

Pressure Antagonism of Barbiturate Anesthesia

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The losses of righting reflex produced by various doses of phenobarbital in mice at 1 atm O₂ versus 1 atm O₂ plus 102 atm He were determined. The resulting dose-response curve at pressure gave an ED₅₀ that was 64 per cent larger than the ED₅₀ at 1 atm. This increment is essentially the same as that found for gaseous anesthetics under similar test conditions. The quantitative similarity of the results of pressure reversals of barbiturate and inhalational anesthetics suggests that the mechanisms or sites of action of these agents are similar. However, the dose-response curve at 103 atm was steeper than that at 1 atm. This raises an alternative possibility that anesthetics and pressure bear no mechanistic relationship to each other, but rather that pressure produces a generalized central nervous system stimulation that would antagonize any depressant effect. (Key words: Theories of anesthesia, critical volume; Hyperbaria, reversal of anesthesia; Hypnotics, barbiturates, phenobarbital.)

JOHNSON AND FLAGLER were the first to demonstrate that the effect of inhalation anesthetics is antagonized by the application of high hydrostatic pressures.¹ This "pressure reversal of anesthesia" was subsequently shown for all gaseous anesthetics tested (e.g., halothane, ether, chloroform, SF₆, CF₄). Kent and Halsey further demonstrated that the increases in partial pressures of nitrous oxide² and isoflurane (personal communication) required to produce anesthesia in mice bore a

linear relation to the total pressure applied by helium.

The phenomenon of pressure reversal has been used as evidence to support the "volume expansion" theory of anesthesia, which suggests that anesthetics act by expanding a crucial neuronal site. This expansion is countered by the force of high hydrostatic pressures. If a mechanistically meaningful relationship exists between high hydrostatic pressures and anesthesia, then it should be qualitatively and quantitatively similar for all anesthetics acting by similar mechanisms. This study was undertaken to determine whether barbiturate anesthesia is antagonized by pressure, and, if so, whether the reversal is quantitatively similar to that found for gaseous anesthetics. A positive finding would be compatible with a common mechanism of action for barbiturates and inhaled agents.

Methods

Experiments were performed in a 20-liter chamber with a working pressure of 300 atmospheres. Chamber gases were mixed and pulled through a CO₂ absorbent by an induction motor-driven fan, and temperature was thermostatically regulated. Lighting was provided both by internal, battery-powered bulbs, and by external lights applied to Plexiglas ports on both sides of the chamber. The animals were viewed through a large (12.5 cm) plexiglas window at the chamber end. Samples of gas in the chamber were removed through a bleed valve and analyzed by gas chromatography. The righting response to a rolling stimulus was used as the criterion of narcosis. Normal animals can right themselves continually for long periods, and the partial pressures of gaseous anesthetics required to abolish this response are highly consistent.³

Groups of eight 20–30-g male Swiss-Webster mice were tested. Each was placed in an individual wire mesh cage. Eight such cages were held in a rotator that could be

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revolved in either direction at 4 rpm. In each experiment, three further mice served as temperature controls. These were restrained individually in non-rotating cages and temperatures measured with rectal thermistors. Rectal and chamber temperatures were read at intervals of one hour or less throughout the experiment. Chamber temperature was adjusted to maintain rectal temperatures within 37–38°C.

Phenobarbital[†] was chosen as a representative barbiturate because its action is stable over a period that allowed pressurization without appreciable change in drug level as indicated by constancy of effect in unpressurized control mice. Preliminary studies demonstrated that 0.120 mg/g of phenobarbital given intraperitoneally resulted in a sleep time of 16.3 ± 0.5 (SE) hours. At the beginning and toward the end of this period, animals would alternate between wakefulness and sleep, but from the third through the tenth hour after injection the degree of narcosis appeared stable. Subsequent observations were confined to the fourth through the seventh hour after injection.

Phenobarbital (130 mg/ml) preserved in propylene glycol and 5 per cent benzyl alcohol solution was measured in a calibrated tuberculin syringe. This was diluted in physiologic saline solution so that each animal received approximately 1.0 ml total volume. Exact dose was corrected for animal weight and injected intraperitoneally with a tuberculin syringe, through a 27-gauge needle, into the left lower quadrant. There was no evidence of intravascular injection. The mice then were placed inside the cages and into the chamber. After the chamber door was closed, 100 per cent oxygen was flushed through at high flow for 10 minutes. The animals were then exposed to either 1 atm of 100 per cent oxygen or 103 atm consisting of approximately 1 atm of oxygen and 102 atm helium. In the high-pressure series, helium was introduced from tanks pressurized to 400 atm.** Since the rate of compression affects performance at high pressure,⁴ we increased helium pressure at a rate of 1 atm/

min. Observations were made at hourly intervals. The rotator was turned on 3 minutes prior to an observation. To pass the rolling-response test, an individual animal had to remain upright for four of five revolutions. Each animal thus received one quantal score each hour. In the 103-atm experiments, chamber oxygen was determined periodically with a Beckman E-2 analyzer, and always exceeded 0.6 atm.

A total of eight dose levels was tested, five at 1 atm and three at 103 atm. Sixteen animals (two experiments) were observed at each dose. The hourly scores for each group of 16 animals were averaged over the four-hour observation period.

Quantal dose-response curve analyses were obtained after the method of Waud.⁵ To test whether the slopes of the two dose-response curves were parallel, further statistical analyses were performed. A quantal regression program applying a logistic transformation (logits) of the percentage responses was used to fit the data by two methods. In one we assumed independent slopes and in the second test the curves were fitted assuming a common slope.

Results

We found that high helium pressures antagonized the effect of barbiturate anesthesia (fig. 1). The results four hours after the injection did not differ from those at seven hours, indicating that the antagonism persisted over the four hours of measurement. One dose (0.120 mg/g) was given in both the 1 atm and 103 atm conditions. At 1 atm, a rolling response score of 22 per cent was obtained, while at 103 atm it was 94 per cent ($P < 0.001$). The dose of phenobarbital giving a 50 per cent score (ED_{50}) at 1 atm was 0.086 mg/g, with a standard error of ± 0.006 . At 103 atm, the ED_{50} was 0.141 ± 0.004 mg/g. The increase in ED_{50} is 64 per cent, which equals that found for isoflurane (63 per cent) and is only slightly higher than that for N_2O (46 per cent).⁷ Chi-square tests about regression of the quantal regression analysis using the first assumption (independent slopes) indicated a nominally significant departure from linearity at 1 atm ($P < 0.05$). However, we performed

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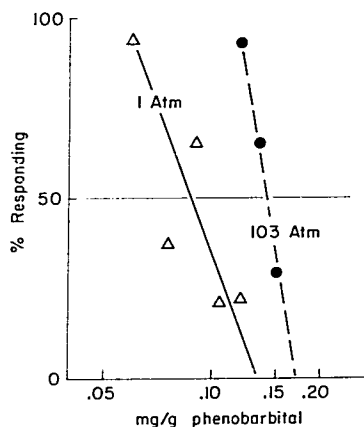


FIG. 1. Alteration of phenobarbital requirement by helium pressure. Per cent retention of the righting response to a rolling stimulus in mice is plotted against the dose of phenobarbital given intraperitoneally under two conditions: in 1 atm of O_2 and in the presence of 103 atm consisting of 0.6–1.0 atm O_2 balance helium. Each point represents 16 animals. The dose of phenobarbital giving a 50 per cent response (ED_{50}) was increased 64 per cent by the addition of helium pressure.

additional experiments at 1 atm after it became clear that the first experiments did not define the ED_{50} with precision. Thus the "true" p value for this deviation from linearity is almost certainly smaller. The regression analysis based on the second assumption (common slopes) revealed that the slopes at 1 atm and 103 atm were significantly different ($P < 0.01$). The departure from linearity discovered in the first test may not have any bearing on the second test, but it must be added as a caveat that the significance of the slope difference is probably less than indicated by our test.

Discussion

Meyer and Overton were the first to observe that anesthetic potency correlates closely with lipid solubility. The remarkable consistency of this correlation has focused attention on

hydrophobic structures such as lipid membranes as potential sites of anesthetic action. Deviations from the correlation are of particular importance since they might preclude any simple theory of action in such membranes. Poorly soluble gases such as N_2 do deviate from the Meyer-Overton predictions.

An explanation for the deviation was suggested by research in problems associated with deep-sea diving to great depths. To avoid the anesthetic effect of N_2 , breathing mixtures of helium and oxygen were used. However, mice and other mammals breathing such mixtures at very high pressures (60 to 80 atm) developed a "high-pressure neurologic syndrome" consisting of a progression of increasing tremors, clonic seizures and death.⁶ In experimental chamber "dives" human volunteers also experienced such tremors.⁷ Since the compression of amphibians in a fluid medium produced similar results, the symptoms seen are not related to helium but rather to high hydrostatic pressure.¹ From such a synthesis of observations has come a mechanistic theory of anesthetic action. Described by Miller as the "critical volume hypothesis,"⁸ this theory states that "General anesthesia or pressure-induced convulsions occur when a hydrophobic region is expanded or compressed, respectively, by critical amounts. . . ." The absolute expansion necessary for abolition of the rolling response in newts and the compression associated with the convulsion threshold in mice have been calculated. The expansion and compression were similar in magnitude (though obviously opposite in direction), suggesting that the combination of the two would abolish convulsions and antagonize the anesthetic effect. This accounts for the observed deviation from predicted anesthetic requirements based on lipid solubility for agents such as N_2 . The high N_2 pressures required to achieve anesthesia also work to oppose that anesthetic effect.

Does this interaction and explanation apply to nongaseous anesthetics? Lever⁹ found that pressure attenuates barbiturate-induced narcosis in newts and perhaps mice, but gave no indication of the consistency or magnitude of the effect. If the critical-volume hypothesis is correct—if general anesthesia does re-

sult from altered membrane dimensions—then the pressure reversal of barbiturate-induced narcosis might indicate a similarity in sites or mechanisms of action of inhalation and barbiturate anesthetics.

Our study demonstrates that 103-atm helium pressure does reverse phenobarbital anesthesia in mice. Furthermore, the degree of antagonism, represented by the 64 per cent increase in ED_{50} , produced by pressure is of the same magnitude as that seen with gaseous anesthetics. This offers further support to a similar mechanism of action. However, the lack of parallelism between the 1-atm and 103-atm dose-response curves raises the question whether, despite the unequivocal antagonism of barbiturate anesthesia by pressure, this interaction may not be exclusively "competitive" at the anesthetic site of action and may even involve an indirect and general central nervous system stimulation.

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Transfusion

AUTOLOGOUS TRANSFUSION AND CARDIOPULMONARY BYPASS Will autologous transfusion for open-heart surgery reduce demands on the blood bank? This question was examined in 122 patients more than 10 years old undergoing elective open-heart surgery. In every other patient, approximately 30 per cent of the patient's blood volume was removed prior to institution of cardiopulmonary bypass. The blood was restored after the end of bypass. The other half of the patients served as controls and received only banked blood. Both groups were divided into cyanotic and acyanotic patients. After cardiopulmonary bypass had been terminated, acyanotic control patients received 2.3 ± 2.5 (mean \pm SD) units of banked blood. The acyanotic autologous-transfusion group received only 0.4 ± 0.9 units each. Post-

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bypass requirements for blood were significantly less in the acyanotic autologous-transfusion group. In the cyanotic patients blood requirements in the two groups were equal. However, removal of autologous blood frequently caused decreased venous return during bypass, resulting in additional transfusion of the pump oxygenator with whole blood and crystalloid. Thus, more blood was actually administered in the autotransfusion group than in the control group, a difference that was significant in the cyanotic patients. Platelet and fresh-frozen plasma utilizations were the same in the two groups. (Plian MB, McCoon DC, Tarhan S: Failure of transfusion of autologous whole-blood to reduce banked blood requirement in open-heart surgical patients. *J Thorac Cardiovasc Surg* 70:228-343, 1975.)