Synergistic and Antagonistic Effects of Premedication on General Anesthetics in Mice

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A NUMBER of drugs are used as preanesthetic medication to relieve fear and anxiety, suppress pain, inhibit secretions and autonomic reflexes, and promote smoother and easier induction. There is, however, considerable uncertainty as to what effect premedication has on the amount or concentration of anesthetic required.

Cohen and Beecher [1] could find no significant reduction in blood levels of cyclopropane or diethyl ether required for surgical anesthesia in man when morphine and atropine, pentobarbital and atropine, or atropine alone in therapeutic doses were given as premedication. Potter and co-workers [2] were unable to find differences in blood concentrations of diethyl ether from patients during surgical anesthesia whether morphine was given prior to anesthesia or not. On the other hand, Taylor *et al.*[3] found that premedication with combinations of morphine or meperidine and atropine, pentobarbital alone, or chlorpromazine alone allowed surgical anesthesia to be obtained with significantly smaller concentrations of diethyl ether than with atropine alone. Animal experiments have proven no more helpful in resolving this question. Calderone [4] found no reduction in blood levels of ether required for surgical anesthesia or respiratory arrest in dogs after premedicication with morphine or subanesthetic doses of amobarbital. Others report quite different results. For example, Robbins and associates [5] concluded that with small doses of morphine, barbital, or amobarbital in dogs there was a decrease in blood concentration of cyclopropane necessary for surgical anesthesia but no decrease in the amount required for respiratory arrest. With larger doses of these agents, however, there was not only a further reduction in blood concentration of cyclopropane needed for anesthesia but also a decrease in the amount of cyclopropane required for respiratory arrest. The results of Seevers and co-workers [6] showed that morphine premedication in dogs lowered the amount of cyclopropane in the inspired air needed for anesthesia and also for respiratory arrest. The latter concentration, however, was not lowered to as great a degree. From experiments in rats, Stormont *et al.*[7] concluded that barbital, phenobarbital, and amobarbital diminished the time of induction, prolonged the duration of action, and allowed the use of smaller concentrations of nitrous oxide for anesthesia.

The present study attempts to resolve the controversial status of the effects of preanesthetic medication on the anesthetic and lethal concentrations of general anesthetics. In addition, the influence of premedication on the time to anesthesia and to death was re-examined. Mice were used to achieve adequate numbers of animals for statistical analysis of the data.

Methods

Albino mice of both sexes, weighing from 15 to 35 g., were placed in 1.95 l. mason jars. A hole was made in each jar cover to accommodate a rubber stopper. The volatile anesthetic was introduced from a syringe onto a piece of cotton gauze suspended from the cover. Experiments were conducted with untreated, saline control, and drug treated groups of 12 mice each. Drug dosage was expressed as the salt. Each agent was dissolved in 0.9 per cent saline and given to treated mice in volumes of 0.01 ml./g. Control mice were used simultaneously with treated animals and received equal volumes of 0.9 per cent saline. All injections were given by the intraperitoneal route. Criteria for anesthesia was the loss of the righting reflex and for death the cessation

Accepted for publication October 2, 1961. The authors are in the Department of Pharmacology, University of Michigan, Ann Arbor, Michigan.

TABLE 1. The Effects of Pentobarbital Premedication on the Anesthetic and Lethal Concentrations in Inspired Air of Three Volatile Anesthetics in Albino Mice

Anesthetic Agent	Rate of Administration (ml./5 minutes)	Level of Anesthesia	Treatment*			P Value†
			None	Saline	Pentobarbital	
Diethyl Ether	0.1	AC_{100}	4.8 ± 0.1	5.1 ± 0.2	4.3 ± 0.2	<.02
		LC_{100}	10.5 ± 0.7	10.3 ± 0.6	11.7 ± 0.5	<.1
Chloroform	0.05	AC_{100}	1.7 ± 0.2	1.6 ± 0.2	0.9 ± 0.2	<.001
		LC_{100}	3.4 ± 0.2	3.0 ± 0.2	3.1 ± 0.2	>.2
Halothane	0.05	AC_{100}	1.7 ± 0.1	1.3 ± 0.1	0.7 ± 0.1	<.001
		LC_{100}	3.7 ± 0.2	3.5 ± 0.1	3.4 ± 0.1	>.6

* Data recorded as the mean volumes per cent ± S.E.

† Group comparison student *t* test between saline and pentobarbital pretreatments.

Pentobarbital, 30 mg./kg. intraperitoneally, and an equivalent volume of saline was given 7 minutes prior to the anesthetic.

AC_{100} = anesthetic concentration required to produce loss of righting reflex in all animals.

LC_{100} = anesthetic concentration required to kill all animals.

of all visible movements. The time interval to anesthesia or death was measured from the start of the administration of the anesthetic. To determine the concentration of anesthetic vapor in the jar the following formula was used:

$$\frac{WAT_1}{MVT_0}P = \text{partial pressure in mm. of mercury}$$

where W is the weight of anesthetic in the jar; A is the molar volume of a gas at STP, and P is the standard pressure. T_1 and T_0 are the room and standard temperature in absolute units. M is the molecular weight of the anesthetic, and V is the volume of the jar.

By administering a constant amount of anesthetic every 5 minutes to untreated mice, the mean anesthetic and lethal concentrations for diethyl ether, halothane, and chloroform were obtained. The effect of sodium pentobarbital on these concentrations was determined by administering 30 mg./kg. of this agent 7 minutes prior to the anesthetic. The effects of 30 mg./kg. sodium pentobarbital and 65 mg./kg. sodium phenobarbital on times to anesthesia and to death were then measured for diethyl ether and chloroform using a single total dose of either 2.0 ml. (24 vol. per cent) of diethyl ether or 0.4 ml. (6 vol. per cent) of chloroform. Pentobarbital was given 7 minutes prior to the anesthetic and the phenobarbital 1 hour before the anesthetic.

For a more accurate method of administering the anesthetic agents, a 'copper kettle' connected to two 2.2 l. desiccator jars was also used. Oxygen was bubbled through the 'kettle' and the resultant vapor mixture delivered to 8 mice simultaneously, 2 treated and 2 control mice in each desiccator jar. The temperature of the vapor mixture in the 'kettle' was monitored continuously with a thermometer so that a constant concentration of anesthetic could be given. The effects on times to anesthesia and death during delivery of 25 per cent diethyl ether, 10 per cent halothane, and 10 per cent chloroform were then determined for each of the following drugs all given 15 minutes prior to the anesthetic: 20 mg./kg. sodium pentobarbital, 1 mg./kg. atropine sulfate, 1 mg./kg. scopolamine hydrobromide, 5 mg./kg. morphine sulfate, 5 and 15 mg./kg. of chlorpromazine, and 5 and 15 mg./kg. of promethazine. In all instances the dosage for premedication chosen was based upon objective evidence of a definite overt pharmacological depressant effect but not sufficient to cause loss of the righting reflex. In all experiments the student *t* test for group comparisons was used to analyze results between treated and control groups.

Results

In general, doses of 30 mg./kg. of intraperitoneal pentobarbital produced ataxia without the loss of the righting reflex. Seven minutes

after premedication the animals were placed in mason jars and given either diethyl ether, chloroform, or halothane. The diethyl ether was administered at a rate of 0.1 ml./5 minute, while chloroform and halothane were given at a rate of 0.05 ml./5 minute. As shown in table 1, pentobarbital premedication reduced the concentration of anesthetic producing anesthesia over control animals given saline from a mean ± S.E. of 5.1 ± 0.2 to 4.3 ± 0.2 volumes per cent with diethyl ether, from 1.6 ± 0.2 to 0.9 ± 0.2 volumes per cent for chloroform, and from 1.3 ± 0.1 to 0.7 ± 0.1 volumes per cent for halothane. The reduction in anesthetic concentration for all agents was significant with a P value of $< .02$. There was no significant change from control animals in the lethal concentrations for any of the three anesthetic agents.

Possibly, because of the short duration of action of pentobarbital in mice, this agent was not acting at the time of death of these animals, and therefore, one might account for the lack of a significant change in anesthetic lethal concentration on this basis. To rule out this possibility, the premedication period was lengthened to 35 minutes and all other factors were kept constant, resulting in production of anesthesia in a new group of animals at the same time death was occurring in the previous groups. In spite of a prolonged premedication period, pentobarbital reduced the anesthetic concentrations over controls from a mean ± S.E. of 1.7 ± 0.2 to 1.2 ± 0.2 vol. per cent for chloroform and from 1.5 ± 0.1 to 1.3 ± 0.1 vol. per cent for halothane. Both reductions were significant with a P value of $< .05$. As was true for a shorter premedication period, there was no significant change in lethal concentrations in the treated versus control animals. These results indicate a selective action of pentobarbital on the anesthetic concentrations of these agents. In both series chloroform produced death at a lower concentration than halothane; the mean ± S.E. for lethal concentrations in control animals were, in the first and second groups respectively, 3.0 ± 0.2 and 3.7 ± 0.2 vol. per cent for chloroform and 3.5 ± 0.1 and 4.2 ± 0.2 vol. per cent for halothane.

By measuring times to anesthesia and to death with a constant concentration of 24 vol. per cent diethyl ether or 6 vol. per cent chloroform, somewhat similar findings were obtained with 30 mg./kg. pentobarbital and 65 mg./kg. phenobarbital premedication. Pentobarbital given 7 minutes prior to the induction of the anesthetic reduced the mean time to anesthesia ± S.E. over controls from 70 ±4 to 43 ± 2 seconds with diethyl ether and from 62 ± 2 to 48 ± 2 seconds with chloroform. Both reductions were significant with a P value $< .001$. However, there was no significant difference in time to death with either agent. Phenobarbital given 60 minutes prior

TABLE 2. The Effects of Barbiturate Premedication on the Times to Anesthesia and Death with Diethyl Ether and Chloroform in Albino Mice

Premedication (intraperitoneal)	Dose (mg./kg.)	Level of Anesthesia	24 Per Cent Ethyl Ether Treatment		P Value*	6 Per Cent Chloroform Treatment		P Value*
			Drug	Saline		Drug	Saline	
Pentobarbital	30	AT_{100}	43 ± 2	70 ± 4	<.001	48 ± 2	62 ± 2	<.001
		LT_{100}	348 ± 20	384 ± 17	>.2	316 ± 55	305 ± 22	>.8
Phenobarbital	65	AT_{100}	49 ± 3	72 ± 5	<.01	45 ± 3	57 ± 3	<.01
		LT_{100}	407 ± 27	410 ± 50	>.9	347 ± 34	363 ± 56	>.8

* Group comparison student t test.
Data recorded as mean ± S.E. in seconds.
Pentobarbital given 7 minutes prior to anesthetic.
Phenobarbital given 60 minutes prior to anesthetic.
AT_{100} = time required to produce loss of righting reflex in all animals.
LT_{100} = time required to kill all animals.

TABLE 3. The Effects of Premedication on Times to Anesthesia and Death with Three Volatile Anesthetics in Albino Mice

Premedication (mg./kg.)	Level of Anesthesia	25 Per Cent Ethyl Ether Treatment		P Value*	10 Per Cent Halothane Treatment		P Value*	10 Per Cent Chloroform Treatment		P Value*
		Drug	Saline		Drug	Saline		Drug	Saline	
Pentobarbital 20	AT_{100}	61 ±6	95 ±5	<.001	50 ±2	59 ±3	<.01	77 ±2	92 ±2	<.001
	LT_{100}	16.0±1.3	13.0±0.9	>.5	12.5±2.3	13.8±2.4	>.7	7.8±0.5	8.3±0.6	>.5
Atropine 1	AT_{100}	130 ±13	121 ±9	>.5	76 ±2	74 ±3	>.5	163 ±5	161 ±6	>.8
	LT_{100}	20.0±1.4	22.5±2.9	>.1	5.1±0.2	5.4±0.2	>.2	23.1±2.2	20.5±2.9	>.4
Scopolamine 1	AT_{100}	122 ±3	117 ±5	>.3	59 ±2	55 ±2	>.1	126 ±4	121 ±5	>.4
	LT_{100}	20.5±1.5	17.5±1.1	>.1	4.5±0.2	4.7±0.2	>.5	7.7±0.4	8.5±0.8	>.3
Morphine 5	AT_{100}	105 ±6	108 ±7	>.7	70 ±3	68 ±3	>.6	127 ±7	117 ±5	>.2
		129 ±7	115 ±4	>.1	64 ±2	60 ±2	>.2			
	LT_{100}	26.0±3.4	17.0±2.0	<.03	7.0±0.6	5.0±0.3	<.01	10.0±0.9	8.3±0.6	>.1
		27.1±3.7	15.0±1.4	<.02	7.1±0.5	5.0±0.2	<.01			

* Group comparison student *t* test.
Data recorded as mean ± S.E., AT_{100} in seconds, LT_{100} in minutes.
All premedications given intraperitoneally 15 minutes prior to anesthetic.

to anesthesia reduced the mean time to anesthesia ± S.E. from 72 ± 5 to 49 ± 3 seconds with diethyl ether and from 57 ± 3 to 45 ± 3 seconds with chloroform. The reductions from controls were significant with a *P* value < .01. Again there was no significant change in times to death (table 2).

When the 'copper kettle' was used, the anesthetic agents were administered in concentrations approximately three times the minimal lethal concentration to reduce the variability which was marked with small amounts. All premedications were given 15 minutes prior to the induction of the anesthetic. A 20 mg./kg. dose of pentobarbital, intraperitoneally, which was sufficient to produce ataxia but not coma reduced the time to anesthesia for all three agents. The decrease was significant with a *P* value of < 0.01. There was no significant change in times to death. The reduction in mean times to anesthesia ± S.E. over control animals was from 95 ± 5 to 61 ± 6 seconds with ether, from 59 ± 3 to 50 ± 2 seconds with halothane and from 92 ± 2 to 77 ± 2 seconds for chloroform. Mean times to death ± S.E. for control animals were 16 ± 1.3 minutes for ether, 12.5 ± 2.3 minutes for halothane and 7.8 ± 0.5 minutes for chloroform. Neither atropine nor scopolamine produced any significant change in either time to anesthesia or to death with any of these agents. Morphine had no significant effect on the time to anesthesia with any anesthetic. It also had no effect on the time to death with chloroform, but it did significantly increase the mean time to death ± S.E. from 17 ± 2.0 to 26 ± 3.4 minutes with ether and from 5.0 ± 0.3 to 7 ± 0.6 minutes with halothane. Because of the unexpected results with morphine premedication, this portion of the series was repeated with similar results (table 3).

To test the hypothesis that respiratory depression resulting in decreased intake of anesthetic vapor was the cause of the increase in time to death with morphine premedication, 5 mg./kg. of morphine was given intraperitoneally 40 minutes prior to the induction of diethyl ether anesthesia. There was no significant change in time to anesthesia between control and treated groups of mice, the average times to anesthesia ± S.E. being respectively 119 ± 8 and 113 ± 8 seconds. For this series of mice, the time of anesthesia corresponded to the time of death of the prior series of mice treated with morphine at a shorter preinduction period. The lack of significant change in time to anesthesia between the groups indicates there probably was no significant change in respiratory depression, if any, between the two periods.

The results with the phenothiazine derivatives, chlorpromazine and promethazine were inconsistent, particularly with the smaller dosage of 5 mg./kg. intraperitoneally. At 15 mg./kg. chlorpromazine, intraperitoneally, significantly lowered the mean time to anesthesia ± S.E.

from 111 ± 3 to 93 ± 3 seconds for ether, from 69 ± 2 to 43 ± 3 seconds for halothane and from 89 ± 6 to 58 ± 3 seconds for chloroform. The larger dose of chlorpromazine also significantly increased the mean time to death ± S.E. under ether from 12.0 ± 0.8 to 21.7 ± 1.3 minutes and under halothane from 5.6 ± 0.2 to 14.1 ± 1.2 minutes. There was no significant difference in the times to death under chloroform. A 15 mg./kg. dose of intraperitoneal promethazine did not significantly affect the time to anesthesia with all 3 anesthetic agents. Its only significant effect was to increase the mean time to death ± S.E. under ether from 15.3 ± 0.9 to 21.5 ± 1.2 minutes, the P value being < 0.01. There was no significant change in times to death with either halothane or chloroform (table 4).

Discussion

The determination of anesthetic and lethal concentrations of the volatile anesthetics utilizing the mason jar apparatus is rather crude since one must assume: (1) total evaporation of the anesthetic from the gauze, (2) sufficiently large volume of the jar to prevent a significant change in anesthetic concentration to O_2 consumption and CO_2 production by the mouse, and (3) no significant loss of anesthetic vapor as the stopper is removed for injection of the agent onto the gauze. Despite these inadequacies in the apparatus, the degree of reproducibility which was obtained indicates a fairly constant set of conditions for each group of animals and allows a reliable comparison between groups to judge the effects of premedication.

The single large injection of an anesthetic agent minimizes the loss of anesthetic vapor from the jar but does not improve any of the faults inherent in the apparatus, hence the 'copper kettle' device was used. We believe that times to anesthesia and to death as an index of potency of the anesthetic agents could be used since one can assume that the dose of anesthetic is proportional to its concentration multiplied by the time the animal is exposed to the vapor. One may question the presence of another factor influencing times to anesthesia and to death, that is, the rapidity of onset of action of the anesthetic agent. This must be taken into consideration in order to compare the responses of the animals to different anesthetics. Comparison between anesthetics is further complicated by the marked day to day variability in responses to the same anesthetic. It is difficult to conceive that inadequacies in the apparatus or technique could account for such discrepancies and that more probably some unrecognized and uncontrolled factor (or factors) is involved. This does not detract from the results obtained with premedication, however, since control and treated mice were observed simultaneously.

To reduce some of the variability in each group and to provide a more constant set of

TABLE 4. The Effects of Chlorpromazine and Promethazine Premedication on the Times to Anesthesia and Death With Three Volatile Anesthetics in Albino Mice

Dose (mg./kg.)	Level of Anesthesia	25 Per Cent Ethyl Ether Treatment		P Value*	10 Per Cent Halothane Treatment		P Value*	10 Per Cent Chloroform Treatment		P Value*
		Drug	Saline		Drug	Saline		Drug	Saline	
Chlorpromazine										
15	AT_{100}	93 ±3	111 ±3	<.001	43 ±3	69 ±2	<.001	58 ±3	89 ±6	<.001
	LT_{100}	21.7±1.3	12.0±0.8	<.001	14.1±1.2	5.6±0.2	<.001	13.1±1.2	15.7±2.2	>.3
Promethazine										
15	AT_{100}	131 ±5	130 ±4	>.8	62 ±1	61 ±1	>.4	103 ±2	102 ±2	>.7
	LT_{100}	21.5±1.2	15.3±0.9	<.01	7.0±1.0	4.9±0.2	>.05	13.3±2.2	12.4±2.4	>.9

* Group comparison student t test.
Data recorded as mean ± S.E., AT_{100} in seconds, LT_{100} in minutes.
Chlorpromazine and promethazine were given intraperitoneally 15 minutes prior to the anesthetic.

experimental conditions, we used a supralethal concentration of anesthetic and measured times to anesthesia and to death. The results with pentobarbital, the only premedication which was used under all experimental situations, indicate that the indices for potency of the anesthetic are valid with either measurement of anesthetic concentrations or times to anesthesia and to death with supralethal concentrations.

From the data obtained in these experiments, the selective effect of pentobarbital of increasing the anesthetic but not the lethal potency of diethyl ether, chloroform, and halothane appears to be a real one. The only other premedication tested with similar effects was phenobarbital. One might suspect from these results that clinically pentobarbital premedication would improve the therapeutic index of these anesthetic agents although no actual calculation of this index can be made from our data. The effects of morphine premedication on lengthening times to death without changing times to anesthesia with diethyl ether and halothane may be the result of a stimulating action of this agent in the mouse. The variable results seen with the phenothiazine derivatives, particularly with the smaller doses, make it difficult to draw definitive conclusions as to their effects as preanesthetic medications. Large doses of chlorpromazine lowered the times to anesthesia and prolonged the time to death for ether and halothane. The lack of significant differences with chloroform would seem to confirm the generally accepted notion that cause of death with chloroform was different from the other anesthetic agents, probably being cardiac rather than respiratory in origin. The negative results with atropine and scopolamine are consistent with their known peripheral actions of inhibiting salivary secretions without marked central nervous system depression. It is interesting that although scopolamine has greater central nervous system depressant effects than atropine both were relatively ineffective in altering the times to anesthesia or death.

Summary

Using albino mice as the test subjects, premedication with subanesthetic doses of pentobarbital (20 to 30 mg./kg.) and phenobarbital (65 mg./kg.) given intraperitoneally had a synergistic effect on the anesthetic concentration of diethyl ether, halothane, and chloroform but no significant effect on their lethal concentration. Atropine or scopolamine premedication (1 mg./kg.) had no synergistic or antagonistic effect on the anesthetic or lethal actions of diethyl ether, halothane, or chloroform.

Morphine premedication in a dose of 5 mg./kg. prolonged time to death with diethyl ether and halothane but had no effect on times to anesthesia with these agents. The phenothiazine derivatives, chlorpromazine and promethazine, produced variable results with doses of 5 mg./kg. In large doses of 15 mg./kg. chlorpromazine reduced time to anesthesia and prolonged the time to death for diethyl ether and halothane, but not chloroform. In equal doses promethazine was less effective.

The investigation was supported (in part) by Grant MY-2653, United States Public Health Service. Mr Carson is a Predoctoral Fellow, No. C-3425, National Science Foundation.

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