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THE EFFECTS OF ETHANOL AND CHLORPROMAZINE ON THE PASSIVE MEMBRANE PERMEABILITY TO Na^+

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SUMMARY

1. Chlorpromazine and ethanol each reduced the passive influx of Na^+ into resting sartorius muscle (frog) incubated in a medium containing 1 mM Ca^{2+}, ouabain (to preclude active fluxes) and isotonic choline chloride (to preclude membrane depolarization).

2. Chlorpromazine also reduced the passive influx of Na^+ into human erythrocytes incubated in the presence of 1 mM Ca^{2+}, ouabain and isotonic choline chloride. In the absence of Ca^{2+}, however, chlorpromazine increased the passive influx of Na^+ into the erythrocytes. Ethanol (up to 1 M) did not alter the passive Na^+ influx in erythrocytes.

3. It is hypothesized that chlorpromazine and ethanol reduced the passive Na^+ influx in muscle by expanding the sarcolemma, not by altering the surface charge of this membrane.

INTRODUCTION

It is known that anesthetics reduce the rate of rise of the action potential of excitable membranes[1-4]. This is generally interpreted as meaning that the anesthetic reduces the electrical conductance of the action potential associated with the entry of Na^+ into the cell[1-4] in accordance with the HODGKIN–HUXLEY[5] hypothesis.

There is no direct chemical evidence, however, that anesthetics actually reduce the passive influx of Na^+ during the action potential. SHANES AND BERMAN[6] found that cocaine depressed the passive efflux of K^+ in resting (*i.e.* non-stimulated) sciatic nerves of the toad. In order to do these experiments on the passive flux, SHANES AND BERMAN[6] suppressed the active ion fluxes by the simultaneous use of anoxia and high concentrations of iodoacetate. As a result of that work and other experiments indicating that local anesthetics penetrate lipid monolayers[7], SHANES[8] developed the hypothesis that all local anesthetics reduce the passive cation permeability of the membrane by forming a "diffusion-limited subfilm" adjacent to the cell membrane. Although the phenothiazine local anesthetics are present in the membrane at concentrations high enough (30 mmoles/kg dry membrane; see ref. 9) to form such a subfilm or a monolayer (having 150 A^2 of membrane per molecule; see refs. 10, 11), there is no evidence that this high membrane concentration actually limits passive diffusion across the membrane.

In order to test SHANES' hypothesis of reduced passive permeability, the effects of chlorpormazine (a potent local anesthetic[9,10]) and ethanol were tested on the passive Na^+ influx of resting skeletal muscle and human erythrocytes, using ouabain to preclude the active Na^+ fluxes[12]. The results indicate that chlorpromazine reduced the passive influx of Na^+ in both muscle and erythrocytes, if choline was used to prevent depolarization of muscle fibers[13]; ethanol reduced the influx in muscle only.

METHODS

The passive influx of $^{22}Na^+$ into human erythrocytes

Identical results were obtained using erythrocytes from fresh venous heparinized blood[14] and from Red Cross blood which had been stored in acid–citrate–dextrose for 1 month. The stock erythrocyte suspension consisted merely of packed erythrocytes, having a hematocrit of 80 % and which had been washed once in isotonic choline chloride, containing 10^{-4} M ouabain, in 15 mM Tris–HCl buffer (pH 7).

A radioactive stock solution containing 1.25 μC/ml was prepared by dissolving 0.1 mC of $^{22}Na^+$ (Atomic Energy of Canada, Chalk River, Ontario) in 4 ml of 15 mM Tris–HCl (pH 7) and then adding 1 ml of this solution to 19 ml of ouabain–choline medium (isotonic choline chloride, containing 10^{-4} M ouabain, in 15 mM Tris–HCl buffer, pH 7).

The experimental procedure was to mix 1.0 ml of the $^{22}Na^+$ stock solution, 0.5 ml of ouabain–choline solution containing different chlorpromazine or ethanol concentrations, and 0.5 ml of the packed erythrocytes. The contents were incubated at room temperature (23°) for 90 min, and then centrifuged for 5 min at 1500 × *g*. The erythrocytes were washed 3 times with ice-cold ouabain–choline solution, the aliquot of each wash being 3 ml and the total time for each wash being about 4 min. The washed erythrocyte pellets were then counted directly in a γ-counter (Picker-Nuclear or Chicago Nuclear instruments). Of the various methods which have been used to determine the intracellular Na^+ content of erythrocytes, the procedure of washing with choline chloride has been shown to give reliable results[15,16]. The passive influx of Na^+ in the presence of drug was expressed as a percentage of the influx of Na^+ in the absence of any drug.

Chlorpromazine·HCl was kindly donated by Poulenc Laboratories, Montreal.

The passive influx of $^{22}Na^+$ into frog sartorius muscles

Frogs were pithed and both sartorius muscles were carefully removed from origin to insertion without cutting into the muscle itself. The muscles were kept in frog Ringer's solution (ref. 17) at room temperature. Immediately before starting the experiment the muscles were washed once in the Ringer solution used for that experiment.

The following were added into each 16 mm × 100 mm glass test tube: (1) 0.2 ml of 1.25 μC/ml of $^{22}Na^+$ dissolved in frog Ringer with 10^{-4} M ouabain; (2) 0.2 ml of drug dissolved in frog-Ringer with 10^{-4} M ouabain; (3) 0.2 ml of frog-Ringer with 10^{-4} M ouabain; (4) one frog sartorius muscle which had been gently blotted and weighed.

The test tubes were capped and incubated in a shaker at room temperature for 3 h. The muscles were then rinsed twice in Ringer's solution where all the Na^+ had

been replaced by choline (choline–Ringer). The muscles were blotted and then placed into empty glass test tubes for γ-counting in a Nuclear Chicago γ-counter.

The most consistent and reproducible results were obtained by comparing the passive uptake of $^{22}Na^+$ by one muscle with that by the contralateral sartorius of the same frog. The results are expressed, therefore, as

$$\frac{^{22}Na^+ \text{ (muscle + drug, in disint./min per g muscle)}}{^{22}Na^+ \text{ (contralateral muscle control, in disint./min per g muscle)}}$$

The average of these ratios are presented in the results.

RESULTS

Effects of ethanol and chlorpromazine on the passive influx of Na^+ in frog muscle

Figs. 1 and 2 show that high concentrations of ethanol and chlorpromazine increased the passive influx of Na^+ in frog sartorius when frog Ringer's solution was used. In choline–Ringer, however, which prevents membrane depolarization[13], these compounds reduced the passive influx of Na^+ at all drug concentrations. The ethanol concentrations chosen (Fig. 1) are those which cause local anesthesia, the threshold for ethanol being between 600 and 1000 mM (ref. 19). The muscles weighed the same (within 5 %) before and after exposure to ethanol, indicating that the ethanol had equilibrated across the sarcolemma and did not act as a hyperosmolar solution to withdraw water from the muscle (refs. 10 and 11 cite further references on the rapidity of transmembrane alcohol equilibration).

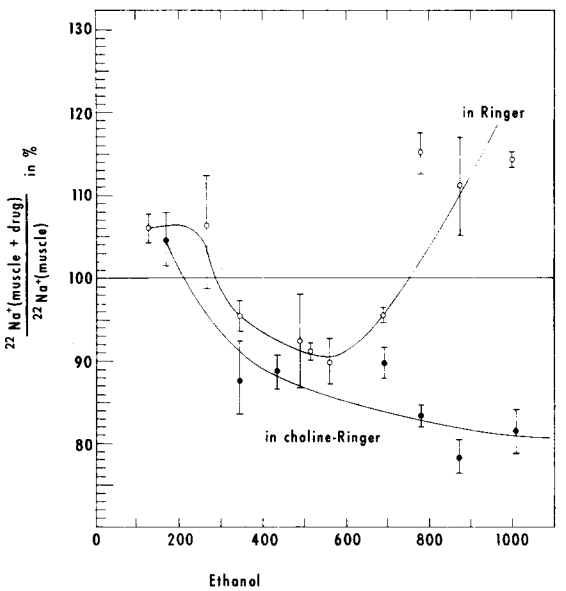

Fig. 1. Effect of ethanol (mM) on passive influx of Na^+ into sartorius (in presence of ouabain). Low concentrations of ethanol reduced the passive Na^+ influx, and high concentrations increased the passive Na^+ influx in frog sartorius muscle in Ringer's medium containing 10^{-4} M ouabain. In choline–Ringer–ouabain, where Na^+ has been replaced by choline, ethanol depressed the passive Na^+ influx at all concentrations. Vertical bars indicate standard error for 6–10 muscle pairs.

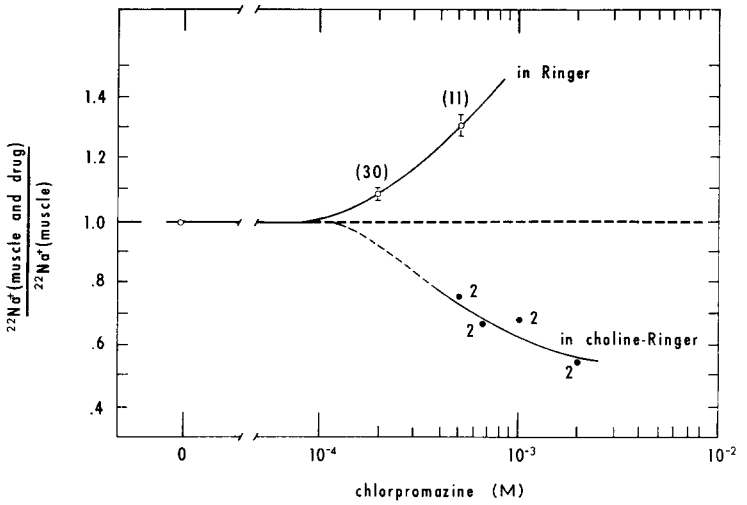

Fig. 2. Effect of chlorpromazine on passive influx of Na^+ into sartorius (ouabain present). Chlorpromazine decreased the passive Na^+ influx in muscles suspended in choline–Ringer–ouabain, but increased the influx in NaCl–Ringer–ouabain medium. The numbers indicate the number of muscles used. Vertical bars are standard errors.

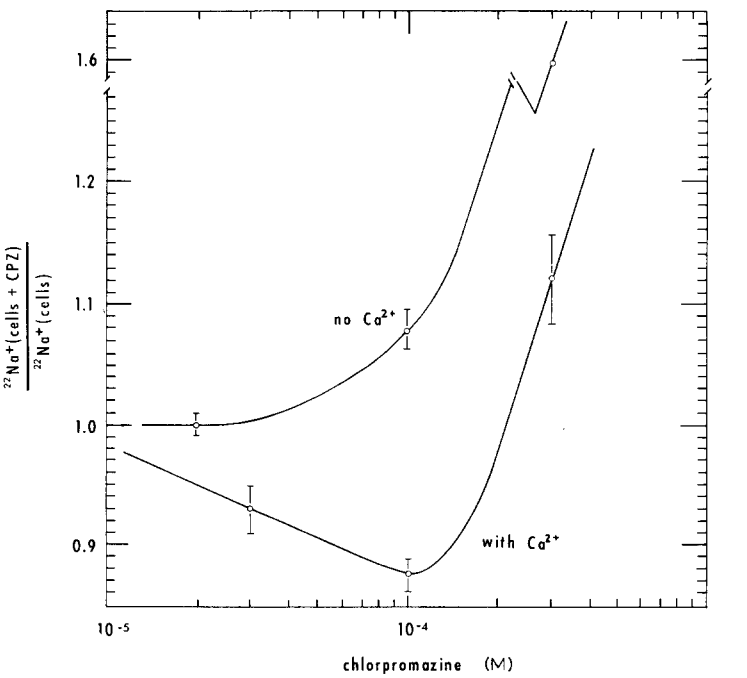

Fig. 3. Human erythrocytes. Chlorpromazine decreased the passive Na^+ influx, when the cells were suspended in isotonic choline chloride, 10^{-4} M ouabain and 1 mM Ca^{2+}. In the absence of Ca^{2+} chlorpromazine increased the passive Na^+ at all drug concentrations. The vertical bars indicate standard deviation.

Effects of ethanol and chlorpromazine on the passive influx of Na^+ into erythrocytes

In the presence of Ca^{2+} and ouabain, chlorpromazine also reduced the passive Na^+ influx in erythrocytes (Fig. 3). If Ca^{2+} was omitted, however, chlorpromazine increased the passive Na^+ influx. Although the concentrations of chlorpromazine appear to be rather high (Fig. 3), it is known that the majority of the chlorpromazine molecules adsorb to the erythrocytes[9,10,18]. Because of the high hematocrit (20–25 % final), the final free concentrations of chlorpromazine were about 10 % of the concentration values plotted in Fig. 3.

Very high concentrations of chlorpromazine increased the passive Na^+ influx whether or not Ca^{2+} was present. This is in accordance with the hemolytic effects of this surface-active drug; these invariably occur in the concentration region approaching 10^{-3} M. These lytic effects have been thoroughly reviewed[10].

Ethanol in concentrations up to 1 M did not depress the passive influx of Na^+ in the erythrocytes, regardless whether Ca^{2+} was present or not.

DISCUSSION

The main observation was that the passive influx of Na^+ in frog sartorius muscle was reduced by ethanol and by chlorpromazine, under conditions where the active transport of Na^+ was precluded by ouabain, and where the cells were presumably prevented from being depolarized by the use of the choline–Ringer medium. Chlorpromazine, in the presence of Ca^{2+}, also reduced the passive influx of Na^+ in erythrocytes.

These results provide direct chemical support for the predictions made by THESLEFF[1], STRAUB[2] and SHANES and co-workers[6–8], on the basis of their electrophysiological observations, that anesthetics reduce the passive permeability (influx) of Na^+.

It is known that the tertiary amine anesthetics, which include procaine and chlorpromazine, depolarize frog sartorius muscle at high concentrations (i.e. drug concentrations which raise the threshold for the action potential by over 10 mV)[20]. Concentrations of procaine below $2 \cdot 10^{-4}$ M, however, hyperpolarize frog sartorius fibers by 1.5 mV (ref. 17). In the absence of any drugs it is known that frog sartorius muscle fibers retain their resting membrane potential of —90 mV up to 24 h (ref. 21). The situation is similar to that for ethanol, where concentrations up to 0.33 M have no effect on the membrane resting potential[22], but cause depolarization at higher levels[13,22–24]. The difference between the enhanced passive Na^+ influx in Ringer's solution and the reduced passive Na^+ influx in choline–Ringer (Figs. 1 and 2) might, therefore, be explained on the basis that high concentrations of ethanol and chlorpromazine depolarized the frog muscle fibers only in Ringer.

Chlorpromazine's requirement for Ca^{2+} in order to depress the passive Na^+ influx in erythrocytes is similar to that reported by PAPAHADJOPOULOS[25] for the action of anesthetic amines on the passive Na^+ efflux from phospholipid vesicles. Other workers[12,26,27] have reported that local and general anesthetics increase the passive loss of K^+ or the passive influx of Na^+ into erythrocytes, but they did not include Ca^{2+} in the incubation medium. WHITTAM[28] has suggested that passive membrane permeability is regulated by the level of intracellular or membrane-bound Ca^{2+}. The situation must be more complicated than this because chlorpromazine displaces the membrane-bound Ca^{2+} (ref. 29) while the alcohols and neutral anesthetics increase it[30].

Very high concentrations (near the mM region) of the local anesthetic amines (chlorpromazine, procaine, *etc.*) disrupt most membranes, including erythrocyte[10,11,31,32] and phospholipid membranes[25,27,33]. This is presumably a result of the high surface activity of these drugs[11,34].

Possible mechanisms of the anesthetic-induced depression of Na^+ influx

It is known that all local and general anesthetics hitherto tested expand cell membranes[35–37]; although tetrodotoxin appears to be an exception to this general rule, it is known that tetrodotoxin does not always anesthetize excitable membranes[38]. Most, but apparently not all, of the anesthetics cause membrane fluidization[39–41]. The anesthetic amines adsorb to phospholipid vesicles and make the surface more positively charged[25–27].

Since ethanol is neutral, it is not immediately obvious that it should alter the ζ-potential of the muscle cell, unless there is a conformational change in the membrane proteins. A possible explanation for the anesthetic-induced reduction of the passive Na^+ influx in muscle, therefore, might be the phenomenon of membrane expansion[42,43].

In order to test the Thesleff–Straub–Shanes hypothesis of reduced passive permeability in a more rigorous way, further experiments must be done where only the passive Na^+ influx is measured during the action potential under conditions of repetitive electrical stimulation. Experiments along these lines have recently been done[44] and the results tend to support but do not yet clearly confirm the hypothesis.

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