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The General Anesthetic Potency of Propofol and Its Dependence on Hydrostatic Pressure

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Although plasma concentrations of propofol during anesthesia are well known, the free concentration remains unknown because of uncertainties regarding plasma protein binding, interaction with other protein-bound substances, the level of binding to its lipid carrier, and the use of adjuvants. At elevated surrounding pressure, all general anesthetics require higher concentrations to reach adequate levels of anesthesia. To determine the anesthetic potency of propofol at equilibrium conditions and to study the effects of pressure on propofol-induced anesthesia, *Rana pipiens* tadpoles were exposed to different concentrations of pure, not emulsified, propofol in aqueous solution. Anesthesia was defined as loss of the righting reflex. Ten animals per concentration were used, and each experiment was conducted twice. Pressure experiments were performed with nonanesthetized tadpoles and urethane-anesthetized tadpoles as control groups. Propofol concentrations were measured spectrophotometrically. At 1 atmosphere absolute (atm abs), a semilogarithmic sigmoidal concentration–response curve was obtained with a half-maximal effect of propofol at 2.2 ± 0.22 μM (EC_{50}; mean ± SE). Increased pressure shifted the concentration–response curve to the right. The EC_{50} increased linearly with increasing pressure up to 121 atm abs (EC_{50} at 121 atm abs = 4.1 ± 0.41 μM). For pressure greater than 121 atm abs, an increased excitability of the tadpoles made it difficult to distinguish the righting reflex from involuntary movements. The saturated solubility of propofol in aqueous solution was found to be 1.0 ± 0.02 mM (mean ± SD), and the octanol/water partition coefficient was 4,300 ± 280. Propofol adhered to the correlation between anesthetic potency and octanol/water partition coefficient exhibited by other general anesthetics. Thus, it behaves like a typical general anesthetic in that it obeys the Meyer-Overton rule and its effects are reversed by increased pressure. (Key words: Potency, anesthetic: Meyer-Overton correlation; octanol/water partition coefficient; pressure reversal. Anesthetics, intravenous: propofol.)

PROPOFOL (2,6-diisopropylphenol) is a new intravenous anesthetic agent with a wide range of clinical indications from induction to maintenance of anesthesia.[1] Propofol exhibits a rapid onset and a short duration of anesthesia, and it lacks excitatory side effects. These properties have led to a wide-spread adoption of propofol into clinical practice, where it represents a new class of intravenous anesthetics structurally unrelated to the other major classes of general anesthetics used.

The molecular structure of propofol endows it with a very low solubility in water, and for this reason the compound is administered clinically in an emulsion of soybean oil. Studies determining the anesthetic activity of propofol in humans by measuring whole blood concentrations in infusion-based studies showed that the clinical potency of propofol apparently varied considerably.[2,3]§ To evaluate the potency of propofol under well-controlled laboratory conditions and to determine the effect of hydrostatic pressure on propofol-induced anesthesia, we measured the anesthetic effect of an aqueous solution, rather than a soybean oil emulsion, of propofol on tadpoles. For a century, this aquatic animal has been a standard for anesthetic potency determinations. Its sensitivity to general anesthetics is similar to that of mammals.[4] It was chosen for our study because it allows for the determination of anesthetic potency without the uncertainties attendant on measurements of blood levels in mammals. Furthermore, as aquatic animals tadpoles are easily exposed to hydrostatic pressure, and therefore the pressure reversal of anesthesia can be studied without the complications associated with the use of high pressures of helium.[5,6]

This study aimed to answer two questions. Does the anesthetic potency of propofol correlate with its lipid solubility, and is it possible to antagonize propofol anesthesia by hydrostatic pressure? Positive findings suggest that propofol shares common mechanisms of action with other general anesthetics.

Materials and Methods

All propofol solutions (Stuart Pharmaceuticals, Wilmington, DE) were made up at a temperature of 22.5 ± 0.5° C as saturated aqueous solutions of pure, not emulsified, propofol by adding an excess amount of pro-

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pofol to deionized distilled water (*p*H 7.0). Care was taken that droplets of propofol did not mix with the water, which was gently stirred to aid dissolution. By preparing solutions in this manner, the formation of microspheres of propofol was avoided. After stirring the solutions overnight, the saturated aqueous solutions of propofol were carefully removed and then diluted to the appropriate concentrations. All propofol concentrations were checked spectrophotometrically at a wavelength of 272 nm, against gravimetrically prepared standard solutions.

To measure the octanol/water partition coefficient, octanol was carefully added to aqueous solutions of propofol (10 and 500 μM) to form a single layer on top of the water. The solution was gently stirred for 2 days to ensure that full equilibration between the two phases had occurred. There was no difference between concentrations after 1 or 2 days of equilibration. Before and after the addition of octanol, samples of the aqueous phase were withdrawn and the propofol was extracted into hexane. Absorbances of the propofol in hexane samples were measured, and the partition coefficient was calculated after the proper dilution factors were taken into account. Each of the experiments was performed in triplicate.

Experiments determining the anesthetic potency of propofol were performed by equilibrating *Rana pipiens* tadpoles in the early pre–limb-bud stage of development (approximately 1.0–1.5 cm in length; Carolina Biological Supply Company) for 15 min with aqueous solutions of pure propofol (0.1–100 μM) in covered 100-ml beakers. For each concentration of the anesthetic, two experiments with five animals each were performed. More than 100 tadpoles were used per concentration–response curve (table 1). Experiments were performed at a temperature of 22.5 ± 0.5° C. Anesthesia was defined as loss of righting reflex after manual inversion of a tadpole with a flame-polished pipette. An animal that did not correct its orientation within 5 s of the stimulus was considered anesthetized. Based on the response of the tadpoles at different concentrations of propofol, a concentration–response curve was generated according to the method of Waud for quantal biologic responses,[7] and the concentration at which half of the tadpoles were anesthetized (EC_{50}) and the slope of the curve were calculated. The resulting EC_{50} values are reported as mean ± SE; all other results are reported as mean ± SD.

Anesthetic solutions for the pressure experiments were prepared as described above. Tadpoles were placed in a 0.34-l stainless steel cylindrical pressure chamber completely filled with anesthetic solution. All solutions were preoxygenated to provide enough oxygen for the tadpoles during the course of the experiment. Hydrostatic pressure was applied with a hand-driven pump (Boston Hydraulics, Boston, MA) that was connected to the pressure system. Applied pressure was measured using a 250-atm gauge (Heise, Newton, CT) that was accurate to within 0.1% of the full-scale reading. Pressure was recorded in atmospheres absolute (atm abs; 1 atm abs = 101.3247 kPa). The pressure chamber was equipped with an internal division to keep the tadpoles close to the Plexiglas window at one end of the cylinder and was illuminated by an external fiber optic. Tadpoles were placed in the chamber in groups of five (two trials per concentration) and were equilibrated with the anesthetic solution for at least 10 min before application of pressure.

Pressure was increased in steps of 30 atm abs. After increasing the pressure over approximately 30 s, the chamber was disconnected from the system and was manually rotated several times over the course of 2–3 min. Anesthesia was evaluated as the loss of righting reflex under pressure, which was defined as the inability of a tadpole to right itself after being inverted by the rotation of the disconnected pressure chamber. Tadpoles anesthetized with various concentrations of urethane (Sigma Chemical Company, St. Louis, MO) and tadpoles in pure water were used as control groups.

Results

The saturated concentration of pure propofol in water at 22.5 ± 0.5° C was found to be 1.0 ± 0.02 mM. To our knowledge this value has not been reported in the literature before. Measurements of the octanol/water partition coefficient yielded a partition coefficient of 4,300 ± 280 (log P = 3.63). This result is consistent with a previous report by Skues and Prys-Roberts.[1]

Rana pipiens tadpoles were equilibrated with different concentrations of aqueous solutions of propofol. After exposure of the tadpoles to the anesthetic, anesthesia (loss of righting reflex) occurred within 5 min, and the number of anesthetized animals did not then vary significantly over 15 min, indicating that equilibrium had been attained. The fraction of anesthetized animals increased with increasing propofol concentrations. The data could be fitted to a logistic function[7] with an EC_{50} of 2.2 ± 0.22 μM and a slope of 2.6 ± 0.52 (fig. 1). The experiments performed in a beaker or in the pressure chamber at 1 atm abs yielded about the same EC_{50} values and slopes for loss of righting reflex and loss of righting reflex under pressure, although the experimental design and the anesthetic endpoint were somewhat different (table 1).

The effect of pressure on tadpoles anesthetized with fixed concentrations of propofol at different pressures is shown in figure 2. At 0.1 μM propofol, all of the tadpoles responded at pressures up to 121 atm abs, and all of the animals showed the same reaction as those not exposed to an anesthetic. However, the tadpoles not exposed to propofol started to lose their righting reflex at pressures greater than 91 atm abs, whereas those at 0.1 μM propofol

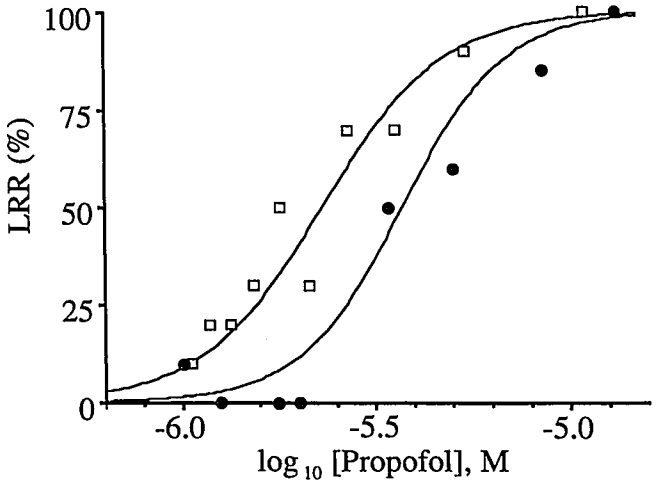

FIG. 1. Concentration–response curves for propofol-induced anesthesia. Data for loss of righting reflex (LRR) in a beaker at 1 atm abs (squares) are compared to data for loss of rolling reflex at 91 atm abs (circles). Curves are logistic functions fitted to the data as described in the text, with parameters given in table 1.

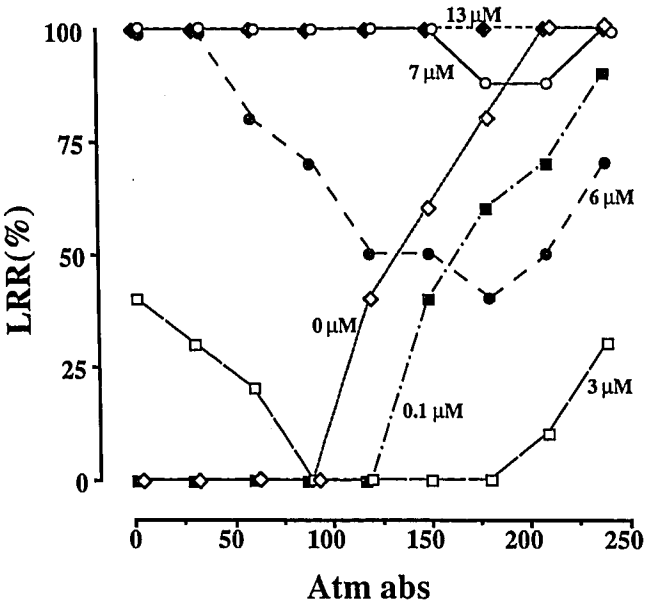

FIG. 2. Loss of righting reflex of tadpoles plotted against pressure in the presence of varying concentrations of propofol (open diamonds = 0 μM; filled squares = 0.1 μM; open squares = 3 μM; filled circles = 6 μM; open circles = 7 μM; filled diamonds = 13 μM propofol). Each of the curves consists of the results obtained from experiments with ten animals.

lost their righting reflex at pressures greater than 121 atm abs. At higher pressure, the fraction of responding tadpoles decreased in parallel for both groups until none of the tadpoles responded at 211 atm abs in the control group. Of the tadpoles exposed to 0.1 μM propofol, 10% responded at 241 atm. At 3.0 μM propofol 40% of the tadpoles were anesthetized at 1 atm abs; this percentage decreased in a linear fashion with pressure until at 91 atm abs none was anesthetized. At pressures of 91–181 atm abs, all of the tadpoles responded to the applied stimulus. At higher pressure, the response decreased progressively until 30% of the tadpoles were unresponsive at 241 atm abs (fig. 2). Tadpoles exposed to concentrations of 6 and 7 μM behaved in a similar fashion, but the pressure necessary to get the largest fraction of tadpoles responding increased. At a propofol concentration of 13 μM, no response of the tadpoles was observed within the range of applied hydrostatic pressure.

At pressures exceeding 121 atm abs, tadpoles exposed to propofol began to demonstrate a twitching motion that rendered further quantification of the loss of righting reflex under pressure difficult. This twitching of the tadpoles qualitatively resembled the excitatory effects of high pressure on tadpoles not exposed to an anesthetic. When the pressure was increased in steps of 30 atm abs, the control tadpoles responded normally at pressures of 31 and 61 atm abs. At 91 atm abs the tadpoles swam more actively and exhibited sudden bursts of activity, a behavior not noticed at lower pressure. This behavior was even more pronounced at 121 atm abs. At pressures of 121 atm abs and greater, an increasing fraction of the tadpoles did not respond to the applied stimulus (fig. 2). At 211 atm abs, none of the control tadpoles responded and no spontaneous activity was observed. Some of the tadpoles at pressures of 181 atm abs and greater bent their tails at the proximal end. The bending of the tail has been described as a sign of pressure paralysis in tadpoles.[8] Propofol protected the tadpoles from entering the excitatory state, as indicated by the fact that tadpoles not exposed to propofol first showed twitching motions at 121 atm abs, whereas tadpoles exposed to an EC_{50} of propofol began to twitch at 151 atm abs.

Because of the actions of pressure *per se*, only the data up to 121 atm abs were grouped by pressure to yield concentration–response curves, which were analyzed according to the method of Waud[7] for quantal biologic responses. The EC_{50} values obtained at each pressure were

TABLE 1. Half-maximal Effective Concentrations and Slopes of Propofol Concentration–Response Curves for Loss of Righting Reflex of Tadpoles

Pressure (atm abs)	n	EC_{50} ± SE (μM)	Slope ± SE	Remark
1.0	150	2.2 ± 0.22	2.6 ± 0.52	Bench experiment
1.0	120	2.6 ± 0.21	3.1 ± 0.54	Pressure chamber
31.0	120	3.1 ± 0.16	6.1 ± 1.20	Pressure chamber
61.0	120	3.6 ± 0.27	3.5 ± 0.61	Pressure chamber
91.0	120	3.8 ± 0.33	2.8 ± 0.47	Pressure chamber
121.0	120	4.1 ± 0.46	1.9 ± 0.34	Pressure chamber

EC_{50} = half-maximal effective concentration.

plotted for propofol and urethane and the slopes compared. The problem of multiplicity was addressed using Bonferroni's inequality.[9] Table 1 lists the EC_{50} values and slopes of these curves. The slopes of the concentration–response curves ranged from 1.9 ± 0.34 to 6.1 ± 1.20, but no systematic trends (for example, as a function of pressure) were observed. Similar degrees of variation in the slopes of the concentration–response curves for tadpoles have been found previously in our laboratory.[10] Figure 1 illustrates the rightward shift of the concentration–response curves with increasing pressure and, with table 1, clearly demonstrates the degree to which propofol-induced anesthesia is reversed by pressure.

The control experiments with urethane yielded results similar to those obtained for propofol. At 1 atm abs we obtained an EC_{50} value of 16.4 ± 1.03 mM and a slope of 4.5 ± 0.85. Sigmoidal semilogarithmic concentration–response curves were obtained for pressure up to 121 atm abs. The curves were shifted to the right with increasing pressure.

Discussion

Anesthetic Potency of Propofol

We found the anesthetic potency of propofol (as defined by the EC_{50} for the loss of righting reflex of tadpoles) to be 2.2 ± 0.22 μM under controlled laboratory conditions. Because this figure is for the free concentration of propofol in the aqueous phase in equilibrium with tadpoles, it will be useful as a standard of comparison for *in vitro* studies. It can also be directly compared to the large body of data for other intravenous and volatile anesthetics in tadpoles. In table 2, it can be seen that propofol is an order of magnitude more potent than any other commonly used general anesthetic that has been studied in the tadpole.

Propofol is thus one of the most potent general anesthetics currently in clinical use. The EC_{50} of propofol measured in this study is about one-sixth that of propofol serum levels determined in clinical studies (humans: 13–14 μM[2,¶]; tadpole: 2.2 μM). *A priori*, this difference could be caused by several factors, including species difference; variation in anesthetic potency with temperature; overestimation of the free propofol concentration in the plasma because of binding to plasma proteins and lipid delivery vehicle; and the nonequilibrium (or steady-state) nature of the measurement in humans. An idea of how important these factors might be can be obtained by comparing propofol data to those for thiopental, an intravenous anesthetic that resembles propofol in having a large volume of distribution. The EC_{50} of thiopental in humans is 35 μM in plasma,[11] and in tadpoles at 20° C it is 20 μM (unionized concentrations), a difference in the same direction as, but smaller than, that for propofol.

TABLE 2. Half-maximal Effective Concentrations of Selected Anesthetics in Tadpoles

Anesthetic	EC_{50} Tadpole (μM)
Propofol	2.2
Thiopental[22]	30
Halothane[22]	230
Isoflurane[22]	290

EC_{50} = half-maximal effective concentrations.

Classical Predictors of Anesthetic Potency and Propofol

How well do classical predictors of anesthetic potency work with propofol? Ferguson found that most anesthetics, including volatile and intravenous anesthetics, cause anesthesia at constant thermodynamic activity, which may be approximated by the ratio of their EC_{50} in the aqueous phase to their solubility in water.[12] This ratio ranges for most clinical anesthetics, including the volatile anesthetics halothane and isoflurane or intravenous anesthetic methohexital, from 0.01 to 0.03. Propofol differs from most other anesthetics in that it has a lower ratio of anesthetic potency/water solubility of 0.002. Propofol is thus about ten times more potent than would be predicted from the Ferguson rule.[12]

Meyer[13] and Overton[14] were the first to correlate anesthetic potency with lipid solubility. They proposed that the anesthetic potency of a compound is determined by its solubility in lipid.[13,14] For this reason, the partition coefficient (the ratio of the concentration of a compound in the lipid phase to that in the aqueous phase) is a useful tool in describing and predicting the anesthetic potency of a compound determined as the concentration in an aqueous phase. Thus, the Meyer-Overton rule would predict that the product of the EC_{50} of an anesthetic and its partition coefficient (λ) is constant. For practical purposes, the octanol/water partition coefficient is used, because octanol provides a good model of the physicochemical properties of lipids in the nervous system.[15–17] The anesthetic potency of propofol is consistent with the Meyer-Overton rule, as indicated in figure 3a). Propofol lies close to the regression line drawn through the data points for a wide range of anesthetics, including gaseous, volatile, and intravenous anesthetics (solid line: slope = 1.2 ± 0.08), and also lies close to the theoretical line with a slope of −1 (dashed line). Comparison of the slopes shows that they are not significantly different from each other ($P > 0.02$). As shown in figure 3b, the anesthetic

¶ Schüttler J, Schwilden H, Stoeckel H: Pharmacokinetic-dynamic modeling of Diprivan (abstract). ANESTHESIOLOGY 65: A549, 1986.

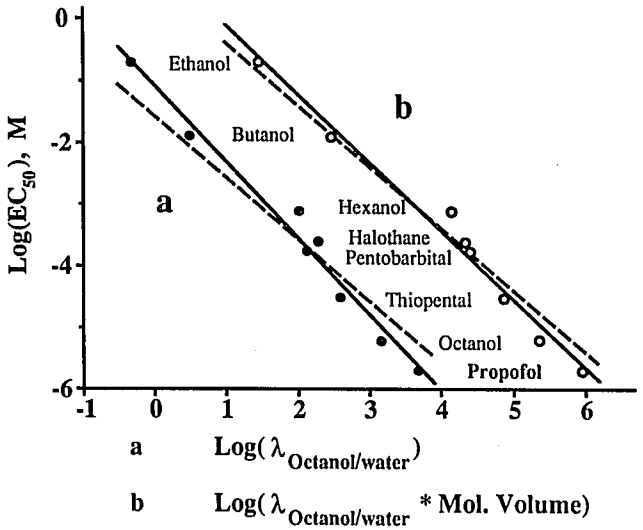

FIG. 3. The general anesthetic potency of propofol is consistent with the Meyer-Overton rule and the Critical Volume Hypothesis. *a:* The logarithms of EC_{50} values are plotted against the logarithm of their respective octanol/water partition coefficients (for source of data, see text). The solid line represents the line of best fit through the points (r = 0.99, slope = -1.2 ± 0.08). The dashed line shows the regression line in accordance with the Meyer-Overton correlation with a slope of -1. Comparison of the slopes with Student's *t* test showed that they were not significantly different ($P > .02$). *b:* The logarithms of EC_{50} values are plotted against the logarithm of their respective octanol/water partition coefficients corrected for molar volume ($\log\lambda_{octanol/water} \cdot$ molar volume). The solid line represents the line of best fit (r = 0.99, slope = -1.1 ± 0.06), and the dashed line shows a regression with the slope fixed to -1, in accordance with the Critical Volume Hypothesis.

potency of propofol also adheres to the critical volume hypothesis,[6] which predicts that the anesthetic potency is dependent on the octanol/water partition coefficient and the molar volume of a substance. The propofol point lies close to the regression line (slope -1.1 ± 0.06) and the theoretically predicted line. The difference between the slopes of these lines is also not significant ($P > 0.1$).

Pressure Reversal of Propofol-Induced Anesthesia

The anesthetic potency of propofol is reduced by the application of hydrostatic pressure. The value of the EC_{50} increases from 2.55 μM at 1 atm abs to 4.1 μM at 121 atm abs, as shown by the rightward shift of the concentration–response curve in figure 1. Figure 4 shows the increase in EC_{50} with increasing pressure, where the EC_{50} values are normalized to their respective EC_{50} values at 1 atm abs. The EC_{50} values of propofol and urethane increase linearly with pressure until 121 atm abs. The slope of the linear regression of the propofol data (slope = 0.0050 $\pm$ 0.00062 EC_{50}/atm abs) is significantly different from zero ($P < 0.001$), suggesting that propofol-induced anesthesia is reversed by hydrostatic pressure.

Comparison of the slopes of the regression lines for propofol and urethane (fig. 4) reveals that they are significantly different ($P < 0.0001$; urethane: slope = 0.0134 $\pm$ 0.00070 EC_{50}/atm abs). Propofol-induced anesthesia is about 2.7-fold less readily reversed by pressure than is urethane-induced anesthesia. Different levels of pressure reversibility of intravenous anesthetics have been reported before; only a few of these studies, however, examined the effect of hydrostatic pressure *per se* on the organism.[18,19] This is of importance because it has been shown that elevated pressures of gases such as hydrogen and helium have an intrinsic anesthetic effect when applied at pressure.[18] In a study involving tadpoles exposed to hydrostatic pressure, Halsey *et al.*[19] reported that the anesthetics Althesin (a mixture of alphaxalone/alphadolone in cremophor) and methohexital pressure-reversed nonlinearly up to pressures of 70 atm abs. At a pressure of 70 atm abs, the EC_{50} of Althesin was increased 4.1-fold compared to the EC_{50} at 1 atm abs. The EC_{50} of methohexital increased 6.9-fold at 70 atm abs. The increases in the EC_{50} values of propofol and urethane at 70 atm abs are 1.3 and 2.0, respectively. However, Halsey *et al.* reported that both Althesin and methohexital exhibited a plateau in their EC_{50} values for pressures greater than 70 atm abs,[19] an effect not observed in our study with propofol and urethane.

The difference between these results may be due to a variety of factors. Althesin was administered in a cremophor formulation and not in pure form, as was the

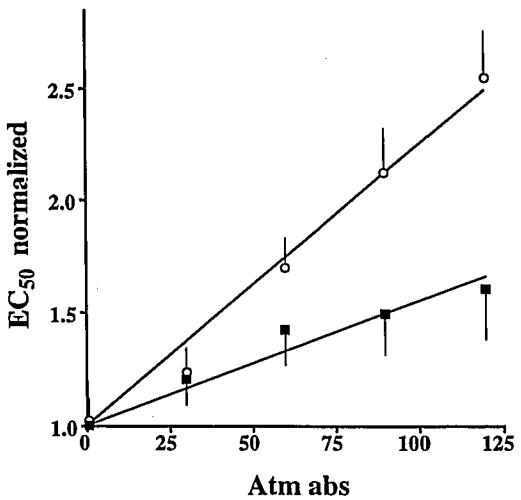

FIG. 4. Pressure reversal of propofol- and urethane-induced anesthesia. The EC_{50} values at different pressures are normalized to their EC_{50} at 1 atm abs and plotted against pressure (squares = propofol; circles = urethane). The data were fitted by linear regression. Because the intercept of the regression lines were not significantly different from 1, they were fitted with fixed intercepts of 1. The slopes of this analysis were significantly different ($P = .0001$).

propofol in this study. The *p*H of the solution containing methohexital was not checked during the pressure experiments. It is known that pressure can change the ionization of a charged molecule and consequently affect its anesthetic potency.[20] It is very unlikely that the differences in pressure reversibility were caused by the effect of pressure on the partition coefficient of the compounds because the difference in partial molar volumes of anesthetics in water and in lipid bilayers is generally too small.[15] However, the important point is that the anesthetic potencies of all of these drugs were reduced by pressure.

Propofol Protects Against the High-Pressure Neurologic Syndrome

Figure 2 shows the response of tadpoles that were exposed to fixed concentrations of propofol. All of the tadpoles exposed to 0, 0.1, and 3.0 μM responded at 91 atm abs. Of the tadpoles not exposed to propofol, 40% did not respond at 121 atm abs, whereas tadpoles exposed to 0.1 and 3 μM propofol first showed a decrement in response at 151 and 211 atm abs, respectively. The description of the behavior of the tadpoles with increasing pressure closely resembles the symptoms of the high-pressure neurologic syndrome. Typically, the high-pressure neurologic syndrome involves an initial excitation phase, with a further pressure increase leading to spontaneous muscle contractions and paralysis and finally death.[21] The reported findings show that propofol delays the onset of the pressure paralysis, indicating that it protects against the high-pressure neurologic syndrome as do most other anesthetics to varying degrees.[18,19,21]

This study shows that propofol behaves as a typical general anesthetic in that its anesthetic potency can be predicted according to the Meyer-Overton rule and in that propofol-induced anesthesia is reversed by high applied hydrostatic pressure.

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