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BRIEF COMMUNICATION

Interactions of thiopentone, light mineral oil, and pressure at frog sciatic nerve

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Armon C, Kerem D. Interactions of thiopentone, light mineral oil, and pressure at frog sciatic nerve. Undersea Biomed Res 1981; 8(2):121–126.—Combinations of thiopentone, light mineral oil, and pressure to as much as 160 ATA were applied to the isolated, thermostated (18°C) frog sciatic nerve. Amplitude and rise rate of the compound action potential were examined and compared to the response in Ringer's solution. Immersion in mineral oil caused no change in the response. Pressure, whether applied by helium or after immersion in mineral oil, caused mainly reversible slowing of the response, as did thiopentone by itself, up to at least 5.1 m*M*. Thiopentone (3.2 m*M*) and helium pressure up to 114 ATA were reversibly synergistic, in that the response was markedly slowed and reduced. Nerves that had been immersed in 3.2 m*M* thiopentone were placed in mineral oil; this procedure caused a reduction in the size of the response. Application of pressure then eliminated the response, and decompression did not lead to its recovery until the nerve was repeatedly rinsed in Ringer's solution. The findings question the inertness of mineral oil to excitable membranes and indicate that caution should be exercised in its use as a compression medium.

thiopentone
pressure-anesthetic interaction
compression medium

Thiopentone sodium is a potent anesthetic used for the induction and maintenance of general anesthesia (1), as an anticonvulsant (1, 2), and in advanced brain resuscitation (3). We decided to study the interaction of this anesthetic with pressure at the frog sciatic nerve. In some experiments the pressure was provided by helium, whereas in others light mineral oil served as a compression medium. The apparent augmentation by the latter of the thiopentone-induced depression of neural activity, both at atmospheric and at high pressures, prompted this communication.

MATERIALS AND METHODS

The sciatic nerves were isolated from the frog *Rana ridibunda*, immersed in Ringer's solution (NaCl, 115 m*M*; KCl, 2.5 m*M*; $CaCl_2$, 2.0 m*M*; Tris buffer, 2.5 m*M*; pH 7.4) and allowed to equilibrate for at least 1 h. Sodium thiopentone solutions were freshly prepared by dissolving the powder (Pentothal, Abbott Labs.) in Ringer's solution to the required concentrations. Nerves exposed to thiopentone were placed in these solutions for 15 min.

After equilibration the nerves were removed from the solution, threaded through four external annular silver electrodes, and tied in place. They were then placed in a small pressure vessel that contained either air saturated with water vapor or liquid mineral oil (analytical grade, Merck), which was not intentionally aerated. A thin film of the equilibration solution served in both instances to make continuous contact with the electrodes. Compound action potentials (CAP) in response to stimuli applied by two of the four electrodes were recorded from the other two, displayed on an oscilloscope together with their electronically produced derivatives, and photographed with a polarographic camera (Tektronix).

Compression and decompression between stations was continuous at a rate of 1 ATA/min, and was affected by helium applied either directly or over the liquid mineral oil (LMO). The sequence of stations was: 1) 1 ATA; 2) maximal pressure (to as much as 160 ATA); 3) intermediate pressure; 4) 1 ATA. At each station the pressure was held constant for at least an hour until the temperature stabilized at a predetermined control value of 18°C ± 0.03°C and until there was no discernible change in the CAP, at which time it was recorded.

Temperature control was achieved by a thermostated water bath around the vessel, with an input from a thermistor sensitive to changes of 0.008°C, placed next to the nerve. Compression heat during LMO immersion was minimal and was markedly reduced in helium compression by cooling the helium cylinder to 4°C. In trials preceding the experiments reported here, the preparation was shown to be stable for more than 24 h in both vapor-saturated air and LMO at temperatures up to 30°C. These limits were not approached in our experiments.

RESULTS

The experimental design and choice of controls yielded the effects of all eight (2^3) qualitative combinations of thiopentone, LMO, and pressure on the CAP of at least two nerves per combination. These effects are listed in Table 1.

Details of the changes in several characteristics of the responses under some of these conditions are presented in Fig. 1. The overshoots of B,d and E,d in the figure did not exceed 1.5, were not apparent in all preparations, and were not accorded any particular significance.

DISCUSSION

If not exposed concurrently to thiopentone, the frog sciatic nerve did not change its response upon immersion in liquid mineral oil. Pressure, whether applied by helium directly or to the nerve immersed in liquid mineral oil, caused mainly slowing of the response. This has been described previously for single fibers (4–6) as well as for compound nerves (7). Thiopentone up to at least 5 m*M* caused mainly slowing of the response, which was reversible after repeated rinsing in Ringer's solution. Most experiments were done at concentrations of 3.2 m*M*. These concentrations are about ten times higher than that used for general anesthesia (1) or to control convulsions in humans (2), about twice as high as that used to narcotize tadpoles (reversible

TABLE 1

POSSIBLE COMBINATIONS OF THIOPENTONE, LIGHT MINERAL OIL, AND PRESSURE: EFFECTS ON COMPOUND ACTION POTENTIAL OF FROG SCIATIC NERVE

No.	Thiopentone Concentration	Immersion in Mineral Oil	Pressure	Qualitative Effect	Relevant Part of Fig. 1
1	0	No	No	Base line	
2	0	Yes	No	Identical to base line	
3	0	No	Yes (helium)	Mainly reversible slowing of response	A
4	0	Yes	Yes	Mainly reversible slowing of response (similar to No. 3)	B
5	up to 5.1 m*M*	No	No	Mainly reversible slowing of response	D
6	3.2 m*M*	Yes	No	Reduction in size, additional to No. 5	E,a–c
7	3.2 m*M*	No	Yes (helium)	Reversible synergism of Nos. 3 and 5, leading to marked reduction in size in addition to slowing of response	C
8	3.2 m*M*	Yes	Yes	Elimination of response, persisting after pressure is removed	E,d
				Recovery of response possible by repeated rinsing of the decompressed nerve in Ringer's solution	

on application of pressure) (8), and of the order of magnitude used in the initial stage of brain resuscitation (3). The changes produced by the lower concentrations were slight. The synergism between 3.2 m*M* thiopentone and helium pressure suggests that the activity of thiopentone at this concentration is similar to that of a charged local anesthetic (7).

We did not expect the additional reduction in the size of the CAP caused by immersing the thiopentone-exposed nerve in LMO, nor its subsequent gradual elimination upon the addition of pressure, which was not reversible merely by removing the pressure. Indeed, this was the only instance in which a certain change in the experimental conditions was not readily reversible upon restoration of conditions before the change.

Most possible explanations of these phenomena—such as the low Po_2 of the LMO, impurities in the LMO or thiopentone, interaction of some formed metabolite with the applied agents—could be ruled out, since the effects were not apparent in any of the other combinations.

We were left with two most likely possibilities, which could only be verified by further experimentation.

1. The interface between LMO and Ringer's solution and a very thin film of Ringer's solution could alter the distribution or orientation of the thiopentone molecules, or both, and thus change the immediate milieu of the nerve (i.e., local pH changes).

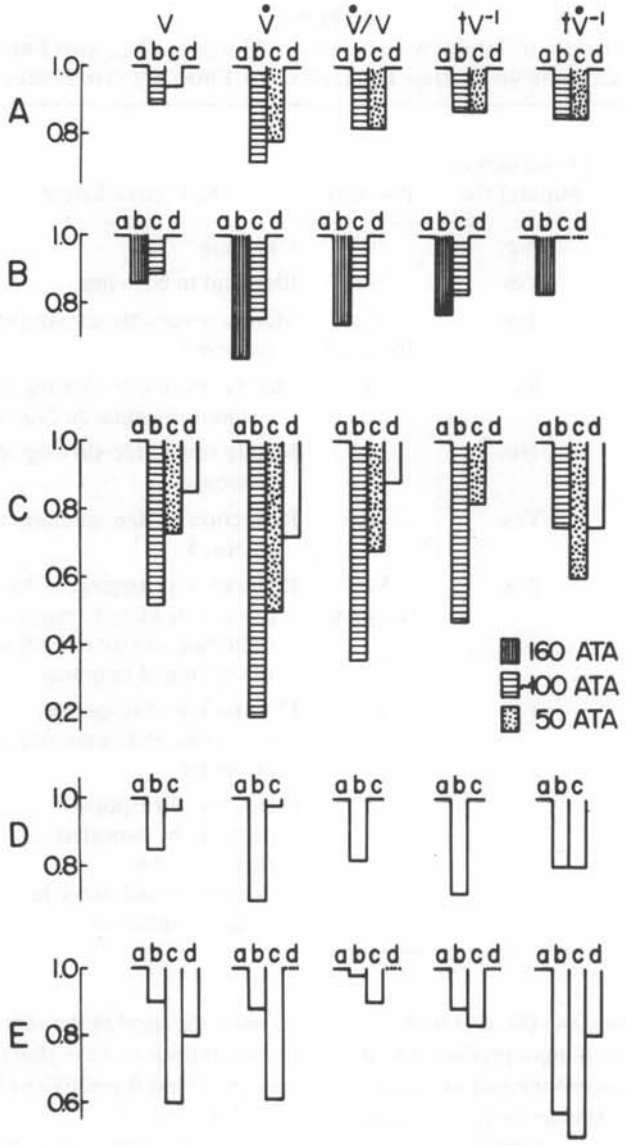

Fig. 1. Interactions of thiopentone, light mineral oil, and pressure, and their effects on several characteristics of compound action potential of frog sciatic nerve. Each row (A, B, C, D, E) is an experiment on a single nerve. A. Effect of helium pressure: a, 1 ATA air; b, 100 ATA helium; c, 50 ATA helium; d, 1 ATA helium. B. Immersion in light mineral oil at following pressures: a, 1 ATA; b, 160 ATA; c, 100 ATA; d, 1 ATA. C. Immersion in 3.2 m*M* thiopentone: a, 1 ATA air; b, 114 ATA helium; c, 51 ATA helium; d, 1 ATA helium. D. Nerve at 1 ATA in various substances: a, Ringer's solution; b, 5.1 m*M* thiopentone; c, Ringer's solution, after multiple rinsing. E. Nerve at 1 ATA: a, in Ringer's solution; b, in 3.2 m*M* thiopentone; c, in 3.2 m*M* thiopentone during immersion in light mineral oil; d, reappearance of response after decompression and multiple rinses in Ringer's solution, following its gradual elimination by compression. V, maximal value of compound action potential; $\dot{V}$, maximal value of derivative; tV^{-1}, time of rise to V^{-1}; $t\dot{V}^{-1}$, time of rise to $\dot{V}^{-1}$. Effects are depicted as reductions relative to the initial condition in each instance (a, defined as 1.0). · · · ·, Values greater than 1.0, meaning an "overshoot" of recovery values. Compression and decompression at 1 ATA/min.

2. Thiopentone somehow allowed the LMO to act as an uncharged local anesthetic that mainly reduces the size of the CAP (7). Ordinarily, long-chain alkanes cannot gain access to the sites whereby uncharged anesthetics act.

Further clarification could be gained by testing other nonaqueous liquids such as silicone oil and local anesthetics other than thiopentone. The addition of pressure and its role in complete elimination of the CAP further complicated the picture. Simple synergism with the anesthetic should have been reversible, as with helium pressure.

A recent study used mineral oil as a compression medium to study pressure anesthesia interaction on invertebrate neurons (9). Although the volume of Ringer's solution around the preparation was larger (e.g., 5 ml), an effect similar to what we found may bear on the failure of pressure to antagonize or otherwise alter the anesthetic (halothane) effect.

These results add to the increasing number of instances where pressure does not reverse anesthetic actions on excitable tissue, question the use of mineral oil as an inert compression medium in conjunction with at least one anesthetic, and may provide some insights into mechanisms of excitability and anesthesia.

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Armon C, Kerem D. Phénomènes d'interaction entre la thiopentone, les huiles minérales légères et la pression sur le nerf sciatique de la grenouille. Undersea Biomed Res 1981; 8:(2):121–126. — Les huit combinaisons qualitatives possibles de thiopentone, d'huiles minérales légères, et de pression atteignant 160 ATA furent appliquées au nerf sciatique isolé, thermostaté (18°C) de la grenouille. L'amplitude et le taux d'augmentation du potentiel d'activité de l'élément furent analysés et comparés à la réponse dans la solution de Ringer. L'immersion dans l'huile minérale ne provoqua aucun changement dans la réponse. La pression, qu'elle soit appliquée par l'hélium ou après l'immersion dans l'huile minérale, engendra surtout un ralentissement réversible de la réponse, comme l'avait fait seule la thiopentone, jusqu'à au moins, 5,1 m*M*. La thiopentone (3,2 m*M*) et la pression d'hélium jusqu'à 114 ATA furent réversibles synergiquement, en ce que la réponse était beaucoup plus ralentie et diminuée. Les nerfs qui avaient été immergés dans 3,2 m*M* de thiopentone furent placés dans de l'huile minérale; cette procédure provoqua une diminution de la dimension de la réponse. L'application de cette pression élimina ensuite la réponse, et la décompression n'entraîna pas sa restitution avant que le nerf ne fût lavé constamment dans une solution de Ringer. Les résultats remettent en cause l'inertie de l'huile minérale aux membranes excitables et ceux-ci démontrent que la prudence est de rigueur quant à son application comme milieu de compression.

la thiopentone
interaction entre la pression et l'anesthésie
le milieu de compression

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