Ca^{2+} concentration and interaction of long-lasting local anaesthetics with the squid axon membrane

The action of local anaesthetics on nerve membrane in depressing or abolishing the action potential has been considered to be short-lasting in the case of procaine and lidocaine, whereas it has been considered to be long-lasting with dibucaine (Ritchie, Ritchie & Greengard, 1965a; Strobel & Bianchi, 1970). These considerations are based on the ease with which the drugs are washed out from the membrane phase of the tissue when they are present in minimal blocking concentrations. Depending on the pK_a of the anaesthetic and the pH of the solution, the anaesthetic exists as uncharged species or as positively charged species and acts as such in depressing the Na^+ and K^+ currents in excitable membranes. Although the uncharged form of the drug penetrates the membrane more easily than the charged form (Ritchie, Ritchie & Greengard, 1965b), the charged form is more effective in abolishing the action potential (Ritchie & Greengard, 1966; Narahashi, Frazier & Yamada, 1970; Strobel & Bianchi, 1970).

It is generally considered that the negative sites on the nerve membrane are occupied by Ca^{2+} ions (stabilized state of the membrane). The charged form of the anaesthetics, procaine (Blaustein & Goldman, 1966) and dibucaine (Suarez-Kurtz, Bianchi & Krupp, 1970) has been shown to compete with calcium for the negative sites on the membrane. In the voltage-clamp experiments described by Blaustein & Goldman (1966), the competitive actions of procaine and Ca^{2+} were demonstrated using single giant axons of lobster and procaine (4 or 7·3 mM) in a bathing medium whose Ca^{2+} concentration was varied (pH ranged from 6·2 to 6·5). In the experiments described by Suarez-Kurtz & others (1970), desheathed frog sciatic nerves were exposed for 25 min to 15 μM dibucaine in frog Ringer solution at pH 9·0. Then the depression and recovery of the action potential was followed using anaesthetic-free Ringer solution whose Ca^{2+} concentration was varied at pH 7·2. Although the competition of Ca^{2+} and positively charged form of the drug has been clearly demonstrated, an aspect of Ca^{2+} action which has not been recognized because of the presence of normal Ca^{2+} concentration in the solution in which the tissue is bathed, is now examined.

The action of Ca^{2+} described below is of theoretical interest. It allows the long-lasting drug to adhere to the membrane of the excitable tissue only when the distance of separation of the drug from the surfaces of the biopolymeric chains of the membrane is reduced to that level at which short range forces of the Heitler-London type could exert their influence. Otherwise the drug would not 'stick' to the membrane. The long-lasting drugs chosen for this purpose were dibucaine and chlorpromazine. The biological preparation used was the single giant axon of the squid, as other multifibre preparations such as frog nerve were found unsuitable.

The giant axons from the hindmost stellar nerve of the squid *Loligo pealii* were carefully cleaned of the surrounding fibres and were placed in a single sucrose gap of the type described by Strobel & Bianchi (1970). The temperature of the cell containing the gap was maintained at 15°.

The artificial sea water (ASW) of the following composition was used: (all concentrations are mM) NaCl, 455, KCl, 15, $CaCl_2$, 44 and tris buffer, 30. The pH of the ASW was adjusted to either 7·2 or 9·0 as required. 0-Ca ASW was prepared by replacing $CaCl_2$ with an equivalent quantity of tris-Cl. The solution for the application of the drug at any given pH was prepared by adding the required quantity of the drug to ASW. The drug concentration used was 50 μM.

With the axon in ASW, application of an electrical stimulus of sufficient strength evoked an action potential which was biphasic initially and became monophasic with the continued flow of isotonic sucrose solution. The height of the spike (action

potential) was usually about 30 mV. When the solution was changed to 0-Ca ASW, repetitive firing was observed. This was eliminated when normal ASW was restored.

When the local anaesthetic, 50 μM dibucaine in ASW, was applied at pH 9·0, the action potential was blocked but it was reversed on increasing the strength of the stimulus. However, at this level, on changing the solution of ASW to pH 7·2 (no drug present), the block of action potential occurred. This was reversed when the solution was changed to ASW solution of pH 9·0 (no drug). This blocking and unblocking of the action potential could be repeated by simply changing the ASW solution of different pH, 7·2 and 9·0 respectively.

In Fig. 1 are given the results obtained with dibucaine when the experimental conditions were as follows.

When the axon was in 0-Ca ASW, pH 9·0, the results of Fig. 1A show that the height of the action potential slowly decreased and repetitive firing appeared. On adding 50 μM dibucaine (pH 9·0) still in 0-Ca ASW, there was further depression of the spike followed by suppression of the repetitive activity resulting finally in block of action potential. However on increasing the threshold stimulus, the spike was restored to about 40% of its original height. The action potential stayed at that level. Application of ASW (44 mM Ca) but at pH 7·2 restored about 80% of the spike height. There was some lowering of the threshold stimulus for excitation. Now changing the ASW solution to pH 9·0 or 7·2 had no effect on the spike height. Obviously the drug has not been incorporated into the membrane phase.

In Fig. 1B are given similar results except for the fact that instead of restoring 44 mM Ca ASW at pH 7·2, 44 mM Ca was restored at pH 9·0. Later when the ASW was changed to pH 7·2, the block of action potential occurred. Changing the solution to ASW pH 9·0 restored the spike as described already indicating the presence of the drug in the membrane.

Application of the drug at pH 7·2 in 0-Ca ASW also led to block of the action potential which was however reversed by 44 mM Ca ASW (pH 7·2) (results not given).

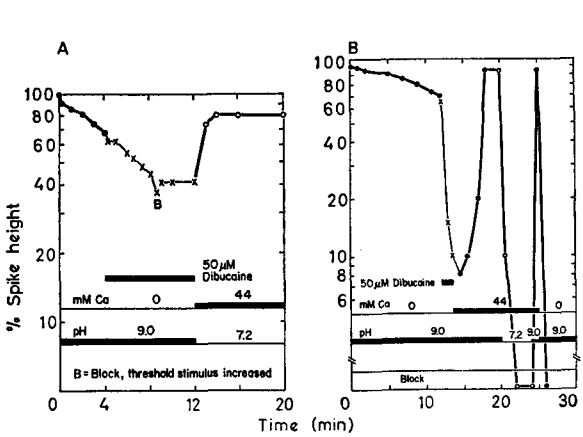

FIG. 1. Effects of dibucaine and Ca^{2+} on squid axon membrane as revealed by the depression of action potential (spike height).

A. The drug was applied in 0-Ca ASW at pH 9·0. Restoration of Ca at pH 7·2 with no drug present in ASW restored the spike height to about 80% of its original value indicating non-incorporation of the drug into the membrane phase.

B. The drug was applied in 0-Ca ASW at pH 9·0. Restoration of Ca at pH 9·0 with no drug present in ASW restored the spike height to its original value. However change of pH to 7·2 with no drug present in ASW abolished the spike indicating the presence of the drug in the membrane.

Under these conditions also the drug was removed from the membrane phase as the cycle of block and unblock of action potential could not be generated.

Application of 50 μM of chlorpromazine, the other long-lasting drug, also gave results similar to those shown in Fig. 1. Identical experiments carried out using another divalent ion (Mg^{2+}) in the place of Ca^{2+} gave similar results. The minimum concentration of the divalent ion required to produce the results given in Fig. 1 has been found to be about 6 mM.

The membrane of the giant axon of the squid in 0-Ca ASW may be considered to exist in a highly labile state and on excitation undergoes repetitive firing (Huxley, 1959). This repetitive discharge can be abolished by restoring the normal Ca^{2+} level. The results of Fig. 1 show that the drug, charged or uncharged, will not adhere to the nerve membrane in 0-Ca ASW. A limiting value of 6 mM of Ca is essential for the interaction of the drug with the biopolymers of the membrane. Below this limiting value, the nerve membrane is still labile and drug incorporation into the membrane is prevented by the strong electrostatic repulsion exhibited by the positively charged form of the drug at pH 7·2 and Ca^{2+} associated with the negative sites on the membrane. The force of repulsion is inversely proportional to the square of the distance of separation of the interacting species, whereas the short range force of the Heitler-London type is inversely proportional to the sixth power of the distance of separation (Moelwyn-Hughes, 1961). Consequently, in the presence of normal Ca^{2+} and the drug in uncharged form (i.e. absence of long range repulsive force on the drug molecule), the biopolymeric chains of the membrane are brought closer together to reduce the distance of separation of the interacting species (drug and the biopolymers of the membrane) to a level at which the Heitler-London forces take over and fix the drug to the nerve membrane.

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