Nonspecific Effect of Bis(2-ethylhexyl) Phthalate on Hexobarbital Sleep Time

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Abstract □ The data presented suggest that the intravenous or intraperitoneal administration of bis(2-ethylhexyl) phthalate prolongs hexobarbital sleep time in mice and rats by enlarging the lipophilic pool, a phenomenon that can be replicated by pretreatment of the animals with olive oil. These results call attention to the importance of considering the physical characteristics of a substance when evaluating its effect on a pharmacologically active agent.

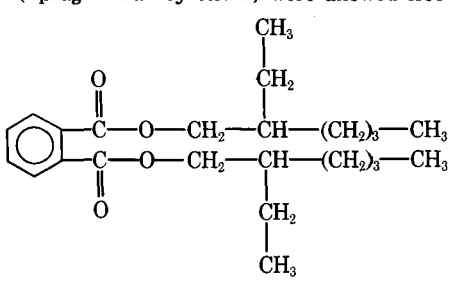
Keyphrases □ Phthalate ester—bis(2-ethylhexyl) phthalate, effect on hexobarbital sleep time, mice, rats □ Hexobarbital—sleep time, effect of bis(2-ethylhexyl) phthalate, mice, rats □ Bis(2-ethylhexyl) phthalate—effect on hexobarbital sleep time, role of phthalate, physical properties, mice, rats

Previous studies (1, 2) showed that the increased hexobarbital sleep time induced in mice and rats by pretreatment with bis(2-ethylhexyl) phthalate (I) is unrelated to the increased sensitivity of the central nervous system to the barbiturate or to an effect of the plasticizer on the rate of hepatic metabolism of the hexobarbital. Confirmation of these findings in this laboratory prompted the investigation of the mechanism responsible for the potentiation of hexobarbital sleep time caused by I.

Although I is absorbed very slowly from the peritoneal cavity (25% of a given dose remains in the body of rats after 13 days) (3), it is rapidly cleared from the blood ($t_{1/2}$ = 4.5–9 min and is dose dependent) when administered intravenously (4). These observations and a report (5) indicating that a significant amount of I associates with the lipoprotein fraction of the blood suggested that I-induced prolongation of hexobarbital sleep time results from its physical properties and is a nonspecific function characteristic of lipophilic substances. The present report substantiates this hypothesis.

EXPERIMENTAL

Male albino mice (Carworth Farms, CF No. 1 strain) and male albino rats (Sprague–Dawley strain) were allowed free access to both food[1] and water except when they were removed from their cages for the experimental procedures. Compound I or olive oil was sonicated in either mouse or rat serum (81% of particulate < 2.7 μm) and administered intraperitoneally or intravenously in doses of 300 and 500 mg/kg in mice and rats, respectively. The volume administered was limited to 1 ml/100 g of body weight in mice and 1 ml/1000 g of body weight in rats.



I

For the determination of hexobarbital sleep time, groups of randomly selected mice or rats were injected intravenously or intraperitoneally with I, olive oil, or the requisite volume of serum. At appropriate time intervals after the administration of I, olive oil, or serum, hexobarbital sodium was injected intravenously in a dose of 100 mg/kg in mice and 80 mg/kg in rats. The sleep time, in minutes, was measured from the end of the hexobarbital injection to the time each animal regained its righting reflex. The righting reflex was considered present when an animal placed on its back was able to right itself in three consecutive trials in 5 sec. The increases in sleep time in the drug-treated groups compared to the corresponding control group were calculated, and the significance of the differences was evaluated by an analysis of variance.

To determine the effect of the treatment on serum hexobarbital levels, groups of eight rats each were injected intravenously with I, olive oil, or the requisite volume of serum followed immediately by hexobarbital sodium, also given intravenously. Five minutes after the hexobarbital injection, blood was drawn from each animal and centrifuged. Hexobarbital was extracted from the serum with 1.5% isoamyl alcohol in heptane and measured in pH 11 buffer at 245 nm[2]. Samples containing I were also measured at pH 2, since I interfered with the hexobarbital reading at 245 nm in pH 11 buffer.

RESULTS AND DISCUSSION

The effect of I on hexobarbital sleep time in mice and rats is shown in Table I. In mice administered I intraperitoneally, hexobarbital sleep time increased significantly at all time intervals. The percent increase varied from 31 to 51% and was unrelated to the time interval between the administration of I and the hexobarbital. Indeed, 24 hr after I administration, hexobarbital sleep time was increased 46%. Similarly, hexobarbital sleep time was increased in rats by 23 and 44% 0.5 and 6 hr, respectively, after the intraperitoneal administration of I.

This prolonged effect after the intraperitoneal administration of I is undoubtedly related to its slow absorption from the peritoneal cavity and, hence, the maintenance of a rather constant level of I in the blood. In marked contrast to the results obtained by the intraperitoneal route, intravenously administered I in mice failed to increase the sleep time when hexobarbital was administered 1 or 4 hr later. In view of the short half-life of I (4), this result was attributed to its rapid clearance from the blood.

These observations, coupled with the confirmation of previous studies (1, 2) that I does not significantly affect the rate of hepatic metabolism of hexobarbital, emphasized the importance of the finding of Stern *et al.* (5) that when I is added to plasma a significant amount of the plasticizer becomes associated with the lipoprotein fraction. It was, therefore, hypothesized that the I increases the lipophilic reservoir for the hexobarbital and, consequently, prolongs hexobarbital sleep time by means of its physical

[1] Wayne Lab-Blox.
[2] Gilford 240 spectrophotometer.

Table I—Effect of I (300 mg/kg in Mouse Serum or 500 mg/kg in Rat Serum) on Hexobarbital (100 mg/kg iv in Mice or 80 mg/kg iv in Rats) Sleep Time in Mice and Rats

Time Interval between I and Hexobarbital, hr	Route of Administration of I	Mice				Rats			
		Sleep Time, min				Sleep Time, min			
		I Treated	Serum Control	% Increase	p Value	I Treated	Serum Control	% Increase	p Value
0.5	Intraperitoneal	39.0	25.9	51	<0.01	23.3	18.9	23	<0.03
1	Intraperitoneal	34.4	24.5	40	<0.03				
2	Intraperitoneal	35.3	24.5	44	<0.04				
6	Intraperitoneal	32.1	24.5	31	<0.01	25.7	17.9	44	<0.03
24	Intraperitoneal	28.9	19.9	46	<0.01				
1	Intravenous	24.0	23.5	—	—				
4	Intravenous	32.2	32.2	—	—				

characteristics. If this hypothesis is correct, I should also prolong sleep time when given intravenously, provided the hexobarbital is given before I is cleared from the blood. Furthermore, other lipophilic substances, such as olive oil, also should prolong hexobarbital sleep time.

To test this hypothesis, mice were injected intravenously with either I or olive oil in serum and immediately given hexobarbital by the same route. Olive oil in serum was also given by the intraperitoneal route of administration, and hexobarbital was administered intravenously 1 hr later. The results are shown in Table II;

Table II—Effect of I or Olive Oil (300 mg/kg in Mouse Serum) on Hexobarbital (100 mg/kg iv) Sleep Time in Mice

Drug	Route of Administration	Time Interval between Drug and Hexobarbital	Sleep Time, min		p Value
			Drug Treated	Serum Control	
I	Intravenous	Immediate	58.3	32.5	<0.01
Olive oil	Intravenous	Immediate	49.6	32.5	<0.01
Olive oil	Intraperitoneal	1 hr	44.9	30.9	<0.01

Table III—Effect of I or Olive Oil (500 mg/kg in Rat Serum) on Hexobarbital (80 mg/kg iv) Levels in Rat Serum

Drug	Micrograms of Hexobarbital per Milliliter of Serum	p Value
I	90	<0.01
Olive oil	80	<0.01
Control	70	<0.01

hexobarbital sleep time was increased by 79 and 53% after the intravenous administration of I and olive oil, respectively.

If the mechanism by which I and olive oil increase hexobarbital sleep time is by enlarging the lipophilic pool, a significantly greater amount of hexobarbital should be present in the serum of I- and olive oil-treated animals as compared to the controls. To evaluate this point, three groups of eight rats each were injected intravenously with I, olive oil, or the requisite volume of serum and then immediately injected with 80 mg/kg iv of hexobarbital sodium. Five minutes after the hexobarbital injection, blood was withdrawn from each animal and the concentration of hexobarbital was determined.

As shown in Table III, the concentration of hexobarbital was significantly increased in both I- and olive oil-treated animals. Thus, these data support the concept that the increase in hexobarbital sleep time induced by I is due to its physical characteristics, which enlarge the lipophilic reservoir for hexobarbital, and not to its pharmacological properties *per se*. These observations call attention to another factor that must be considered when evaluating the effect of substances on hexobarbital sleep time.

REFERENCES

(1) R. J. Rubin and R. J. Jaeger, *Environ. Health Perspect.*, **3**, 53(1973).

(2) J. W. Daniel and H. Bratt, *Toxicology*, **2**, 51(1974).

(3) R. J. Jaeger and R. J. Rubin, *Environ. Health Perspect.*, **3**, 95(1973).

(4) C. O. Schulz and R. J. Rubin, *ibid.*, **3**, 123(1973).

(5) I. Stern, J. Miripol, and R. Izzo, *Pharmacologist*, **16**, 283(1974).

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