Bull. Environ. Contam. Toxicol. (1991) 46:108–112

Environmental Contamination and Toxicology

Effects of Nitrogen Compounds with Hexobarbital Induced Sleep in Swiss Albino Mice

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Many heterocyclic nitrogen compounds are utilized as industrial solvents and synthetic intermediates. Presence of alkyl substituted pyridines in food flavors, and foods have been identified (Suyama and Adachi 1980). Buttery et al. (1977) separated 12 different pyridines from roasted lamb. Additionally, pyridine compounds are formed endogenously in the organisms by the reactions between amino acids and carbonyl compounds with ammonia (Smith 1976). Certain pyridines like 4-amino pyridine and analogues are used as reversal agents for non-depolarizing muscle relaxants, and also in neuromuscular disorders (Biessels et al. 1984 and 1985). Inspite of the wide occurrence and utility of nitrogen containing chemicals, little data is available on their toxic effects and interactions with biochemical and physiological processes in mammalian species. Therefore, this investigation was conducted to evaluate in vivo effects of nitrogen containing chemicals on the duration of sleep induced with a commonly used sedative hypnotic-hexobarbital. The hexobarbital is known to be exclusively metabolized by the microsomal mixed function oxidase system in mammalian liver (Gillette et al. 1973) and any chemical interfering with its metabolism may produce changes in its pharmacological responses.

MATERIALS AND METHODS

White female Swiss Albino mice weighing 22-30 g were housed under ambient conditions in our animal facility. They were allowed rat purina chow diet and water ad libitum. Animals were acclimated to laboratory environment for 2 hours, weighed individually, marked and then injected with pretreatment chemicals.

The following nitrogen containing chemicals: p-amino salicylic acid, acetyl salicylic acid, methyl salicylate, nicotinamide, methyl nicotinate, 3-ethyl pyridine, 3-methyl pyridine, 3-pyridyl carbinol, phenylbutazone, chlorpromazine and pyrazinamide were obtained from commercial sources. Hexobarbital and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemical Company, St. Louis, Missouri. These chemicals were dissolved in 0.9% saline or DMSO to contain the experimental dose in 0.1 ml of solution, weight by volume.

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All 11 pretreatment nitrogen compounds were injected intraperitoneal (i.p.) in the lower abdominal part of a group of mice, either as 0.1 ml total volume of physiological saline or DMSO. The highest dosage of these chemicals shown in the tables 2 and 3 corresponds to their 24 hour LD25 dose. The LD25 for each chemical was calculated by the method of Litchfield and Wilcoxon (1949) from the mortality data obtained on 5-6 groups of 10 mice/group injected with varying doses of nitrogen compounds.

After 30 minutes of pretreatment chemical injections, each experimental mice received i.p. injection of 75 mg/kg dose of hexobarbital dissolved in 0.1 ml saline. Hexobarbital injected mice were allowed to sleep until they regained their reflexes. They were considered awake when able to move their hind legs and could walk away on prodding with a sharp pencil. The time required for each animal to regain their reflexes after hexobarbital injections, was noted as the duration of their sleep.

Data was analyzed by t-test to determine significant differences in the mean sleeping time responses of treated and untreated groups of mice. One-way analysis of variance was used to assess the effect of drug pretreatment among and within the experimental groups of mice.

RESULTS AND DISCUSSION

Results in Table 1 shows the duration of sleep elicited by different dosages of hexobarbital in control groups of mice. The data suggests that higher doses of hexobarbital induced longer sleeping time response as compared to lower dosages. Regression analysis of the data revealed a high level of correlation (r = 0.99) between the

Table 1. Effect of various hexobarbital doses on sleeping time response of mice.

Hexobarbital i.p. Dose (mg/kg)	Mean Animal Weights in grams[a]	Sleeping Time in minutes + SD[b]
25	23.9 ± 1.4 (15)	19.7 ± 4.62 (17.8)[c]
50	24.6 ± 1.5 (15)	25.0 ± 4.0 (27.3)
75	24.3 ± 1.4 (55)	35.7 ± 1.8 (36.9)
100	25.1 ± 1.7 (25)	48.0 ± 6.0 (46.4)

a = White Swiss Albino female mice individually weighed to nearest of gram ± Standard Deviation (number of animals).
b = Minutes slept before the mice were able to straighten their hind legs and could walk on prodding with sharp pencil ± Standard Deviation (SD).
c = Numbers in parenthesis shows predicted sleeping times calculated from the linear regression analysis of data (r = .99).

sleeping time of mice and administered hexobarbital dosage, and that the dose-response curve is linear for similar weights and age groups of these animals.

Table 2 shows the acute effects of nicotinamide, methyl salicylate, acetyl salicylate, p-amino salicylate, and dimethyl sulfoxide. Nicotinamide at the highest dosage level of 1940 mg/kg increased hexobarbital sleeping time by more than 197% of the control group while the dosage level of 940 mg/kg increased the sleeping time by more than 169% of the control value. The increased responses to higher dosages of nicotinamide is highly significant ($P < 0.001$). The mice treated with 485 mg/kg nicotinamide showed no change in their sleeping responses indicating no interaction between nicotinamide and hexobarbital at this dosage. The mice treated with 40 mg/kg methyl salicylate slept somewhat longer (120%, $P < 0.05$). No significant differences were observed when the mice were treated with 20 or 10 mg/kg methyl salicylate, acetyl salicylic acid, and p-amino salicylic acid. Dimethyl sulfoxide which was used to dissolve some of the treatment chemicals had no effect on hexobarbital induced sleep.

Table 2. Acute effect of pretreatment chemicals on hexobarbital (75 mg/kg) sleeping time of mice.

Chemicals[a]	Dosage mg/kg	Mean Sleeping Time[b] ± SD (Number of Animals
Nicotinamide	1940.0	71.0 ± 5.0 (10)[c]
Nicotinamide	970.0	62.0 ± 6.0 (10)[c]
Nicotinamide	485.0	36.0 ± 3.5 (10)
Methyl Salicylate	40.0	43.0 ± 4.0 (10)[d]
Methyl Salicylate	20.0	35.0 ± 2.2 (10)
Methyl Salicylate	10.0	32.0 ± 2.5 (10)
Acetyl Salicylate	800.0	34.0 ± 4.0 (10)
Acetyl Salicylate	400.0	31.0 ± 5.0 (10)
p-amino Salicylate Acid	2500.0	34.0 ± 4.0 (10)
Dimethyl Sulfoxide	0.1 ml	36.0 ± 4.4 (10)
Control (Saline)	0.1 ml	36.0 ± 2.0 (55)

SD = Standard Deviation
a = Pretreatment chemicals were injected 30 minutes prior to hexobarbital injections.
b = Mice were considered awake when they could straighten their hind legs and walk away on prodding.
c = Significantly different from control ($P < .001$).
d = Significantly different from control ($P < .05$).

Table 3 summarizes the acute effects of 7 different types of nitrogen containing chemicals and drugs on hexobarbital sleeping time responses of mice. At the highest dose tested, chlorpromazine produced

the most drastic change in the sleeping time response followed by 3-pyridyl carbinol > 3-ethyl pyridine > 3-methyl pyridine > pyrazinamide > phenylbutazone > nicotinamide. Chlorpromazine pretreatment at 75 mg/kg increased the sleeping time of mice by more than 900% of control value. The percent changes in hexobarbital induced sleeping times were: 1500 mg/kg 3-pyridyl carbinol 690%; 200 mg/kg 3-ethyl pyridine 667%; 250 mg/kg 3-methyl pyridine 386%; 700 mg/kg pyrazinamide 314%; 210 mg/kg phenylbutazone 219%; and 620 mg/kg methyl nicotinate 167%. These values are highly significant ($P < .001$) when the mean values are compared using the t-test. Highly significant ($P < .001$) differences in the sleeping time responses were observed, when mice were pretreated with the lowest dosages of chlopromazine, 3-ethyl pyridine, 3-methyl pyridine,

Table 3. Acute effect of pretreatment chemicals on hexobarbital (75 mg/kg) sleeping time of mice.

Chemicals[a]	Dosage mg/kg	Mean Sleeping Time[b] ± SD (Number of Animals)
Methyl Nicotinate	620.0	60.0 ± 5.8 (10)[c]
Methyl Nicotinate	310.0	31.5 ± 6.0 (10)
3-Ethyl Pyridine	200.0	240.0 ± 25.0 (10)[c]
3-Ethyl Pyridine	100.0	167.0 ± 13.0 (10)[c]
3-Ethyl Pyridine	50.0	61.0 ± 8.0 (10)[c]
3-Methyl Pyridine	250.0	139.0 ± 8.0 (10)[c]
3-Methyl Pyridine	125.0	86.0 ± 6.0 (10)[c]
3-Methyl Pyridine	62.5	49.0 ± 5.0 (10)[d]
3-Pyridyl Carbinol	1500.0	248.0 ± 31.0 (10)[c]
3-Pyridyl Carbinol	800.0	97.0 ± 12.0 (10)[c]
3-Pyridyl Carbinol	400.0	54.0 ± 9.0 (10)[c]
Phenylbutazone	210.0	79.0 ± 7.0 (10)[c]
Phenylbutazone	105.0	56.0 ± 5.0 (10)[c]
Phenylbutazone	52.5	36.0 ± 6.0 (10)[c]
Chlorpromazine	75.0	325.0 ± 12.0 (10)[c]
Chlorpromazine	37.5	204.0 ± 9.0 (10)[c]
Chlorpromazine	18.7	146.0 ± 8.0 (10)[c]
Pyrazinamide	700.0	113.0 ± 9.0 (10)[c]
Pyrazinamide	350.0	50.0 ± 5.0 (10)[c]
Dimethyl Sulfoxide	0.1 ml	37.0 ± 3.0 (20)

SD = Standard Deviation
a = Pretreatment chemicals were injected 30 minutes prior to hexobarbital injections.
b = Mice were considered awake when they could straighten their hind legs and walk away on prodding.
c = Significantly different from control ($P < .001$).
d = Significantly different from control ($P < .05$).

3-pyridyl carbinol and pyrazinamide; while the lower dosages of phenylbutazone and methyl nicotinate exhibited no signficant changes. When the data was analyzed using one-way analysis of variance, it indicated significant differences ($P < .01$) among and within the drug pretreatments in eliciting changes of hexobarbital induced sleeping responses of mice.

Hexobarbital is rapidly metabolized by cytochrome P-450 dependent microsomal mixed-function oxidase system in mammalian liver (Gillette et al. 1973; Storer and Conolly 1985). It has also been shown that methylene dioxyphenyl compounds like piperonyl butoxide form complexes with cytochrome P-450 in vivo or in vitro (Franklin 1977) and can increase hexobarbital sleeping time (Storer and Conolly 1985). Present study clearly demonstrated that nitrogen containing compounds may profoundly interfer with the duration of action of hexobarbital in vivo. Many heterocyclic chemicals such as pyridines, pyrroles, pyrazoles, pyranzines, pyrazidines, pyrimidines and triazenes are known to undergo P-450 mediated N-oxidation or N-hydroxylation (Damani and Crooks 1982; Ziegler 1984). It appears that test chemicals in our study are capable to inhibit and prolong the metabolism of hexobarbital, resulting in increased sleeping responses of the mice.

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Received March 20, 1990; accepted June 30, 1990.