Biochemical Pharmacology, Vol. 28, pp. 549-552.

0006-2952/79/0215-0549/$02.00/0

DEVELOPMENT OF ACUTE TOLERANCE TO PENTOBARBITAL: DIFFERENTIAL EFFECTS IN MICE

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(Received 26 October 1978; accepted 6 November 1978)

Recently, two strains of mice, C57BL and DBA, have been utilized as model systems in studies of the pharmacological effects and mechanisms of action of ethanol and pentobarbital [1-3]. These mouse strains differ in their voluntary intake of alcohol [4] and have been shown to possess different central nervous system (CNS) sensitivity to the acute effects of ethanol [1-3, 5-7]. C57BL mice prefer to drink ethanol solutions rather than water, whereas DBA mice prefer water over ethanol. It has been demonstrated that C57BL mice are less sensitive than DBA animals to ethanol. In contrast, we have shown that the C57BL strain is more sensitive than DBA mice to pentobarbital [2]. Tabakoff and Ritzman [8] reported that C57BL mice exhibited acute tolerance to an acute dose of ethanol but DBA mice did not. In C57BL animals, the brain ethanol level was higher at the recovery than at the loss of the righting reflex. This was taken as evidence for the development of acute tolerance [8]. The term acute tolerance was derived from the phenomenon observed by Mellanby that dogs receiving a single dose of ethanol showed greater impairment at a given blood ethanol concentration during the ascending portion of the blood ethanol curve than at the same concentration reached during the descending portion of the curve [9].

The present report deals with studies designed to ascertain whether the selective development of acute tolerance in C57BL mice was specific to ethanol or whether it would occur with other hypnotics such as pentobarbital. A demonstration that the difference between the two mouse strains was specific for ethanol would further support the use of

these two strains in studies of tolerance to and physical dependence on ethanol.

Male C57BL/6J and DBA/2J mice (11-12 weeks old) were purchased from the Jackson Laboratories, Bar Harbor, Maine. They were housed singly on a 12/12-hr light/dark cycle in a controlled environmental room (22-23°) and received Teklad mouse diet (Teklad Mills, Winfield, Iowa) and tap water ad lib. for 7 days prior to the experiment. Each mouse was injected intraperitoneally (i.p.) with approximately 2 μCi of sodium pentobarbital-2-^{14}C (New England Nuclear, Boston, Mass.) as a 0.25% w/v solution in saline at a dose of 50 mg/kg. Sleep onset time and sleep time were determined as described previously [2]. A group of mice of each strain was decapitated at the time the righting reflex was lost (sleep onset) and another group at the time the righting reflex was regained (awakening). Blood (50 μl) was collected at those times and brains were removed immediately, weighed and homogenized in 2 volumes of 0.1 M acetate buffer, pH 5, which contained 10% w/v NaCl. Procedures for the extraction and determination of unchanged pentobarbital-2-^{14}C in blood and brain samples have been described previously [2, 10]. Pentobarbital concentrations were computed and statistical analyses were performed using the PROPHET system, a unique national computer resource sponsored by the National Institutes of Health.

The blood and brain levels of pentobarbital at the onset of sleep and at awakening for the two strains of mice are shown in Tables 1 and 2 respectively. It is seen that DBA mice awoke at significantly higher blood (Table 1) and brain (Table 2) pentobarbital levels than C57BL mice. This is in agreement with our previous conclusion that the C57BL mice were more sensitive than the DBA strain to pentobarbital [2].

Table 1. Blood Levels of Pentobarbital at Sleep Onset and Awakening

Mouse Strain	Blood Pentobarbital μg/ml ± S.E.M. Onset	Awakening
C57BL/6J * (N = 12)	34.64 ± 1.35	15.32 ± 0.61
DBA/2J * (N = 12)	33.03 ± 0.67	20.54 † ± 0.64

* Sleep onset times (min ± S.E.M.): C57BL, 3.88 ± 0.07; DBA, 3.95 ± 0.07. Sleep time: C57BL, 73.56 ± 5.96; DBA, 55.32 ± 5.02.

† Significantly different ($p < 0.001$) from C57BL mice.

For DBA mice the brain pentobarbital concentration at awakening (Table 2) was significantly higher than at sleep onset, indicating the development of acute tolerance. In contrast, C57BL mice awakened at a significantly lower brain pentobarbital level than that at sleep onset, suggesting no acute tolerance development. This is directly opposite to the reported [8] response to ethanol.

Table 2. Brain Levels of Pentobarbital at Sleep Onset and Awakening

Mouse Strain	Brain Pentobarbital μg/g wet wt ± S.E.M. Onset	Awakening
C57BL/6J (N = 12)	31.44 ± 0.75	26.95 * ± 1.25
DBA/2J (N = 12)	30.88 ± 0.54	37.25 *† ± 1.34

* Significantly different ($p \leq 0.005$) from levels at onset of sleep.

† Significantly different ($p \leq 0.001$) from C57BL mice.

The lower brain than blood levels of pentobarbital in both strains of mice at the time of onset, and, conversely, the higher brain than blood concentrations of the drug at awakening are probably related to drug distributional factors.

It has been reported that C57BL mice, which have a lower brain sensitivity than the DBA strain to the acute effects of ethanol, showed development of acute tolerance to ethanol [8]. We have now shown that with respect to pentobarbital, only the DBA mice, which have a lower brain sensitivity to the acute effects of the drug, developed acute tolerance. Thus, the development of acute tolerance to a drug might be related to the degree of CNS sensitivity to the drug. However, two strains of mice, the long-sleep (LS) and short-sleep (SS), which were selectively bred (not inbred) for their differences in sensitivities to the hypnotic effects of ethanol [11, 12], both did not develop acute tolerance to ethanol [8]. These two strains of mice showed no difference in sensitivity to the hypnotic effects of pentobarbital [2]. Due to the unavailability of the LS and SS mice, we could not perform the above experiments with these animals. Nevertheless, our present results support the use of C57BL and DBA mice as animal models to determine the relationships between voluntary ethanol intake and neural sensitivity to the acute effects of ethanol, and the rates of development of tolerance to and physical dependence on ethanol. This work is currently in progress.

Acknowledgements: The authors express their sincere appreciation to Olivia Doyle and Patrick George for excellent technical assistance.

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