BRAIN LEVEL OF PENTOBARBITAL IS THE PRIMARY DETERMINANT FOR THE DEVELOPMENT OF HYPNOTIC TOLERANCE IN MICE

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Controlling the duration of hypnotic effect of pentobarbital by simultaneously administered bemegride, the relationship between the duration of hypnosis and the degree of developed tolerance after acute or chronic treatment was investigated in mice.

Bemegride attenuated the hypnotic effect of pentobarbital, but neither the brain level of pentobarbital nor the development of tolerance was modified by bemegride, indicating that the brain level of pentobarbital is the primary determinant for the production of tolerance and full duration of hypnosis is not essential in this mechanism.

Keywords—pentobarbital, hypnotic tolerance, bemegride, brain level of pentobarbital, duration of hypnosis, acute tolerance

It is well recognized that a single dose of pentobarbital developed tolerance to its hypnotic effect and the degree of the tolerance was dependent on the dose at the initial injection and varied by the time elapsed between the first and second injections.^{1,2)} However, few papers have appeared on the precise relationship between the brain concentration of pentobarbital or the duration of hypnosis at the initial dose and the degree of developed tolerance.

The present paper describes this type of acute tolerance after a single dose of pentobarbital and compares the degree of developed tolerance in relation to the duration of hypnosis and also to the level of the drug following the initial injection and comparison was also made with the tolerance after repeated injections.

Mice of dd strain weighing 20 to 22 g were used. The duration of hypnosis was measured by the loss and regaining of the righting reflex. Drugs, 45 mg/kg of pentobarbital and 20 mg/kg of bemegride, were dissolved in saline and administered intraperitoneally in a volume of 0.1 ml/10 g of body weight. For the estimation of brain and blood levels of pentobarbital, animals

were sacrificed 20 min after the injection of ¹⁴C-pentobarbital (45 mg/kg, *i.p.*, specific activity, 0.28 mCi/mmol), then the drug was extracted by the method of Brodie *et al.*³⁾ and the radioactivity was measured by the conventional method using an Aloka liquid scintillation spectrometer (Type LSC-601).

Twenty-four hours after the 1st injection of 45 mg/kg of pentobarbital, the sleeping time induced by the same dose was shortened by about 35% (significantly different from control on day 1, $p < 0.05$) indicating the development of tolerance (Fig. 1). With daily repeated treatment with the same dose of pentobarbital, sleeping time decreased progressively until the 4th day and reached the plateau of about 1/2 of the duration and remained constant thereafter (from day 3 to day 5 significantly different from control on day 1, $p < 0.01$).

Combined injection of bemegride with pentobarbital attenuated the hypnotic effect of the initial dose of pentobarbital by about 50% ($p < 0.01$) and approximately the same duration of hypnosis was observed during 5 daily consecutive treatments. On day 6, this group of animals

was challenged with 45 mg/kg of pentobarbital, but the same degree of tolerance was formed as in the animals treated daily only with pentobarbital (Fig.1). We confirmed that this dose of bemegride administered 24 hr prior to the injection of pentobarbital did not affect the duration of hypnosis (data are not shown).

In a separate group of animals treated with a combined dose of bemegride and pentobarbital on the 1st day, the duration of hypnosis induced by 45 mg/kg of pentobarbital on the 2nd day was shortened to the same extent as in the control group treated only with pentobarbital on the previous day (Fig.1).

Thus, bemegride had no influence on the development of hypnotic tolerance to pentobarbital in acute or chronically treated animals. In other words, the duration of hypnosis induced by the initial dose of pentobarbital did not always affect the degree of tolerance.

In a preliminary experiment, we measured the brain and blood levels of pentobarbital after *i.p.* injection in naive and tolerant mice, rendered tolerant by repeated injections for 1 week. Up to 30 min, no significant difference was observed between both groups. In this study, therefore, we compared the brain and blood levels of pentobarbital 20 min after the injection when the animals treated with bemegride or made tolerant by a single or repeated dose of pentobarbital are ready to awake shortly.

As shown in Table I, the concentration of pen-

tobarbital in the blood and brain areas after the challenging dose of 45 mg/kg showed no signifi-

FIG. 1. Effect of Bemegride on the Development of Hypnotic Tolerance to Pentobarbital in Mice

□ 45 mg/kg of pentobarbital was daily administered intraperitoneally.

▨ 20 mg/kg of bemegride was injected in combination with 45 mg/kg of pentobarbital until day 5 and the sleeping time caused by 45 mg/kg of test dose of pentobarbital alone was measured on day 6.

▧ 20 mg/kg of bemegride was injected intraperitoneally on the 1st day in combination with 45 mg/kg of pentobarbital and the sleeping time of test dose of pentobarbital alone was measured on day 2.

Values are the mean $\pm$ S.E. of 20 to 30 animals.

Significantly different from the sleeping time induced by 45 mg/kg of pentobarbital on day 1 (* $p < 0.05$, ** $p < 0.01$).

Except for the duration of the hypnosis caused by 45 mg/kg of pentobarbital on day 1, no significant difference was obtained between each value through day 1 to day 6.

TABLE I. Effect of Bemegride Treatment on the Concentration of Pentobarbital in Some Brain Areas and Blood in Naive and Pentobarbital Tolerant Mice

Animal	Treatment	Cerebral cortex	Brain stem	Cerebellum	Blood
		(μ g/mg protein)			(μ g/ml)
Naive	PB	0.37 ± 0.04	0.38 ± 0.03	0.39 ± 0.04	23.7 ± 0.9
	PB + BG	0.35 ± 0.03	0.40 ± 0.02	0.40 ± 0.02	23.7 ± 2.0
Tolerant*	PB	0.35 ± 0.02	0.36 ± 0.02	0.42 ± 0.07	24.2 ± 0.5
	PB + BG	0.36 ± 0.02	0.42 ± 0.02	0.39 ± 0.01	23.7 ± 0.6

Brain and blood levels of pentobarbital was estimated 20 min after the *i.p.* injection of pentobarbital (PB, 45 mg/kg) or PB plus bemegride (BG, 20 mg/kg) and each value is presented as the mean $\pm$ S.E. of 4 experiments.

*: Mice were treated with 45 mg/kg of pentobarbital for 5 days and tests were performed on day 6.

cant difference between control and bemegride treated groups in naive and tolerant mice.

Bemegride attenuated the hypnotic effect of pentobarbital, but neither the brain level of pentobarbital nor the development of tolerance was modified by bemegride, indicating that the brain concentration of pentobarbital is more important for the development of hypnotic tolerance rather than the duration of hypnosis during combined treatment. Actually, after treatment with a sub-hypnotic dose of pentobarbital, 22.5 mg/kg, *i.p.*, tolerance was not demonstrated on the 2nd day.

These data also imply the fact that the tolerance developed 24 hr after the initial dose and also by repeated administrations for 5 days may be the result of decreased sensitivity of the CNS since the brain level of pentobarbital after challenging dose showed no significant difference

between control and tolerant animals.

It is widely accepted that the duration of hypnosis induced by pentobarbital parallels with the dose and the blood or brain level of the drug, but as indicated in the present experiments, when the brain level of pentobarbital is maintained at a sufficient level, full duration of hypnosis is not necessarily required for the development of tolerance to its hypnotic effect.

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