Time Courses of Lidocaine Effects on Sodium Membrane Currents in Small and Large Neurons

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Abstract. Time courses of effects of lidocaine on sodium currents and sodium dependent action potentials were studied in somata of small and large neurons. Cultured rat sensory spinal ganglion cells (diameter: 30 μm) and neurons of the buccal ganglion of *Helix pomatia* (diameter: 150 μm) served as the test cells. The latency of the suppressive action of lidocaine was the longer the larger the size of the cells was. Maximal blocking effects occurred within 10 min in sensory spinal ganglion cells and within 40 min in snail neurons. Model calculations based on the assumptions (i) that lidocaine is distributed in the extra- and intracellular space by simple diffusion and (ii) that the drug concentration at the outer surface of the cells is elevated stepwisely, revealed a strong dependency of intracellular concentration changes on the size of the cells. From these findings it is concluded that lidocaine blocks sodium channels primarily from the intracellular side.

Key words: Local anesthetics — Lidocaine — Sodium current — Cell size — Intracellular diffusion

Introduction

Lidocaine is a local anesthetic widely used in medicine and dentistry to block conduction of action potentials in nerve fibers. The amount of lidocaine needed for a complete block of nerve conduction (e.g. for complete pain relief) varies considerably in different patients. This may, at least partly, be attributed to a

number of extracellular factors. For example, changes of extracellular pH, as they occur during inflammation, influence the concentration ratio of protonated and neutral lidocaine molecules and thus the diffusion rate of lidocaine in the extracellular space (Ritchie and Greene 1985). This may alter the time courses and delay or enhance the reaching of final levels of the drug at the site of its action.

Presently, we are unable to answer the question to which extent the intracellular milieu determines the effectivity of local anesthetics. Our knowledge, e.g. concerning the predominant model local anesthetics employ to enter the site of action in the neuronal membranes, is restricted. It is widely assumed that neutral forms of extracellularly given local anesthetics, such as lidocaine, diffuse through the cell membrane into the interacellular space. Inside the cell the cation form enters the internal mouth of the sodium channel blocking there sodium currents (Ritchie and Greengard 1961; Strichartz 1973; Narahashi and Frazier 1975; Hille 1977a, b; Ritchie 1987; Schwarz et al.1977). In addition to this 'classical' hydrophilic way, Hille (1977a, b) has described a hydrophobic pathway which allows lipid soluble forms of local anesthetics intramembraneous access to the sodium channel. The rate of action of local anesthetics via the hydrophilic pathway is assumed to depend on (i) the ratio of permeabilities of the membrane and of the adjacent spaces, and (ii), especially at definite permeability ratios, on the intracellular volume. The latter dependency is assumed to be weak for the hydrophobic pathway. Moreover, the kinetics of interactions between the drug and the target structures may, in principle, be taken into account. Hence, if the time course of action of a local anesthetic depends markedly on intracellular volume of the excitable cell tested, this finding would favour the assumption that (i) the hydrophilic pathway of this drug predominates under experimental conditions, and that (ii) the apparent diffusion coefficient of lidocaine in the membrane differs significantly from that of the adjacent spaces.

The aim of the present investigation was to test the functional significance of the described modes of lidocaine entrance into the sodium channel. The time courses of action of this drug on sodium currents and sodium dependent potentials were studied in somata of small and large neurons. From the results of these experiments and from corresponding findings obtained in nerve fibers a mathematical model was derived which allows estimation of the rates of concentration changes of the drug in intracellular compartments.

Materials and Methods

The experiments were carried out (i) on sensory spinal ganglion (SSG)-cells of neonatal rats, 3—6

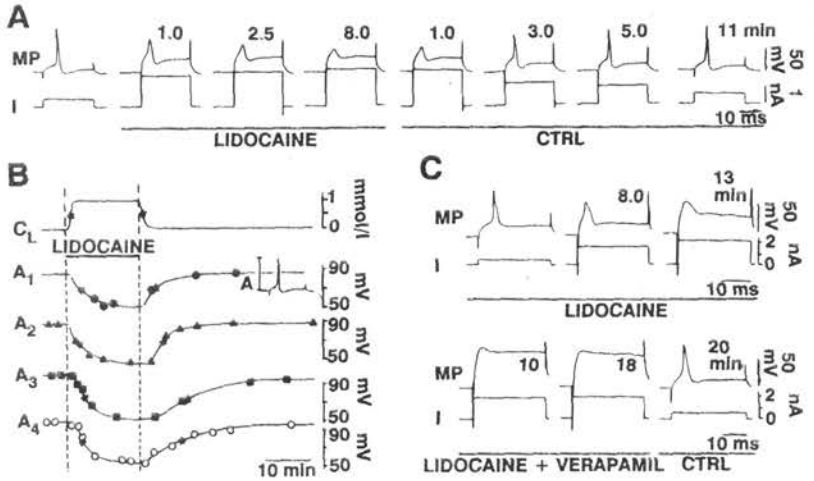

Fig. 1. Effects of lidocaine (final bath concentration 1 mmol/l) on action potentials in cultured sensory spinal ganglion cells. *A*: Reduction of amplitude of action potentials (*MP*) and increase of the threshold current (*I*) injected intracellularly. Duration of exposure to lidocaine is indicated. CTRL: Washing the preparation with control solution. *B*: Time courses of changes of action potential amplitudes (A_1—A_4) and of the lidocaine concentration in the bath (C_L). Half maximal changes are marked by *. *C*: Persistence of components of action potentials during exposure to lidocaine (1 mmol/l) and suppression of these components after addition of the organic calcium antagonist verapamil (final bath concentration 40 μmol/l) to the lidocaine containing superfusate. Exposure times in *A* and *C* are given in minutes.

weeks after explantation of SSG in culture conditions (Bingmann et al. 1977) and (ii) on the identified neurons B1—B3 of the buccal ganglion of the snail *Helix pomatia* (Schulze et al. 1975).

(i) SSG cells were transferred into a chamber (1.5 cm × 4 cm) which was mounted on an inverted microscope, and superfused at a flow rate of 3 ml/min by an approx. 2 mm thick layer of 32 °C saline of the following composition (in mmol/l): NaCl 124, KCl 5, KH_2PO_4 1.24, $NaHCO_3$ 26, $CaCl_2$ 2, glucose 10. The solution was equilibrated with 5 % CO_2 in O_2. The resulting pH value was 7.4 and it did not change significantly after lidocaine (Sigma; 1 mmol/l) addition to the bath. The cells were impaled with microelectrodes (tip diameter less than 0.5 μm) filled with KCl (2 mol/l). The resistance of the electrodes ranged between 20 and 100 MΩ. Membrane potentials were measured conventionally (Axoclamp 2A). Action potentials were elicited by intracellular current injection through the recording microelectrode. In voltage clamp studies two separate microelectrodes were used for measuring membrane potential and for current injection (Axoclamp 2A, Axon Instruments Inc., Burlingame, USA). The holding potential varied between −50 mV and −90 mV, the command potentials (duration 50 ms) between −30 mV and +20 mV. Sodium currents were blocked by tetrodotoxin (Sigma; 1 μmol/l; $n = 3$), calcium currents by cobalt (2 mmol/l; $n = 2$) or by the organic calcium antagonists flunarizine (dissolved in beta dextrin; Janssen, Neuss, FRG; 40 μmol/l; $n = 4$) and verapamil (40 μmol/l; $n = 3$).

(ii) Prior to being placed in the recording chamber neurons of the buccal ganglion of *Helix pomatia* were treated for 1 min with pronase (Merck; 0.1 g in 5 ml bath solution (see below)) which facilitated the dissection of the connective tissue covering the ganglion. In the chamber (volume 2 ml) the ganglion was fixed by needles on the bottom of wax and superfused at a rate of 10—

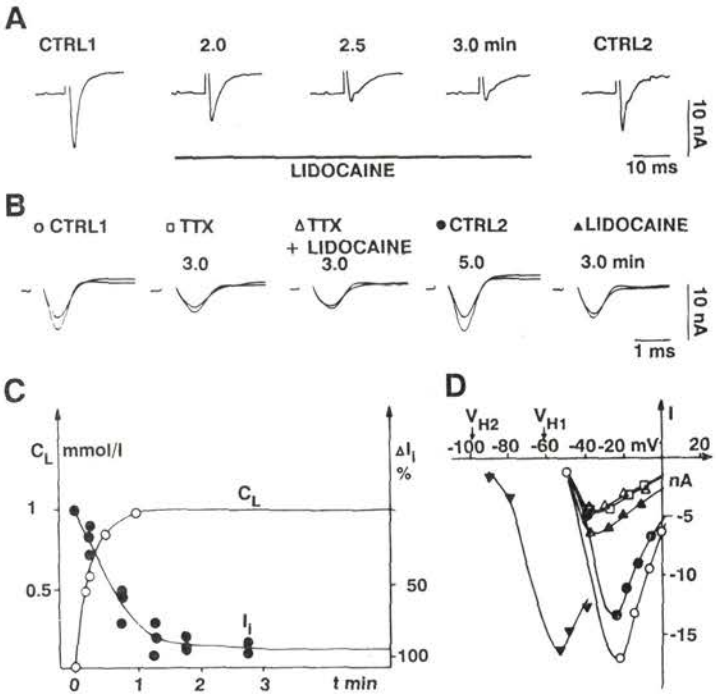

Fig. 2. Action of lidocaine (final bath concentration 1 mmol l) on inward currents in sensory spinal ganglion cells. *A*: Original tracings of inward currents under control conditions and during exposure to lidocaine (*B*) and to lidocaine + tetrodotoxin (TTX). Two tracings were superimposed indicating maximal variations. Holding potential −60 mV, command potential −20 mV. *C*: Time courses of changes of the maximal inward current (ΔI_I 100 %) and of the lidocaine concentration in the bath solution (C_L). *D*: Inward current (I) in a neuron, determined at a holding potential of −60 mV (VH_1) after stepping to various command potentials in control solution (○CTRL1; 3 min), after TTX addition (□ 10 $^{-6}$ mol l) and after the addition of lidocaine to the TTX containing bath (△). Recovery in control solution (●CTRL2; 5 min) and redecline of inward currents in lidocaine containing bath (▲; 3 min). Removal of the blocking effect of lidocaine by shifting the holding potential (VH_2) to −100 mV (▼). Exposure times in *A* are in seconds.

15 ml min by a saline which contained (in mmol l): NaCl 130, KCl 4.5, $CaCl_2$ 9, TrisCl 5. Bath temperature was 20 °C. The pH value was adjusted to 7.4. The cells were impaled by microelectrodes (tip diameter 1 − 2 μm) filled with 3 mol l KCl. The electrode resistance was generally less than 5 MΩ. Membrane currents were recorded using conventional voltage clamp techniques (see above, Klemmverstärker 7503, Hugo Sachs Elektronik, Hugstetten, FRG). The holding potential was −50 mV. Command potentials (duration 50 ms) varied between −20 mV and +40 mV. The calcium currents in snail neurons were suppressed by cobalt (20 mmol l; $n = 7$).

Results

Experimental data

In the experiments carried out on sensory spinal ganglion (SSG) cells the rate of action of lidocaine (1 mmol/l) on action potential amplitude was first tested in 10 spherical neurons with diameters of 28—32 μm (Bingmann et al. 1988). After switching from control solution to the lidocaine containing saline, the drug concentration in the bath centre reached 90 % of its final value within 1 min. This was (i) estimated based on measurements of temperature changes during alternating perfusions of the chamber with warm and cold salines, and (ii) calculated from the flow rate and the chamber volume. The half maximal effects of lidocaine on action potential amplitude (in 10 s intervals) were reached after 2.8 ± 0.19 min (mean ± SEM; $n = 10$; cf. Fig. 1 *A, B*). Maximal suppressive action occurred within 10 min if action potentials had been elicited by currents injected twice per minute. The same pattern was observed in the same cell after repeated exposures to lidocaine. During washing, however, the depressive effect disappeared slowlier after repeated than after single application (cf. Fig. 1 *B*). The recovery half time was 4.3 ± 0.43 min (mean ± SEM).

Even during a 20 min exposure to lidocaine (1 mmol/l) components of action potentials persisted with amplitudes of 20—30 mV, and duration of up to 10 ms. They were blocked by the inorganic as well as organic calcium antagonist cobalt (2 mmol/l) and flunarizine or verapamil (40 μmol/l each; Fig. 1*C*). Therefore, the persisting components were interpreted as calcium spikes. They were blocked by 5—10 mmol/l lidocaine only. In a second series of experiments the action of 1 mmol/l lidocaine on inward currents elicited twice per minute were studied in 3 SSG cells (Fig. 2 *A*). Command pulses were used which elicited maximal inward currents under control condition ($n = 3$). The time course of the lidocaine effect on the currents was similar to that on action potentials: approx. 50 % of the final lidocaine effect developed within 1—2 min (Fig. 2 *A, C*). Recovery occurred at a similar rate. Tetrodotoxin insensitive currents were not blocked by 1 mmol/l lidocaine (cf. Fig. 2 *B, D*). They were abolished, however, by cobalt (2 mmol/l) and hence were interpreted as calcium currents.

In voltage clamp studies carried out on the somata of the buccal snail neurons B1—B3 (diameters ca. 150 μm; Kunze 1921; Schulze et al. 1975), inward currents were elicited by command pulses running from −20 mV to +40 mV ($n = 26$). This protocol was repeated in 10 min intervals during lidocaine application (1 mmol/l) and after washing with control solution. The effects of lidocaine on the total inward current ($n = 19$) and on cobalt insensitive components ($n = 19$) presumably carried by sodium ions ($n = 7$) were tested.

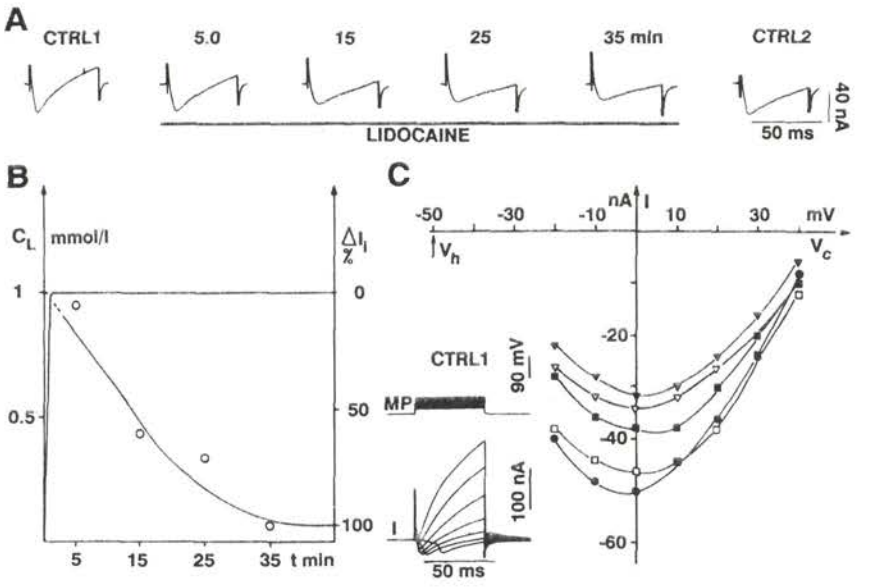

Fig. 3. Action of lidocaine on cobalt (20 mmol/l) resistant inward currents in a neuron (buccal ganglion, *Helix pomatia*). *A*: Original tracings of inward currents (CTRL1, CTRL2: before the commencement and after the end of the drug application, respectively). *B*: Time courses of changes of the maximal inward currents I_i in a neuron and of the lidocaine concentration (C_L). *C*: Maximal inward currents (I) before the commencement of the drug application (CTRL1 ●), and after 5 min (□), 15 min (■), 25 min (▽), 35 min (▼) of lidocaine exposure (1 mmol/l) (V_h = holding potential). Inset: Original tracings, *MP* = membrane potential

Fig. 3 shows a typical experiment performed in a cobalt containing bath. Half-final lidocaine effect occurred within 10 to 15 min, and maximum effect after 30 to 40 min. Recovery of the inward currents was observed after a similar latency. No systematic differences in the time courses of lidocaine effects were observed between total and cobalt insensitive currents.

Model calculations

As compared to the rate of action of lidocaine on action potentials and on inward currents in sensory spinal ganglion (SSG) cells and in snail neurons, the lidocaine concentration in the bath (c_B, which corresponds to the real c_L as shown in the previous chapters) changed rapidly. For the sake of simplicity it is assumed that c_B was stepwisely elevated from $c_B = 0$ up to $c_B = c_0$ at time $t = 0$ and kept constant at this value during the lidocaine exposure. With the flow of lidocaine molecules across the membrane into the intracellular space (ICS) of the neuron the intracellular lidocaine concentration (c_i) increases. The

time course of changes of c_i in the ICS can be calculated under the following assumptions: (i) The molecular flux j_m of lidocaine across the membrane is thought to be proportional to the difference of the actual concentration of this drug at the inner surface of the cell membrane (c_i^s) and that in the bath fluid at the outer surface of the cell membrane (c_0):

$$-j_m = P(c_i^s - c_0) \tag{1}$$

The factor P represents the constant permeability of the membrane for lidocaine.

(ii) In the neuronal ICS lidocaine is distributed mainly by simple diffusion.

Based on reports in the literature (DeVoe 1974; Hodgkin and Keynes 1953; Bunch and Kallsen 1969; Kushmerick and Podolsky 1969) it was assumed that the 'effective' diffusion coefficient D of lidocaine in the neuronal ICS may have a value between 50% and 100% of that in water. Thus, an effective D value of 10^{-7} cm^2/s was chosen for lidocaine diffusion in the ICS.

If lidocaine is distributed in the ICS by diffusion, the molecular flux j inside this space can be described by the law of Fick:

$$j = -D\nabla C_i \tag{2}$$

Suppose that lidocaine diffuses throughout the intracellular volume and that neither sinks nor sources of lidocaine exist at the membrane or in the ICS. Hence, diffusion of lidocaine molecules in the intracellular volume can be described by the following equations:

$$D\nabla^2 c_i - \frac{\delta}{\delta t} c_i = 0 \tag{3}$$

with the boundary conditions at the surface of the cell (cf. Formula 1 and 2)

$$D\nabla c = P(c_i^s - c_0) \tag{4}$$

and the starting condition

$$c_i(t = 0) = 0 \tag{5}$$

The somata of sensory spinal ganglion cells and of the giant snail neurons were supposed to be spheres with radius a_1. Axons distant from the soma regions were described as infinitely extended cylinders with radius a_2. The solution of equation (3) in common with (4) and (5) is given in principle by Crank (1975, pp. 79, formula 5.46 and pp. 96, formula 6.40):

$$c_{ik}(r, t) = c_0(1 - 2L_k \sum_{n=1}^{\infty} Q_{nk}(r) \exp(-\beta_{nk}^2 Dt/a_k^2)) \tag{6}$$

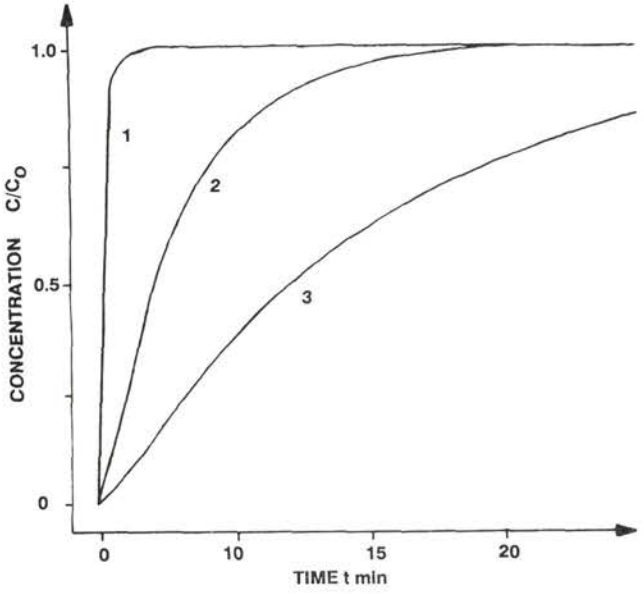

Fig. 4. Model calculations of time courses of lidocaine concentration c at the inner side of the membrane of an infinitely extended cylinder (diameter 5 μm, curve 1), of a 30 μm thick sphere (curve 2) and of a 150 μm thick sphere (curve 3) in relation to the lidocaine bath concentration c_0 (membrane permeability $P = 5 \times 10^{-6}$ cm/s; diffusion coefficient of lidocaine 10^{-7} cm^2/s).

for a sphere ($k = 1$) and a cylinder ($k = 2$) with

$$0 \leq r \leq a_k; \qquad L_k = Pa_k/D$$

and

$$Q_{n1}(r) = \frac{a_1 \sin(\beta_{n1} r/a_1)}{r(\beta_{n1}^2 + L_1(L_1 - 1))\sin(\beta_{n1})}$$

$$Q_{n2}(r) = \frac{J_0(\beta_{n2} r/a_2)}{J_0(\beta_{n2})(L_2^2 + \beta_{n2}^2)}.$$

β_{nk} is the n-th positive root of

$$\beta_{n1} \cot(\beta_{n1}) + L_1 - 1 = 0 \qquad \text{(for a sphere)}$$

and

$$\beta_{n2} J_1(\beta_{n2}) - L_2 J_0(\beta_{n2}) = 0 \qquad \text{(for a cylinder)}$$

($n = 1, 2, 3 \ldots$). J_m ist the Bessel function of order m.

Fig. 4 illustrates time courses of c_i (t) at the inner side of a 5 μm thick infinitely extended cylinder (curve 1), a 30 μm thick sphere (curve 2) and a 150 μm thick sphere (curve 3). A P/D ratio of 50 cm^{-1} was chosen. It represents a P value of 5×10^{-6} cm/s and a D value of 10^{-7} cm^2/s. The curves show that the time course of model c_i at the inner cell surface greatly depends on the intracellular volume. Ninety per cent of the bath concentration c_0 in the cylinder (which represents an axon) is reached within seconds; in the 30 μm thick sphere (which represents an SSG cell) that value is reached in about 10 min, and in a 150 μm thick sphere (which represents a giant snail neuron) 90 % of c_0 is reached within half an hour.

Discussion

Beside the classical hydrophilic pathway of local anesthetics to the sodium channel, Hille (1977a, b) has described a hydrophobic one which allows non charged drug molecules an intramembraneous access to the sodium channel. Studying the functional significance of these alternative modes of entrance, the present experiments aimed at estimating intracellular concentration changes of lidocaine and to relate this estimated time course to that of lidocaine action on sodium channels. A prerequisite of such estimations is that the lidocaine concentration at the outer surface of the membrane increases rapidly in comparison to the rate of action of lidocaine in the sodium channel (Hille 1977a, b). As this action was described to occur in myelinated fibers within seconds, in this preparation only controlled bath concentration changes of lidocaine within less than 100 ms could be regarded as stepwise changes which can hardly be achieved. In the sensory spinal ganglion (SSG) cells and in the giant snail neurons of the buccal ganglion, however, lidocaine blocked inward currents within approx. 3 and 30 min, respectively. Therefore, the t_{90} rise time of the lidocaine concentration in the bath ranging typically below 1 min (especially in experiments on snail neurons) seemed to represent tolerable deviations from the desired stepwise elevation of the lidocaine content in the bath. The extreme slow rate of action of lidocaine on the inward currents especially in snail neurons cannot be attributed to corresponding slow changes of the lidocaine concentrations at the surface of the neuronal membrane. Diffusion barriers between bath and neuronal membranes were destroyed as far as possible by pronase and stagnant boundary layers between bath and neurons were minimized by high flow rates (Lipinski and Bingmann 1987). Hence, after systemic exposure of these preparations to acetylcholine, membrane potential changes occurred within seconds (Witte et al. 1985). The slow depression of inward currents by

lidocaine, observed in the present experiments in neuronal somata, and the comparable slow reduction of the conductivity of the membrane of squid axons found by Narahashi and Frazier (1975) during exposure to procaine indicate that at least an overwhelming majority of these drug molecules have to use the hydrophilic pathway. Whenever reaching the submembraneous compartment, the molecules start to diffuse into the central intracellular compartments. Equilibration in the intracellular space will be reached within seconds if the ICS volume is small, which is the case e.g. in myelinated fibers. In large cells, however, the latency until equilibration may amount up to 30 minutes.

A critical submembraneous lidocaine concentration of approx. 0.5 mmol/l appeared to be a prerequisite for the blocking action of this drug on sodium currents. After 30—60 min exposures a bath concentration of 0.5 mmol/l lidocaine exerted the same effect on sodium dependent potentials of SSG cells as 1 mmol/l. Calcium dependent potentials and currents sensitive to calcium antagonists were blocked only at tenfold bath concentrations of lidocaine (Bingmann et al. 1987).

The discussed concept is supported (i) by the observation of Štolc (1988) who reported the exposure of the outer membrane of intracellularly dialyzed SSG cells (with artificially enlarged intracellular volumes) to the lidocaine derivative carbizocaine to reduce inward currents (approx. tenfold slower as compared with nondialyzed SSG cells in the present study), and (ii) by the present model calculations which were based on the assumption that lidocaine is distributed in the extracellular and intracellular spaces (ECS, ICS) mainly by simple diffusion. In the extracellular spaces of the brain tissue, diffusion of the drug molecules was widely analyzed in vivo and in vitro and found to depend on the geometry of the ECS which is determined by the two parameters 'volume fraction' and 'tortuosity factor' (Nicholson and Phillips 1981). The volume fraction is the quotient of that volume in which extracellular diffusion occurs and of the total tissue volume. The tortuosity factor describes the enlargement of mean diffusion length caused by the complex geometry of the ECS. Unfortunately, there is only little information which allows the estimation of the corresponding parameters in the ICS (DeVoe 1974). The diffusion constants for potassium in squid axons (Hodgkin and Keynes 1953) and for urea as well as for glycerol in barnacle muscles (Bunch and Kallsen 1969) were found to be the same as in free solution. In frog muscles, however, the diffusion of potassium, sodium, ATP and sucrose was cut in half (Kushmerick and Podolsky 1969). These reduced diffusion rates were attributed primarily to the intracellular tortuosity which may be brought about by the specific arrangement of membranes. These findings led to the estimation that the effective diffusion coefficient D of lidocaine is between 50 and 100 % of the D value found in water, i.e. approximately 10^{-7} cm^2/s.

The model calculations revealed significant transient intracellular concentration gradients especially in large cell bodies. These gradients depended primarily on (i) the membrane permeability (the permeability of the membrane was assumed to be in the same order of magnitude as described by Finkelstein (1976) but far lower than in Hille's model calculations (1977a, b)), (ii) on the diffusion coefficient of lidocaine in the ICS, and (iii) on the cell volume.

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