Potentiation of Urethan Anesthesia by Epinephrine. (22413)

SACHINDRA N. PRADHAN,* BETTY ACHINSTEIN, AND MURRAY J. SHEAR.
Laboratory of Chemical Pharmacology, National Cancer Institute,† Bethesda, Md.

During a study of the action of drugs on carotid blood pressure in mice under urethan anesthesia(1), it was observed that they remained under deep anesthesia for a long time following a dose of epinephrine. Further experiments were thereupon conducted to investigate the effect of epinephrine on urethan anesthesia. This report presents the results of such experiments and shows that epinephrine potentiates urethan anesthesia, and that urethan reduces lethal toxicity of epinephrine.

Method and materials. Experiments were conducted in 3 strains of mice (CAF_1, C3H and C57 Black), weighing 18 to 24 g, and of both sexes. Urethan (10% solution) and, usually after an interval of one hour, epinephrine (0.05 and 0.1% solution), were injected in various doses either subcutaneously or intraperitoneally. Phenobarbital sodium and pentobarbital sodium were given in various doses in some experiments. Duration of anesthesia was measured as the time elapsed between the loss of righting reflex and its return(2-4). This interval should really be called "side position time" (SPT) rather than "anesthetic or sleeping time"; but since the latter term implies the presence of a state which is difficult to measure in mice(2), SPT has been used in these experiments to signify "anesthetic time."

Results. Table I summarizes the results of experiments on epinephrine toxicity in the 3 strains of mice. A dose of 5 μg/g killed 24% of CAF_1 mice, 57% of C3H mice, and 100% of C57 Black mice. From the overall picture at all dose levels, the lethal toxicity appeared to be lowest in the CAF_1 mice and greatest in the C57 Black strain.

Toxic manifestations following injection of epinephrine, especially of high doses, were: apprehensiveness, erection of hair, bulged eyeballs, gradually increasing prostration, frothing (sometimes hemorrhagic) through the nostrils, increased respiration, restlessness, and slight excitatory movements before death.

To study the effect of combination treatment, C57 Black mice were given a subcutaneous dose of urethan followed, after one hour, by an intraperitoneal dose of epinephrine. Results of experiments with various dose combinations show that duration of anesthesia in the survivors increased with the dose of either drug (Table II).

Moreover, urethan protected the mice against the lethal toxicity of epinephrine and prolonged their survival time. Under urethan anesthesia, the toxic manifestations of epinephrine were present, but they were less marked. With epinephrine alone, all but one of the mice died within a short time; the interval from injection to death was 2 ± 1 hr., 1.5 ± 0.5 hr., and 1 ± 0.1 hr. for the 3 dose levels used (2.5, 5 and 10 μg/g, respectively). None of these mice, of course, were anesthetized. At all doses of epinephrine, combination with urethan reduced the number of deaths. Moreover, the mice that died, following injection of urethan and the 2 higher doses of epinephrine, survived longer than those that got epinephrine alone. At the highest dose of epinephrine, the survival was prolonged with epinephrine up to a maximum of 5 hr beyond the epinephrine controls.

In other experiments, also with C57 Black mice, epinephrine was given in 3 dose levels (20, 10 or 1 μg/g) alone, or in combination with various dose levels of urethan. At the 2 higher levels of epinephrine, all the mice died. With 1 μg/g of epinephrine no deaths occurred; the anesthetic time was again prolonged by combination with the higher doses of urethan (1.4 and 1.6 mg/g).

The experiment was repeated with CAF_1 mice, in which epinephrine was less toxic.

* Present address: Department of Pharmacology, Howard University School of Medicine, Washington, D.C.

† N.I.H., Public Health Service, U. S. Department of Health, Education, and Welfare.

TABLE I. Lethal Toxicity* of Epinephrine in 3 Mouse Strains.

Strain	Route	Ratio of: No. dead/No. treated					
		1 μg/g	2.5 μg/g	5 μg/g	10 μg/g	15 μg/g	20 μg/g
CAF_1	Subcut.	—	0/ 6	3/12	9/12	6/ 6	6/ 6
"	Intraper.	—	—	5/22	12/12	29/38	21/22
C3H	Subcut.	0/5	1/ 5	19/34	7/ 8	8/ 8	8/ 8
C57 Black	Subcut.	0/6	2/ 6	6/ 6	6/ 6	—	—
" "	Intraper.	0/6	8/12	12/12	12/12	—	—

* Toxicity was studied usually on 5 or 6 mice at each dose level; larger numbers at certain doses signify pooled data from several experiments.

Epinephrine (2.5, 5, 10, 15 or 20 μg/g) was injected intraperitoneally one hour after a subcutaneous dose of urethan (1.5 mg/g).

In this experiment the data on anesthesia and mortality were recorded at 0.5-1 hour intervals up to 6 hours and again after 24 hours. Table III gives the detailed data on combination of a fixed dose of urethan with various doses of epinephrine. It shows that preadministration of urethan completely protected the mice against the lethal toxicity of low doses of epinephrine (*cf.* Table I); as the dose level of the latter increased to 10 μg/g or more, the degree of protection decreased. In Fig. 1, curve A shows the relationship between the dose of epinephrine and the percentage reduction of its lethal toxicity by urethan.

Curve B in Fig. 1 shows the potentiating effect of epinephrine on duration of anesthesia of urethan; the potentiation increased with the dose, as also can be seen from Table III. This lowering of epinephrine lethal toxicity by urethan and the prolongation of urethan

TABLE II. Duration of Anesthesia in Surviving Mice* (C57 Black).

Epinephrine (μg/g)—intraper.	Urethan (mg/g)—subcut.	Hr			
10	0	— (0)	>6 (2)	>24 (1)	>24 (1)
5	0	— (0)	>6 (4)	> 6 (4)	>24 (3)
2.5	0	0 (1)	1.5 ± .5 (6)	4.5 ± 1.0 (5)	6.0 ± .2 (6)
0	0	—	0 (6)	0 (6)	1.5 ± .5 (6)
	0	1.2	1.4	1.6	

* Number in parenthesis indicates No. of survivors in each group of 12 mice.

anesthesia by epinephrine, in CAF_1 mice, is in agreement with the results obtained in C57 Black mice. With these drugs in combination, at the same dose levels, percent survival was higher in CAF_1 mice, but prolongation of urethan anesthesia was less than in the C57 Black strain.

From these curves it is seen that this combination gave an optimum effect at a dose level between 5 and 10 μg/g of epinephrine. At lower doses, prolongation of anesthesia was less marked and the proportion of mice having a long period of anesthesia was less, while higher doses gave increasing mortality.

In the combination treatment, reversal of route of administration, *i.e.* urethan given intraperitoneally and epinephrine subcutaneously, produced similar effects, but they were less marked. Similar experiments were carried out in all 3 strains of mice, with similar results. The most complete set of experiments was carried out in C3H Black mice, in which the largest number of dose variations was employed (urethan at 1.2, 1.4 or 1.6 mg/g; epinephrine at 5, 10, 15 or 20 μg/g). The results obtained in all of these experiments were entirely analogous to those obtained when the urethan was given subcutaneously and the epinephrine given intraperitoneally, except that the reduction in epinephrine mortality and the prolongation of urethan anesthesia were less pronounced.

Phenobarbital sodium (100 μg/g) or pentobarbital sodium (45, 60 or 75 μg/g), injected subcutaneously half an hour before an intraperitoneal injection of epinephrine (5 μg/g) in C3H and C57 Black mice, did not reduce the lethal toxicity of the latter; but the survival period was slightly prolonged. With this combined treatment, however, the right-

TABLE III. Anesthetic Time of Urethan (1.5 mg/g), Alone or Combined with Epinephrine, in CAF_1 Mice.

Dose of epinephrine, μg/g	No. of mice remaining under anesthesia for						
	0 hr	to .5 hr	.5-1 hr	1-2 hr	2-4 hr	4-6 hr	6 hr
0	10				2		
2.5	6	3		2	1		
5		1			1	6	4
10	1	(1)		(1)	(1)		6 (2)
15		(6)		(1)		1	4
20		(6)	(1)	(2)			2 (2)

At each dose level, 12 mice were employed.
Numbers within parenthesis show the mice that died under anesthesia within the respective periods.

ing reflex became negative following the injection of epinephrine and remained unchanged till death. Epinephrine (2.5 μg/g) in these mice increased the anesthetic time of pentobarbital; the increase was greater, the higher the dose of pentobarbital.

Anesthesia produced in C3H mice with urethan (1.5 mg/g-subcutaneous) was markedly prolonged also by nor-epinephrine (20 μg g-intraperitoneal), and the lethal toxicity of nor-epinephrine was somewhat reduced by urethan. Cortisone (50 μg/g) did not potentiate the action of urethan (1.2 mg/g) in C57 Black mice.

Discussion. Increasing interest has been shown recently in the potentiating effect of various sedative and non-sedative agents on anesthesia induced by barbiturates(2-6,8) and by other anesthetics(6,13,9). While the effect of the sedative agents can be explained on the basis of their synergistic depressive action on the central nervous system, the mechanism of action of the non-sedative agents is still obscure. The effect of several anesthetics has been potentiated by non-sedative agents of diverse nature, such as epinephrine(9,11), nor-epinephrine(9), histamine(6), glucose(8,10), intermediary metabolites(8,9,10) and various other organic and inorganic substances(2,5,8). Contradictory findings are, however, not lacking to show that epinephrine shortens the sleeping time (7). A substance found to be an effective potentiating agent with one anesthetic may fail with another(5,10). Sodium chloride potentiated barbiturate anesthesia, but failed in case of urethan(5).

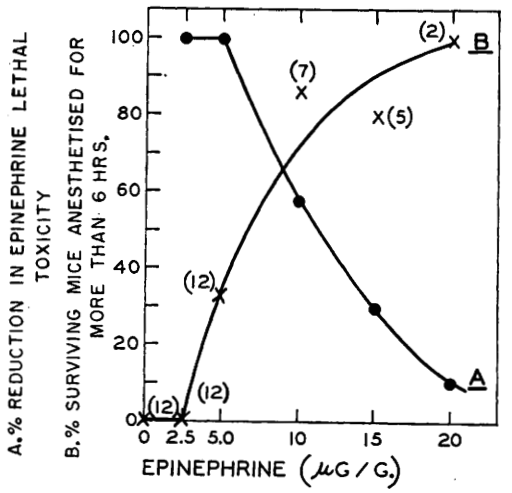

FIG. 1. Reduction of epinephrine lethal toxicity by urethan (1.5 mg/g) (curve A) and potentiation of urethan anesthesia by epinephrine (curve B) in CAF_1 mice. Numbers in parenthesis indicate No. of survivors at the end of 24 hr, in a group of 12 mice each.

In our experiments with mice, potentiation of pentobarbital anesthesia by epinephrine was slight; whereas, anesthetic time of urethan was markedly prolonged by epinephrine and nor-epinephrine. Variation in lethal toxicity of epinephrine in different strains of mice was noticed as in the case of the response to hexobarbital(12). In a given strain of mice the more toxic the dose of epinephrine the more prolonged was the anesthetic time of urethan in combination with epinephrine. For a particular dose of epinephrine, the more susceptible a species to epinephrine toxicity the more marked was the potentiating effect of epinephrine on urethan anesthesia.

Attempts have been made from time to time to explain the mechanism of the poten-

tiation of anesthesia by these non-sedative agents. Experiments(7-9) to investigate whether interference with carbohydrate metabolism, by various agents including epinephrine, is the cause of potentiation of anesthesia have not been conclusive. In an exploratory experiment, we injected insulin (0.4 unit/mouse) intraperitoneally in C3H mice under urethan with or without epinephrine; it did not counteract the effect of epinephrine on urethan anesthesia. This experiment did not indicate that the action of epinephrine on carbohydrate metabolism was responsible for prolongation of urethan anesthesia. Permeability of the brain to barbiturates was found (8) to be increased by lactate, pyruvate and glutamate but not by other substances examined. It has also been shown that potentiating effect of epinephrine is not linked with its action on adrenal cortex(11). On the other hand, barbital anesthesia(18) in mice was found to be prolonged by adrenalectomy; pretreatment with cortisone returned the anesthetic time to normal level, but hydrocortisone decreased it significantly. These effects on anesthesia were found to be correlated with barbital concentration in brain.

The various actions of epinephrine on the central nervous system are difficult to reconcile with each other. The conventional therapeutic dose has little stimulating effect on the central nervous system, but large doses or small doses injected into the carotid artery in animals manifest a stimulating effect(14). Epinephrine was found to potentiate the convulsant effect of electroshock(15). On the other hand, it has some analgesic effect(14) and can cause anesthesia in dogs after intracisternal injection of quite large doses(16,17). In our experience, a few mice were found to lose the righting reflex and appeared to be under anesthesia after a large intraperitoneal dose of epinephrine alone. On the basis of these diverse findings, it is difficult to ascribe the potentiating effect of epinephrine to its action on the central nervous system, and the mechanism of its action on anesthesia remains to be resolved.

Summary. Three strains of mice (CAF_1, C3H and C57 Black) were treated with various dose combinations of urethan and epinephrine. Duration of anesthesia, lethal toxicity and survival time were observed, with the following findings: 1. Epinephrine lethal toxicity was greatest in C57 Black mice and lowest in the CAF_1 strain. 2. In all strains, urethan anesthetic time was greatly prolonged; this effect increased with dose of epinephrine. 3. Urethan reduced lethal toxicity of epinephrine; with increase of dose of epinephrine, reduction of toxicity was less. 4. Urethan anesthetic time was also potentiated by nor-epinephrine; its lethal toxicity was somewhat reduced by urethan. 5. Anesthetic time of phenobarbital sodium and of pentobarbital sodium was slightly potentiated by epinephrine. These agents did not reduce the lethal toxicity of epinephrine.

1. Pradhan, S. N., Achinstein, B., and Shear, M. J., *J. Pharm. Exp. Therap.*, 1955, v113, 44.

2. Kahn, Jr., J. B., *ibid.*, 1953, v109, 292.

3. Cook, L., Toner, J. J., and Fellows, E. J., *ibid.*, 1954, v111, 131.

4. Goldin, A., Dennis, D., Venditti, J. M., and Humphreys, S. R., *Science*, 1955, v121, 364.

5. Richards, R. K., Bertcher, E. J., and Taylor, J. D., *Arch. int. Pharmacodyn.*, 1952, v89, 463.

6. Ambrus, J. L., Ambrus, C. M., Leonard, C. A., Moser, C. E., and Harrison, J. W. E., *J. Am. Pharmaceut. Assn.*, 1952, v41, 606.

7. Westfall, B. A., *J. Pharm. Exp. Therap.*, 1946, v87, 33.

8. Lamson, P. D., Greig, M. E., and Hobdy, C. J., *ibid.*, 1951, v103, 460.

9. Lamson, P. D., Greig, M. E., and Williams, L., *ibid.*, 1952, v106, 219.

10. Lamson, P. D., Greig, M. E., and Robbins, B. H., *Science*, 1949, v110, 690.

11. Fenu, G., *Minerva Otorinolaringologica*, 1954, v4, 91.

12. Jay, G., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 379.

13. Heinrich, M. A. Jr., *Fed. Proc.*, 1951, v10, 307.

14. Goodman, L. S., and Gilman, A., *The Pharmacological Basis of Therapeutics*, The Macmillan Co., New York, 1955, 490.

15. Minz, B., and Domino, E. F., *J. Pharm. Exp. Therap.*, 1953, v107, 204.

16. Leimdorfer, A., and Metzner, W. R. T., *Am. J. Physiol.*, 1947, v150, 588.

17. ———, *ibid.*, 1949, v157, 116.

18. Komiya, A., and Shibata, K., *J. Pharm. Exp. Therap.*, 1956, v116, 98.

Received March 26, 1956. P.S.E.B.M., 1956, v92.