals was i.p. injected 45 mg/kg PB in combination with varying doses of bemegride on the 1st day and 24 h after the treatment the sleeping time induced by test dose of PB was determined. Significantly different from the sleeping time induced by 45 mg/kg i.p. PB on the 1st day (** p < 0.01). ( ): Number of animals which slept in a group.

FIG. 3.  Effect of TRH on the Development of Hypnotic Tolerance to Pentobarbital in Mice
For details see text and legend of Fig. 2.
infusion of TRH with pentobarbital did not modify the development of tolerance (Fig. 4). The hypnosis induced by the test dose of pentobarbital, 30 mg/kg, in the groups treated with saline solution or TRH alone as control was not different from that in non-treated control animals.

During the infusion of pentobarbital, animals became quiet and after termination of infusion they lost their righting reflex for a while and did not respond to external stimuli. But when infused with TRH, animals also became motionless but were more sensitive than the groups infused only with pentobarbital and responded to touch or tail pinch during and after infusion.

**Effect of THC on the Development of Tolerance**

The sleeping time induced by 30 mg/kg of pentobarbital was about 20 to 25 min and occasionally animals failed to sleep, but when combined with THC the hypnosis was doubled up to the duration approximately equivalent to that induced by 45 mg/kg of pentobarbital alone. Under this condition, however, tolerance was not produced to the hypnotic effect of 45 mg/kg of pentobarbital on the 2nd day as well as the group treated with 30 mg/kg pentobarbital alone (Fig. 5). THC induced no hypnosis by itself and did not potentiate the acute hypnotic tolerance to

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**FIG. 4. Effect of TRH Intracarotidally infused with Pentobarbital on the Development of Hypnotic Tolerance in Rats**

PB (20 mg/ml solution) was infused with or without TRH (0.5 mg/ml) for 10 min at the rate of 40 μl/min and 24 h after infusion sleeping time induced by 30 mg/kg i.p. PB was determined. Control animals were infused with saline solution or TRH alone (For details see text). Significantly different from the sleeping time induced by 30 mg/kg i.p. PB in non-treated controls (**p < 0.01**).

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**FIG. 5. Effect of THC on the Pentobarbital Hypnosis and the Development of Acute Hypnotic Tolerance in Mice**

Thirty mg/kg PB was i.p. injected with or without 2 mg/kg i.v. dose of THC on the 1st day and 24 h after the treatment the sleeping time induced by test dose of PB, 45 mg/kg, was determined.
pentobarbital.

DISCUSSION

Twenty-four hours after a single intraperitoneal injection of pentobarbital, the hypnosis induced by the same dose was reduced significantly indicating the development of acute tolerance, and the degree of the developed tolerance to the fixed test dose was dependent on the dose at the initial treatment. In the present experiment, the determinant role of the brain level of pentobarbital at the initial treatment for the tolerance developing mechanism was confirmed by controlling the drug concentration and duration of hypnosis.

Simultaneous intraperitoneal injection of bemegride or TRH with pentobarbital attenuated the hypnotic effect of pentobarbital in a dose dependent manner though the combined treatment did not affect the development of tolerance. Even in the animals which did not sleep at the initial injection by the combination of high dose of bemegride or TRH, tolerance developed to the same extent as that in the control animals treated with pentobarbital alone. Thus, the full duration of hypnosis at the initial treatment is not necessarily required for the development of this type of acute tolerance.

We have reported in the previous paper that simultaneous administration of bemegride did not alter the brain level of pentobarbital. Similarly, Breese et al. have reported the lack of the effect of TRH on the brain concentration of pentobarbital and we also confirmed the results with TRH under present experimental conditions (data not shown). These facts indicate that the attenuation of sleeping time induced by the combination of bemegride or TRH is not the results of the reduction of brain level of pentobarbital and if the brain level of pentobarbital is maintained in a sufficient level at the initial treatment, tolerance will be formed regardless of the duration of hypnosis.

In order to control the drug concentration and the exposure time of the brain to the drug more precisely the intracarotid infusion technique was employed. This method would exclude possible influences of the combined drugs on the brain level of pentobarbital and the duration of exposure time to the drug since brain pentobarbital showed a rapid decline immediately after the termination of infusion. Infusion of pentobarbital, 20 mg/ml solution for 10 min at the constant rate of 40 μl/min, developed acute hypnotic tolerance as in the case of intraperitoneal injection. Simultaneous infusion of TRH antagonized the hypnotic effect of pentobarbital during and after infusion without affecting the development of tolerance.

The importance of the brain concentration of pentobarbital at the initial treatment on the development of acute tolerance was further demonstrated by the combined injection of THC with pentobarbital. Intraperitoneal injection of 30 mg/kg pentobarbital caused short hypnosis and 24 h after the treatment, tolerance was not produced to the hypnotic effect of the test dose of pentobarbital. Concomitant administration of THC, 2 mg/kg i.v., significantly prolonged the hypnosis to the extent that is caused by 45 mg/kg pentobarbital, however, the treatment failed to develop tolerance on the next day.

The hypnosis prolonging effect of THC is supposed to be due to the direct effect of THC and its metabolites on the CNS and not attributable to the alteration in penetration or accumulation of pentobarbital or its metabolites. We did not measure the brain concentration of pentobarbital under the influence of THC, but from the results reported by Watanabe et al. using Δ8-THC, a congener of Δ9-THC of similar pharmacological properties, the influence of THC on the brain level of pentobarbital can be neglected under the present experimental conditions.

It is well understood that there are two types of tolerance to the hypnotic effect of barbiturates, dispositional and functional. For pentobarbital, a short-acting barbiturate, the dispositional tolerance is supposed to be a predominant mechanism which developed very rapidly, however, the functional tolerance developed more gradually during the chronic treatment.
with the drug.\textsuperscript{69} In this study, we only dealt with the acute tolerance so that the difference between acute and chronic tolerance remain unsolved, however, at least for the development of acute tolerance after a single treatment, the brain level of pentobarbital at the initial injection played a determinant role and full duration of hypnosis is not essential in this mechanism.

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REFERENCES


