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Effect of Acetazolamide on Barbiturate-induced Sleeping Time in Mice. II.[1] Effect on Barbiturate Levels in the Plasma and Brain[2]

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In the previous paper,[1] acetazolamide (AZA) was reported to shorten the hexobarbital sleeping time, while prolonging the norhexobarbital (NHB) sleeping time in mice. Since AZA was found to prolong the sleeping time induced with metharbital (MET) and mephobarbital (MB) as well as barbital (BA) and phenobarbital (PHB) in this study, N-methylation of barbiturates does not appear to be a critical factor in the action of AZA. The induction times of the five barbiturates, including NHB, were shortened by AZA pretreatment. At the moment of awakening from sleep, the brain level of BA was the same as that without the AZA pretreatment, which suggests unchanged brain sensitivity to the barbiturate. At that time, however, the plasma level was reduced in the case of AZA pretreatment. Similar results were obtained with PHB, MET, and MB. Thus, the prolongation of barbiturate sleep and shortening of the induction time are probably due to a decrease in the rate of removal of the barbiturates from the brain due to the reduction of cerebrospinal fluid flow and/or the inhibition of a system for acid transport out of the brain.

Keywords——acetazolamide; barbital; metharbital; phenobarbital; mephobarbital; norhexobarbital; brain-plasma barbiturate level; drug interaction; sleeping time

In the preceding paper,[1] the authors reported that the sleeping time induced with hexobarbital was shortened, while the sleeping times induced with pentobarbital, thiopental, and norhexobarbital (NHB) were prolonged by acetazolamide (AZA) pretreatment. The present study was carried out in order to obtain more general information on the effect of AZA on barbiturate sleep, using other barbiturates such as barbital (BA), phenobarbital (PHB), metharbital (MET), and mephobarbital (MB).

Experimental

Animals——Male ddy albino mice, weighing 20—25 g, were used. The mice were kept in a constant temperature room at 23°. The room had a cycle of 12 hr of light and 12 hr of darkness. The light was turned on at 6:00 a.m. Food and water were provided *ad libitum*.

Chemicals——AZA was purchased from Lederle Japan Ltd., Tokyo. Sodium salt of BA and sodium salt of PHB were purchased from Daiichi Pure Chemicals Co., Ltd., Tokyo and Daiichi Seiyaku Co., Ltd., Tokyo, respectively. MET, MB, and allobarbital were purchased from Dainippon Pharmaceutical Co., Ltd., Osaka, Takeda Chemical Industries Ltd., Osaka, and Tokyo Kasei Kogyo Co., Ltd., Tokyo, respectively. All other chemicals used were of analytical grade.

Preparation of Drug Solutions and Procedures for Animal Experiments——Sodium salt of BA and sodium salt of PHB were dissolved in distilled water and administered intraperitoneally at 200 and 120 mg/kg body weight, respectively. MET, MB, and NHB were dissolved in distilled water containing sodium hydroxide (one molar equivalent with respect to the barbiturate), and administered intraperitoneally at doses of 134, 87.5, and 200 mg/kg body weight as the sodium salt, respectively. AZA was administered 30 min before the administration of barbiturates. All other methods for preparation of drug solutions and procedures for animal experiments were as described previously.[1]

1) Part I: J. Sato, K. Ito, M. Sato, T. Aimoto, R. Kimura, and T. Murata, *J. Pharm. Dyn.*, **2**, 133 (1979).
2) Some parts of this study were presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April, 1978.
3) Location: *a) 7-1 Katsuraoka-cho, Otaru 047-02, Japan*; *b) 2-2-1 Oshika, Shizuoka 422, Japan.*

Determination of Barbiturate Concentrations in Plasma and Brain——BA, PHB, MET, and MB in plasma and brain were extracted with chloroform,[1] and then determined by a gas chromatographic method. Gas chromatographic analysis was performed on a Shimadzu GC-4CMPF machine equipped with dual hydrogen flame ionization detectors. Analytical conditions and internal standards for the barbiturates are shown in Table I. The other conditions were as reported previously.[1]

Table I. Conditions for Gas Chromatographic Analysis of Barbiturates

Barbiturates	Column temp. (°C)	Detector temp. (°C)	Internal standard
BA	155	210	Allobarbital
MET	155	210	Allobarbital
PHB	200	250	NHB
MB	180	250	Hexobarbital

Results

Table II shows the effect of AZA pretreatment on the mouse sleeping times induced with five barbiturates; BA, PHB, MET, MB, and NHB. Pretreatment with AZA significantly shortened the induction times and prolonged the sleeping times induced with BA and the other four barbiturates.

Table II. Effect of AZA on Sleeping Times Induced with Barbiturates in Male Mice

Barbiturate[a] (as Na salt)	Induction time (min)[b]		Sleeping time (min)[b]	
	Control	Pretreated[c]	Control	Pretreated[c]
BA	38.0±1.8(10)	28.5±0.9(8)[g]	41.0±2.2(10)	67.9± 2.1(8)[g]
PHB	30.7±2.0(9)	23.5±1.3(10)[f]	46.8±3.0(9)	64.5± 5.9(10)[d]
MET	5.8±0.3(4)	3.0±0.2(6)[g]	72.1±4.7(4)	93.0± 4.4(6)[d]
MB	3.7±0.3(8)	2.7±0.3(8)[d]	44.8±3.2(8)	86.4±10.8(8)[f]
NHB	13.8±0.7(6)	10.0±0.9(5)[e]	24.9±1.6(6)	43.9± 2.2(5)[g]

a) See "Experimental."
b) Figures denote means±S.E. with numbers of observations in parentheses.
c) AZA (25 mg/kg, *i.p.*) was administered 30 min before *i.p.* administration of barbiturates.
d)—*g*) Significantly different from control group; *d*) $p<0.05$, *e*) $p<0.02$, *f*) $p<0.01$, *g*) $p<0.001$.

Table III. Effect of AZA Pretreatment on Plasma and Brain Levels of Unchanged Barbiturates at the Moment of Awakening

Barbiturate[a] (as Na salt)	Treatment[b]	Time of awakening[c] (min after barbiturate administration)	Barbiturate level[c]	
			Plasma (μg/ml)	Brain (μg/g)
BA	Control	59.2± 4.8(5)	209.8±2.5(4)	134.7±2.3(5)
	AZA	74.6± 2.8(5)[d]	201.0±1.2(4)[e]	133.2±4.9(5)
PHB	Control	73.6±12.5(4)	165.5±4.4(4)	110.2±4.6(4)
	AZA	95.4± 5.6(4)	145.5±6.3(4)[d]	111.3±5.6(4)
MET	Control	56.3± 7.2(7)	144.7±2.0(6)	107.5±4.0(6)
	AZA	80.5±11.6(7)	134.9±5.7(6)	106.9±4.6(6)
MB	Control	38.3± 2.0(8)	81.9±3.0(8)	58.3±1.9(8)
	AZA	62.7± 5.3(6)[f]	70.1±1.7(6)[e]	55.8±2.0(6)

a) See "Experimental."
b) AZA (25 mg/kg, *i.p.*) was administered 30 min before *i.p.* administration of barbiturates.
c) Figures denote means±S.E. with numbers of observations in parentheses.
d)—*f*) Significantly different from control group; *d*) $p<0.05$, *e*) $p<0.02$, *f*) $p<0.001$.

Table III shows the barbiturate levels in the plasma and brain at the moment of awakening after administration of the barbiturates. The mice awoke at 59.2 min in the control group and at 74.6 min in the AZA pretreated group after administration of BA; at that time, the plasma level of BA in the pretreated group was significantly lower than that in the control group, but the brain levels were about the same in both groups. Essentially similar results were obtained for PHB and MB. With MET, the control mice awoke at 56.3 min and the AZA pretreated animals at 80.5 min; at that time, the plasma level in the pretreated group was lower than that in the control group, though the difference was insignificant ($t<0.2$). The brain levels were about the same in both groups.

Discussion

In the preceding paper,[1] the sleeping time induced with hexobarbital was found to be significantly shortened, while prolongation of sleep was observed with pentobarbital, thiopental, and NHB after AZA pretreatment. The opposite effects of AZA on hexobarbital and NHB sleeping times might be related to the differences in barbitate structure, including the N-methyl group. In the present study, therefore, other barbiturates such as BA and MET, and PHB and MB were employed.

The sleeping times induced not only with BA and PHB but also with the N-methyl derivatives were prolonged by the AZA pretreatment (Table II). Thus, the N-methyl structure did not account for the opposite effects of AZA on hexobarbital and NHB sleeping times.

At awakening from BA sleep the brain level of BA in mice pretreated with AZA was approximately equal to that in the control mice (Table III). Similar results were obtained in PHB, MET, and MB (Table III). These findings suggest that no change in brain sensitivity to the barbiturates may be produced by the AZA pretreatment.[4] However, the plasma levels were lowered by AZA pretreatment in the cases of BA and the other three barbiturates (Table III).

In the present study, it was also demonstrated that the induction times of the five barbiturates, including NHB, were shortened by the AZA pretreatment (Table II). Wahlström[5] reported that probenecid prolongled the BA sleeping time, with an earlier onset of sleep. He pointed out that the effect of probenecid could be explained by inhibition[6] of a system for acid transport out of the brain. AZA[7] has been reported to inhibit the probenecid-sensitive transport system in the kidney. Thus, AZA may also affect the penetration of the barbiturates across the blood-brain barrier by the inhibition of an acid transport system out of the brain. As reported previously,[1] AZA decreases cerebrospinal fluid flow,[8] and it might decrease the rate of removal of the barbiturates from the brain and thus prolong the barbiturate sleep. Both the inhibition of acid transport and the lowering of cerebrospinal fluid flow may contribute to the prolongation of barbiturate sleep. Further studies on the effects of AZA on other processes, such as renal excretion of the barbiturates, are in progress.

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