Comparison of Effects of Selected Local Anesthetics on Sodium and Potassium Channels in Mammalian Neuron

S. ŠTOLC

Institute of Experimental Pharmacology, Centre of Physiological Sciences, Slovak Academy of Sciences, 842 16 Bratislava, Czechoslovakia

Abstract. Effects of procaine, trimecaine, and a new carbanilate local anesthetic, carbizocaine, on early sodium inward current and fast and slow components of potassium outward current in the membrane of the rat dorsal root ganglion neuron were studied using the internal dialysis and potential clamp techniques. All the currents studied were depressed in the presence of the drugs tested. However, for inhibition of the inward current concentrations lower by approximately one to more than two orders were sufficient compared to those required for similar inhibition of the outward currents. Carbizocaine was the most effective, procaine the least effective drug. Almost identical ratios of the negative logarithms of mean effective concentrations for blocking the inward and the outward current respectively, were found for each of the drugs tested. None of the drugs could be characterized as a specific blocker of sodium or potassium channels. It is concluded that the mechanisms of action of these three local anesthetics in all the three types of ion channels studied in the neuronal membrane are very similar regardless of both the type of the chemical bond in the intermediary chain of the molecules (ester, anilide, carbanilate) and the structure of the aromatic moiety, or the absolute potency of the drug.

Key words: Local anesthetics — Sodium ion channels — Potassium ion channels — Potential clamp technique — Isolated neuron dialysis

Introduction

It is generally accepted that the block of conduction of action potential in excitable cells elicited by common local anesthetics is mainly due to inhibition of activation of sodium channels (Shanes 1958; Strichartz 1976; Covino and

The paper is dedicated to Prof. Helena Rašková on the occasion of her 75th anniversary.

Vassallo 1976; De Jong 1977; Neumcke et al. 1981; Roth 1986). These drugs are able to block also ion channels of other types (Shanes 1958; Douglas and Kanno 1967; Imaizumi and Watanabe 1982; Gage 1984). The affinities of local anesthetics to other channel types, e. g. potassium channels, however, is usually smaller than that to sodium channels. Although a number of observations have confirmed this qualitatively (Shanes 1958; Taylor 1959; Ritche 1971; Narahashi et al. 1972; 1976; Strichartz 1975; Hille 1977; Wang et al. 1982), a more exact quantitative analysis of this phenomenon, e. g. as carried out by Århem and Frnkenhaeuser (1974) and by Borchard and Drouin (1980), are rather scarce. Besides, no drug of a series of new perspective local anesthetics with carbanilate structure (Beneš et al. 1969; Čižmárik et al. 1978; Beneš 1986) has been tested so far as to its effect on sodium and potassium channels. A typical representative of these drugs selected for this study is carbizocaine synthetized by Beneš et al. (1978).

The aim of the present study was to measure quantitatively the effects of selected local anesthetics with different types of chemical bonds in the intermediary chain on three types of ion channels: sodium, fast potassium, and slow potassium channels in dialyzed single neurons of the rat dorsal root ganglion using the potential clamp technique. The study has been expected to contribute to the understanding of the mechanism of membrane action of local anesthetics.

The study showed that regardless of whether the local anesthetics under study were esters, amides or carbanilates, and regardless of their absolute potency, the ratios of their potencies to block the early inward current and the fast and slow outward currents were similar. Obviously, the mechanisms of action on various ion channels in excitable membranes are close in all the drugs tested including the new drug carbizocaine.

A part of this study was published in abstract from elsewhere (Štolc et al. 1984).

Materials and Methods

Single neurons of dorsal root ganglia isolated from 3—6 days old rats were used. Following removal, the ganglia were treated with both trypsin (8 mg/ml) and collagenase (1000 U/ml) dissolved in Eagle's minimal essential medium, for 15 min at 32 °C. Single neurons obtained by gentle mechanical dissection under a dissecting microscope were transferred by a fine pipette into an experimental chamber. The cells were then dialyzed and potential of the neuronal membrane was controlled by essentially the same technique than those used by Veselovsky et al. (1979) and described in detail by Kostyuk et al. (1981a). Briefly, the cell was wedged in a conical hole with an internal diameter of about 12 μm made in the wall of plastic tubing making up the perfusion system (Fig. 1). After

rupturing by suction the part of the membrane oriented toward the perfusion system, communication was established between the intracellular space and the perfusion system. This enabled to easily control the composition of medium in contact with both sides of the membrane. To analyze drug actions on the early sodium inward current extracellular solution of the following composition was used (mmol/l): NaCl 140; $CaCl_2$ 2; $MgCl_2$ 2; TRIS 5. The intracellular solution contained TRIS 120; glucose 20; and EDTA 1. When testing drug effects on potassium outward current the extra- and the intracellular medium composed of TRIS 145; $CaCl_2$ 2; $MgCl_2$, and TRIS 50; KF 70; glucose 20; EDTA 1, respectively. pH of the extracellular medium was adjusted with hydrochloric acid to 7.3. Hydrofluoric acid was used to adjust pH of the intracellular solutions to the same value. All experiments were carried out at 22—24 °C.

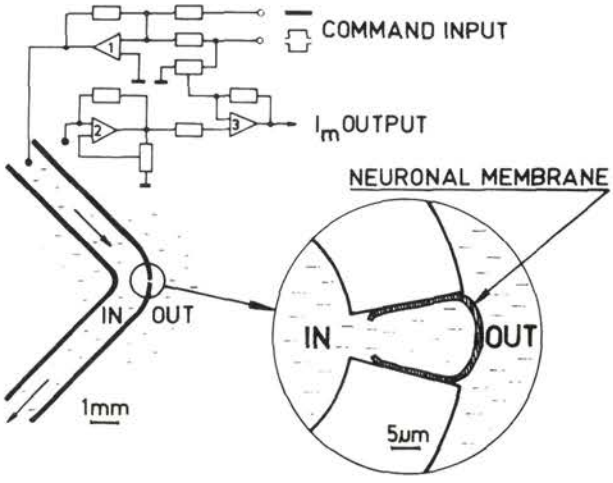

Fig. 1. Schematic representation of the experimental design of dialysis and potential clamping of isolated neuron. IN and OUT mean internal and external perfusion medium, respectively. Amplifiers 1, 2, and 3 are used for current injection and current to voltage conversion. The scheme is essentially the same as that described by Kostyuk et al. (1981a).

The electrical equipment to clamp the neuronal membrane potential and to measure transmembrane currents consisted essentially of a command former and a current-to-voltage converter. The system enabled to subtract electrically leakage and shunting currents and to compensate partially for the series resistance (Kostyuk et al. 1981a). Holding potential was adjusted arbitrarily to 100 mV negative inside. Transmembrane currents were measured during membrane depolarizing pulses of about 15 ms and 300 ms when testing sodium and potassium currents, respectively; typically they were in the nA range. Complete current-voltage characteristics were obtained for each cell repeatedly. The drugs were applied to the external side of the membrane for 20 min.

Chemicals: procaine hydrochloride (PROCAIN® Spofa), trimecaine hydrochloride (MESOCAIN® Spofa), carbizocaine hydrochloride (kindly supplied by dr. L. Beneš, Inst. Exp. Pharmacol., Ctr. Physiol. Sci., Slovak Acad. Sci,. Bratislava), trypsin (TRYPSIN® Spofa), collagenase (bacterial, 2000 U/mg, Merck), Eagle's minimal essential medium (MEM® Sevac). All other chemicals were of analytical grade.

Results

All the drugs tested fit well to the general scheme of local anesthetics proposed by Löfgren (1948, see also Ritchie 1971). In addition to differences in the aromatic moiety of the molecule (determining lipophilicity of the drug) the most remarkable differences concern the intermediary chains (Fig. 2). Procaine was used in this study as the representative of ester local anesthetics; trimecaine, a close congener of lidocaine, as the representative of anilide type; while carbizocaine has a carbamate bond in this part of the molecule.

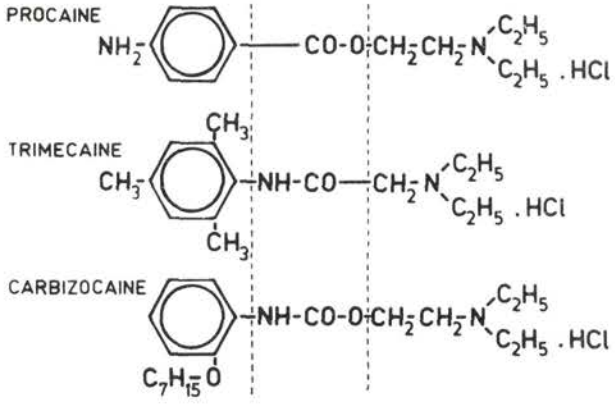

Fig. 2. Chemical structures of procaine, trimecaine, and carbizocaine.

The early inward current appearing after membrane depolarization was depressed following carbizocaine application to the external surface of the neuronal membrane (Fig. 3). A current to voltage plot of data from this experiment is shown in Fig. 4. The effect of carbizocaine was essentially the same as those induced by procaine and trimecaine, except for the different range of effective concentrations. The concentration-effect relationships for all the three drugs tested are illustrated in Fig. 5. The mean effective concentrations (EC_{50}) and factor f expressing relative affinities, with the procaine affinity taken equal to 1, are shown in the inset. It is obvious that carbizocaine is by about two orders more effective in blocking the early inward current than the other drugs.

The threshold potential i. e. the potential at which the early inward current begins activating (E_{thr}) as well as the potential at which this current amplitude reached its maximal value (E_{peak}) were significantly shifted by the local anesthetics to more positive values only at the highest concentrations used. The displacement of E_{thr} and E_{peak} ranged from 2 to 8.4 mV and from 11 to 30 mV, respectively.

The effects of the three drugs on the outward current appearing during the depolarizing pulses were measured in a separate set of experiments. Two phases of the outward current can be distinguished in the rat dorsal root ganglion neurons; a fast inactivating and a slow non-inactivating. They have been ascribed to two distinct populations of potassium channels (Kostyuk et al. 1981b). The slow component is more prominent only during longlasting depolarizations; pulses of about 300 ms were therefore used in these experiments.

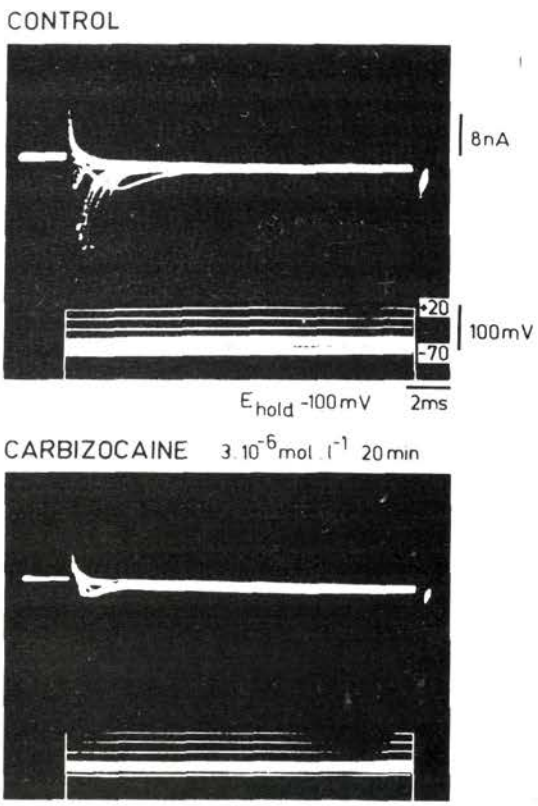

Fig. 3. A typical record of an experiment illustrating the depressing effect of carbizocaine on the early inward current carried by sodium ions. For the sake of clarity some peaks of the inward currents and voltage traces were retouched. Holding potential is out of the oscilloscope screen.

A typical effect of carbizocaine on the outward current is illustrated in Fig. 6. A reduction of both components can be observed. A current-voltage plot from this experiment is shown in Fig. 7. Procaine and trimecaine inhibited the outward current in a similar way except for the different ranges of effective concentrations. This can be seen from the concentration-effect realtionships for the drugs (Figs. 8 and 9). The sensitivities of both components of the outward current to the tested drugs were very similar. The order of potencies was identical with that found in testing the inward current, carbizocaine being the most effective, procaine the least effective. However, for each particular drug the ranges of concentrations effective

in blocking the outward current were uniformly higher than those required to block inward current.

In pharmacology the drug effect/drug concentration relationship is usually expressed by a linear to logarithmic plot. Consequently, to compare the affinities of the local anesthetics tested to two separate membrane functions (inward and outward current), a bilogarithmic plot was considered to be appropriate. The

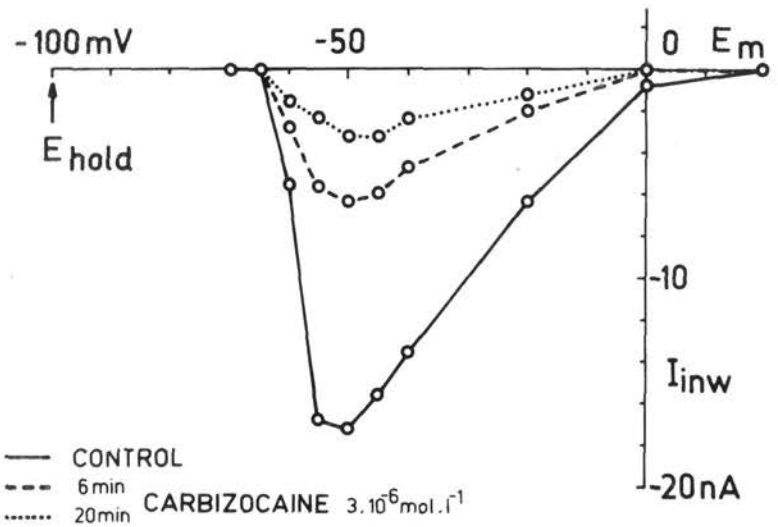

Fig. 4. Current-voltage plot of the experiment illustrated in Fig. 3.

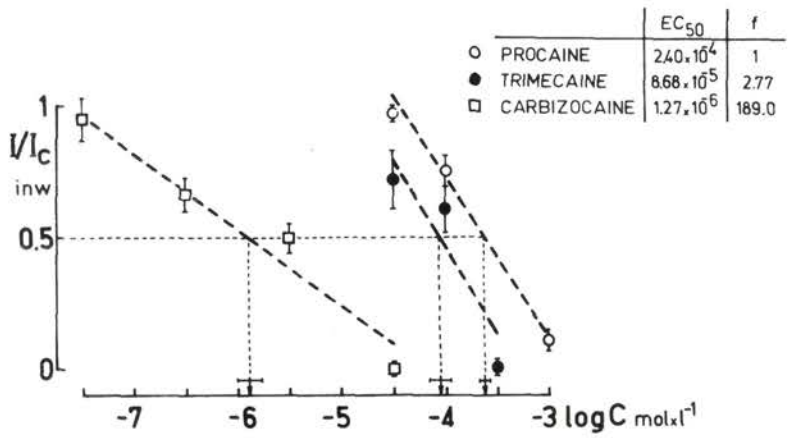

Fig. 5. The dependence of the early inward current amplitude expressed as relative to the control (I/I_o) on the concentration (C) of the local anesthetics. Mean effective concentrations (EC_{50}) are shown. Factor f expresses drug affinities related to that of procaine.

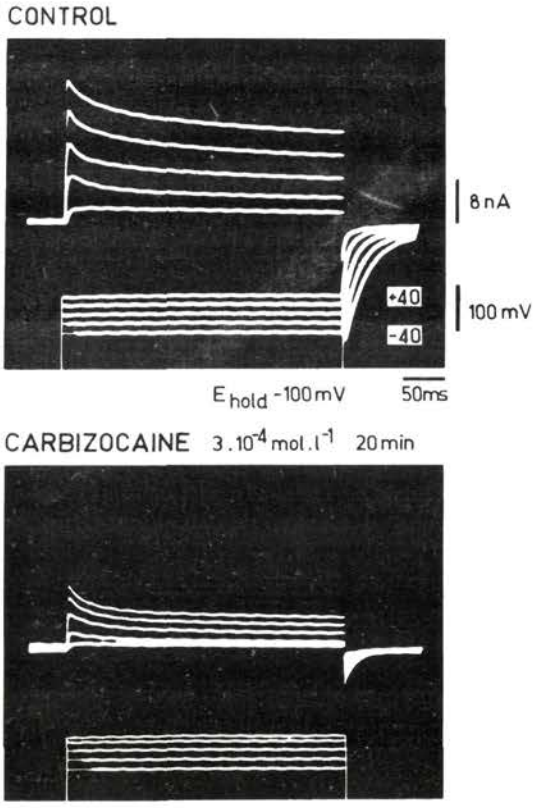

Fig. 6. A typical record of an experiment showing the depressing effect of carbizocaine on both the fast inactivating component and the slow non-inactivating component of the outward current carried by potassium ions. For the sake of clarity voltage traces were retouched. Holding potential is out of the oscilloscope screen.

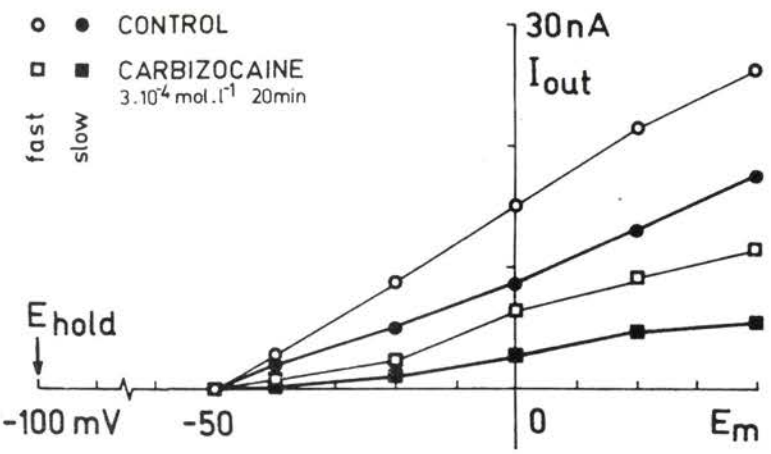

Fig. 7. Current-voltage plot of the experiment illustrated in Fig. 8.

plots of negative logarithms of mean effective concentrations (pD_2) for the inward and the two outward currents for each drug (Fig. 10) show clearly that, although differing remarkably in their absolute affinities to particular currents (EC_{50}), linear correlations can be fitted to these variables, with the correlation coefficients r for I_{inw} versus $I_{out\ fast}$ and I_{inw} versus $I_{out\ slow}$ being 0.971 and 0.966, respectively. The slopes of both regression lines are not significantly different.

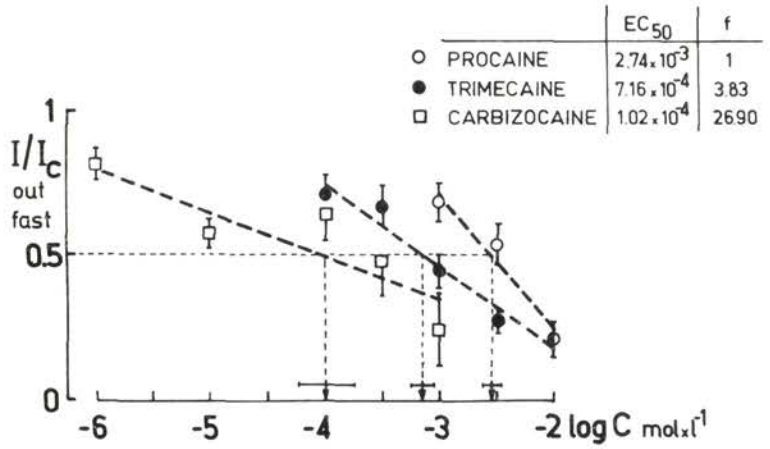

Fig. 8. The dependence of the fast outward current amplitude expressed as relative to the control (I/I_o) on the local anesthetic concentration (C). The same arrangement as in Fig. 5.

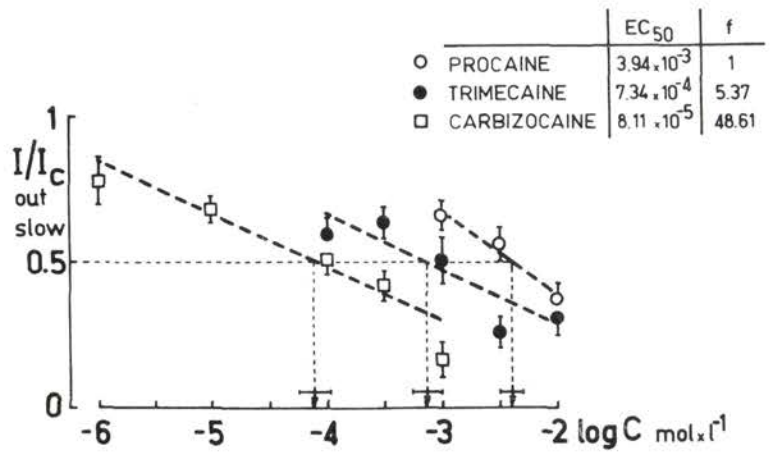

Fig. 9. The dependence of the slow outward current amplitude expressed as relative to the control (I/I_o) on the local anesthetic concentration (C). The same arrangement as in Fig. 5.

Thus, the higher the blocking potency of the tested drugs to the inward current the higher the potency to block both components of the outward current and vice versa.

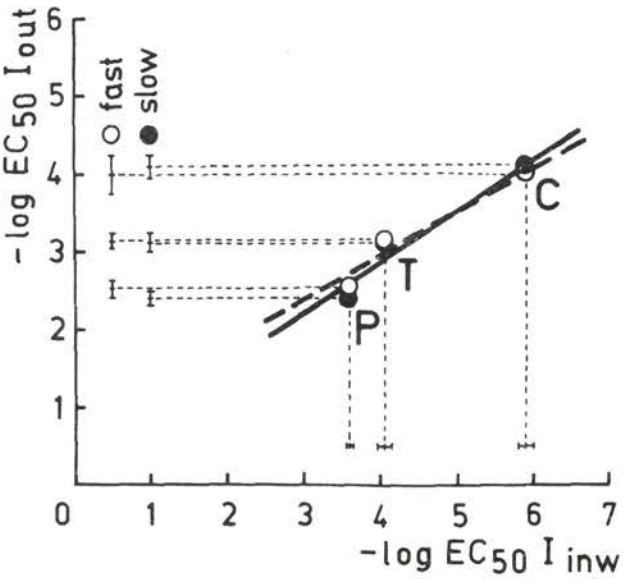

Fig. 10. Plot of negative logarithms of mean effective concentrations (EC_{50}) for the early inward current (I_{inw}) and for the fast and the slow components of the outward current (I_{out}). Note the differences in the affinities between the three drugs and the proportional ratio of the affinities to inward and outward current for each drug. Correlation coefficients of the regression lines for fast (broken line) and slow (full line) outward currents versus inward current are $r = 0.966$ and $r = 0.971$.

Discussion

Kostyuk et al. (1981a, b) have clearly shown that separate populations of ion channels conducting sodium and potassium ions are responsible for the inward, and for each of the two components of the outward current occurring in the membrane of the rat dorsal root ganglion neurons. This model was used for our comparative study of the effects of local anesthetics on different ion channels.

Local anesthetics inhibit excitability of neuronal membranes. The drugs reduce membrane ability to generate action potentials by depressing changes in membrane conductance at an instant decrease in transmembrane potential (e. g. Covino and Vassallo 1976; De Jong 1977).

A comparison of the mean effective concentrations of the tested drugs revealed that the new local anesthetic with the carbanilate structure, carbizocaine, is by about one to more than two orders more effective than procaine or trimecaine, a close congener of lidocaine, depending on the current considered.

Local anesthetics have been clearly shown to block, in addition to sodium channels (Shanes 1958; Shanes et al. 1959; Taylor 1959; Ritchie 1971; Khodorov 1974; Khodorov et al. 1976; Strichartz 1976; Negulyaev and Nosyreva 1979; Schmidtmayer and Ulbricht 1980; Neumcke et al. 1981) also potassium (Ritchie 1971; Narahashi et al. 1972; 1976; Frazier and Narahashi 1975; Strichartz 1975; Hille 1977; Imaizumi and Watanabe 1982; Wang et al. 1982), and calcium channels (Douglas and Kanno 1967, Gage 1984). Although it has been stated that these drugs are stronger in blocking sodium than the other types of ion channels, quantitative comparisons of the differences, as well as attempts to explain their nature, have been but sporadically published so far.

Århem and Frankenhauser (1974) estimated the mean effective concentrations of procaine, lidocaine, and benzocaine on the peak sodium permeability and the steady state potassium permeability in the node of Ranvier. According to their data lidocaine is about 8.7 times more potent in blocking sodium permeability than in blocking potassium permeability. This corresponds well to the ratio 1 to 8.2 found in the present study with the lidocaine derivative trimecaine. However, these authors found higher procaine affinity in blocking sodium permeability (1 to 46.7) than observed in our experiments (1 to 12.5). This may have been due to the fact the above authors estimated middle effective concentration by extrapolation using an insufficient number of points. Borchard and Drouin (1980) showed that also carticaine, a thiophene local anesthetic, blocked sodium channels in the same model at an approximately 2.26 times lower concentration than required to block potassium channels, if mean effective concentrations were compared.

A lower effect in blocking both types of potassium channels as compared to sodium channels was observed for all the drugs tested in the present study. This not only applies to ester or amide type drugs with benzene of thiophene aromatic moieties but also to drugs with carbanilate structure. If negative logarithms of mean effective concentrations ($-\log EC_{50}$ or pD_2) for both effects are compared, the ratio remains constant regardless of both the absolute potency of the drug, the type of their intermediary chain, or the structure of the aromatic moiety of the molecule. None of the drugs tested can be considered a highly specific blocker of ion channels. This suggests a rather non-specific type of interaction beween the local anesthetics and the neuronal membrane.

Blaustein and Goldman (1966) attempted to explain the lower sensitivity of potassium ion channels, as compared to sodium channels, to local anesthetics.

They assumed that membrane phospholipids might serve as ion carriers. Local anesthetics have been suggested to stabilize the membrane by competing with calcium bound to the carriers. The different effects of these drugs on sodium and potassium permeabilities could be formally explained in their model by different dissociation constants of carriers and the corresponding type of ions to be transported. Yet the significance of the antagonism between calcium and local anesthetics in the mechanism of action of local anesthetics is no longer accepted (Shanes et al. 1959; Århem and Frankenhauser 1974; Narahashi et al. 1976). De Jong (1977) simply suggested that „it is perhaps potassium channel's narrower dimensioning that precludes as firm an anesthetic attachement as offered by the more generously proportionated sodium channel".

According to Hille's model (1977) the gating in potential operated channels is connected to changes in channel conformation which in turn are related to changes in charge distribution in the membrane. As sodium channel undoubtedly is more sophisticated than potassium channel, the former has conceivably higher space requirements than the latter one. Hence, even comparatively small changes in charge distribution, in channel configuration, or in flexibility of lipids surrounding the channel protein evoked by a local anesthetic may more readily limit activation of the sodium channel than may be expected in the more simple, hence less space demanding, potassium channels. If however the receptor hypothesis (Covino and Vassallo 1976; Strichartz 1976; Hille 1977) is considered, a close similarity should be admitted to exist between the proposed anesthetic receptor structure present in the three analyzed types of ion channels with different affinities to the tested drugs.

Although Frazier and Narahashi (1975) observed in squid axon a shift of g_{Na} curve to depolarizing potentials in the presence of the local anesthetic tricaine, the possibility cannot be ruled out that the similar shifts of E_{thr} and E_{peak} which occurred in our experiments are, at least partially, related to different precision of potential clamping at high and low currents inherent to the technique used. Alternative explanations might be a gradual alteration of sodium channel properties by local anesthetics or a differential block of populations of sodium channels more sensitive to these drugs leaving the less sensitive channels with different activation properties to be operative. This problem, however, requires additional analysis.

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