Pressure Antagonism to Nerve Conduction Block by Anesthetic Agents

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As an antagonist to anesthesia, hyperbaric pressure is a promising tool for investigating anesthetic mechanisms. We have used it to test the hypothesis that local and general anesthetics may share a common pressure-sensitive site of action in the nerve cell membrane. Excised rat preganglionic sympathetic nerves were equilibrated with one of a series of anesthetic agents at a concentration calculated to depress the amplitude of the compound action potential by approximately 25 per cent. Agents tested were halothane (1 molar); methoxyflurane (2.5 molar); lidocaine (0.01 molar); procaine (0.01 molar); benzoate (1 molar); a quaternary lidocaine derivative, QX572 (0.1 molar); tetrodotoxin (TTX) (2 x 10⁻⁵ molar); and the spin-labeled molecule, TEMPO (5 molar). Drug-treated nerves were exposed to helium pressure over the range from 1 to 137 atmospheres absolute (ATA).

Compression increased the amplitudes of the compound action potential in nerves treated with halothane, methoxyflurane, lidocaine, benzoate, or TEMPO (P < 0.01), but not those treated with procaine, QX572 or TTX. These results support the hypothesis that general anesthetics and uncharged hydrophobic local anesthetics block conduction in part by an action on a common pressure-sensitive membrane site, possibly the lipid bilayer. (Key words: Hyperbaria, pressure reversal; Anesthesia, local; Theories of anesthesia.)

Compressing to pressures above 100 atmospheres absolute (ATA) restores locomotion and orienting reflexes to animals anesthetized at one atmosphere.¹⁻² The excellent correlation between lipid solubility and potency among the volatile general anesthetic agents strongly supports the hypothesis that general anesthetics act on a hydrophobic component of nerve cell membranes, either the lipid bilayer or hydrophobic regions in membrane proteins. Anesthetics have been shown to increase membrane fluidity.³ In phospholipid bilayers, volatile general anesthetics increase lipid fluidity,⁻⁴⁻⁵ lower lipid phase-transition temperatures,⁻⁶ and distort the envelope of gel–sol phase separation in a binary lipid membrane.⁻⁷ Hyperbaric pressure antagonizes the first two effects but not the third.⁻⁸ Local anesthetics also appear to follow the Meyer-Overton correlation between potency and lipid solubility⁻⁹⁻¹⁰ and have been shown to exert a fluidizing effect on membrane lipids.⁻¹¹⁻¹² Thus, the possibility that volatile inhalation agents and local anesthetics may share a common hydrophobic site of action in the membrane suggests itself.

Conduction block by inhalation agents has been shown to be antagonized by pressure.⁻¹²⁻¹³ If it is true that the more hydrophobic local anesthetics act in a fashion similar to inhalation agents, it may be predicted that conduction block by local anesthetics will also be antagonized by pressure. Since there is excellent evidence that even permanently charged hydrophilic compounds are effective local anesthetics when applied to the inner (axoplasmic) surface of the nerve cell membrane,⁻¹⁴ a nonspecific action on membrane lipids can not be considered an exclusive basis for local anesthetic conduction block. It may therefore be further predicted that the extent of pressure antagonism will be correlated with hydrophobicity, and will thus be greatest for a permanently neutral compound such as benzoic acid and for agents with relatively low pK's, in which the uncharged base constitutes a significant fraction of the molecules present at physiologic pH.

To test the hypothesis that neutral local anesthetics and volatile general agents may act at the same site, the interactions between hyperbaric pressure and conduction block were explored for a range of drugs. These included the volatile inhalation agents halothane and methoxyflurane; the permanently uncharged local anesthetic benzoic acid; procaine and lidocaine, two tertiary amines with different pK values; the permanently charged quaternary amine, QX572; and the specific sodium channel-blocking agent tetrodotoxin (TTX). The spin-labelled hydrophobic molecule TEMPO was also tested, since it had been used earlier as a model anesthetic in lipid bilayer studies.⁻¹⁵

Methods

The preganglionic sympathetic nerve of the superior cervical ganglion was dissected from adult male Sprague-Dawley rats under pentobarbital anesthesia. The nerve was left intact in all experiments except those with tetrodotoxin, an agent for which desheathing is necessary to allow equilibration in a reasonable length of time. Experiments on desheathed nerves were repeated with lidocaine in order to ensure that injury consequent to removing the sheath did not alter the response of the nerve to pressure. Recording of the compound action potential and application of pressure were carried

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out as described in a previous report. The nerve was mounted on extracellular stimulating and recording electrodes immersed in a 100-ml volume of Krebs' solution. Pressure was applied by placing the entire bath in a stainless steel chamber with provision for electrical connections, sealing the chamber, and admitting helium from a high-pressure cylinder. All experiments were carried out in Krebs' bicarbonate buffer at pH 7.4; control studies demonstrated that compression to 200 atm reversibly decreased pH to 7.2. Temperature was maintained at 37°C throughout.

Drugs used were halothane (1 mM); methoxyflurane (2.5 mM); lidocaine (0.4 mM); procaine (0.4 mM); benzocaine (1 mM); QX312 (0.1 mM); tetrodotoxin (2 × 10⁻⁶ M) and TEMPO (5 mM). Concentrations were chosen to produce decreases of approximately 25 percent in amplitude of the compound action potential. Drugs were administered by diluting stock solutions to the appropriate concentrations in oxygenated Krebs' solution. Tests with pressure were made when the block was stable as indicated by identical records taken 5 minutes apart.

Experiments were carried out on 4–7 nerves with each anesthetic. Differences between controls, drug-treated nerves at 1 atm, and drug-treated nerves under pressure were analyzed by a t test for paired data.

Results

Compressional Effects on the Untreated Nerve

As in our previous studies, compression to 137 ATA had little effect on the amplitude of the compound action potential of the preganglionic sympathetic nerve. Above 137 ATA, there was a tendency for amplitude to decrease. For this reason, and because previous studies had suggested that pressure antagonism was maximal at 100 ATA, comparisons between agents were made at approximately the latter pressure.

Compression to this level did exert a distinct effect on the latency and duration of the response (fig. 1). Both latency and duration of the compound action potential are increased at high pressure, indicating that conduction velocity is slowed, and perhaps that the duration of individual action potentials are also lengthened.

Pressure and Response Amplitude in Drug-Treated Nerve

Compression to 103 ATA enhanced the amplitude of the compound action potential in nerves treated with 25 percent blocking concentrations of halothane, methoxyflurane, lidocaine, benzocaine, or TEMPO (P < 0.01). Pressure did not significantly change action potential amplitude in nerves partially blocked by procaine, QX312, or tetrodotoxin (fig. 2). Among the agents whose blocking actions were antagonized by pressure, there was no significant difference in the extent of pressure antagonism. The antagonism to block in most nerves was only partial; with an average drug-induced depression of the action potential 75 percent of control, the average restoration at 100 ATA with, e.g., methoxyflurane, was to approximately 85 percent of the control value (fig. 2). An example of partial conduction block by benzocaine and its alleviation by pressure is shown in figure 3.

Temporal Variables: Additive Slowing of Conduction by Both Drugs and Pressure

All drugs tested increased the latency and duration of the response. Exposure to hyperbaric pressures also increases latency and duration, and the effects appear additive (figs. 3 and 4). The increases in latency with blocking agents averaged about 15 percent, and did not differ significantly among agents. Pressure of 103 ATA added an average of 7 percent to the measured latency, and again there was no significant difference among agents or between intact and drug-treated nerves.

Pressure to 103 ATA increased the duration of the response, whether measured at the baseline or at half-maximal amplitude. Conduction-blocking agents also increased duration and added to the effect of pressure on this variable. Again, there appeared to be no difference among agents or between partially blocked and normal nerves.

Discussion

Pressure significantly increases the amplitude of the action potential in nerves partially blocked by
Fig. 2. Effects of 103 ATA pressure on compound action potential amplitude in drug-treated nerves. Bar graph is drawn as percentage of amplitude at 1 ATA; each is the average for 4–12 nerves. Pressure increased amplitudes of responses in nerves partially blocked by halothane, methoxyflurane, benzocaine, lidocaine, or TEMPO (P < 0.05). There was no significant amplitude change in untreated nerves or nerves partially blocked by procaine, QX572, or TTX.

general anesthetics. Pressure also antagonized block by TEMPO, a small lipid-soluble molecule. This antagonism also holds for benzoceaine, which is a completely uncharged local anesthetic, and for lidocaine, which at this pH consists of approximately 25 per cent uncharged base.\textsuperscript{16} It apparently does not hold for procaine, which at a pH of 7.4 is only 3 per cent uncharged base,\textsuperscript{16} or the experimental compound QX572, a quaternary compound with no base counterpart. Nor does it appear to be true for tetrodotoxin, a specific sodium channel-blocking agent. The experiments with tetrodotoxin were carried out on desheathed nerves. It is possible that injury consequent to desheathing may have influenced the results with this agent. However, pressure antagonism to lidocaine block of desheathed nerves could be readily demonstrated.

Neutral versus cation action of local anesthetics is a long-standing problem in pharmacology. Data that demonstrate increased potency of local anesthetics at low pH suggest that the cation is an effective blocking agent,\textsuperscript{17,18} as do studies showing that quaternary derivatives block sodium currents in a manner similar to tertiary local anesthetics.\textsuperscript{14} On the other hand, the correlation between lipid solubility and local anesthetic potency, the effectiveness of benzoceaine in blocking conduction, and the lipid-disordering effects of local anesthetics point to a hydrophobic nonpolar site of action. The apparently conflicting evidence is ably summarized in two excellent recent reviews.\textsuperscript{19,20} The evidence strongly suggests that local anesthetics act on a site reached from the inner axoplasmic surface of the membrane. Since tetrodotoxin is ineffective when

Fig. 3. Effects of 103 ATA pressure and the local anesthetic benzoceaine on neural response. Pressure partially restored the amplitude, but added to the increase in latency and duration produced by benzoceaine.
applied to the inner surface, and does not compete with local anesthetics for binding sites, it is presumed to act at a different membrane site.

In summary, pressure antagonized blocks produced by the more hydrophobic neutral agents but not by the more hydrophilic charged agents. These results support the hypothesis that the lipid-soluble base forms of local anesthetics may exert a conduction-blocking effect similar to that of volatile anesthetics. One would have preferred to see some gradation of antagonism, with lidocaine occupying an intermediate position in its response to pressure. This was not detected in our results. It may be that the relatively limited extent of pressure antagonism observed with all blocking agents in this preparation obliterated small differences between agents.

In our hands, pressure antagonism to conduction block as measured by the compound action potential was never better than partial. The slowing effect of pressure on conduction may have minimized the apparent increase in peak amplitude. Under the conditions of the study, the fibers of smaller diameter were the ones blocked and thus subject to pressure restoration of conduction. They would also be the slowest-conducting and their contribution thus relatively late in the compound response. Greater antagonism has been reported to occur when nerves are exposed to inhalational agents in the vapor phase, rather than dissolved in solution as in the present study. However, in the latter study no antagonism to any agent given in solution was observed. The significance of this difference remains to be resolved.

As was the case with the other neutral hydrophobic molecules, TEMPO conduction block was also antagonized by pressure. A different result has been reported by others. We have no explanation for the discrepancy.

Both pressure and blocking agents exerted clear slowing effects on conduction, and appeared to interact additively. A decrease in speed of events is a consistent observation in pressure studies. The action potential of individual nerve fibers is broadened because of a decrease in the velocity of the membrane permeability changes that underlie the action potential. Intuitively these observations are consistent with the concept that an increase in pressure is in part equivalent to a decrease in temperature.

With respect to pressure antagonism of anesthesia itself, the phenomenon has now been reported for a wide variety of anesthetic agents, including inert gases, clinical volatile agents, barbiturates, and in recent work by Halsey, local anesthetic agents. At the molecular level, the basis established by the work on artificial lipid membranes is satisfyingly economical. Anesthetic-expanded protein components would also be subject to pressure-induced restoration and thus provide a molecular basis for “pressure reversal.” In erythrocyte studies membrane expansion by anesthetics is of the same order as the calculated membrane compression imposed at anesthesia-reversing pressures.

At the cellular level, the present work confirms hyperbaric antagonism to conduction block by a number of anesthetic agents. The limited extent of the antagonism raises the question whether by itself this phenomenon can be the cellular basis for “pressure reversal of anesthesia.” Pressure failed to antagonize the effects of local anesthetics on latency and duration of the response; in fact, it added to the slowing effects of local anesthetics. These temporal properties are surely important factors in information processing within the nervous system. This result adds to our growing awareness that “pressure reversal of anesthesia” is not a straightforward restoration of anesthetic-expanded or anesthetic-disordered membranes to their preanesthetic “null” state and that the awake animal at 100 ATA with anesthetic on board is not the same as the awake animal at 1 ATA without anesthetic.

References


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