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Biophysical Chemistry 116 (2005) 227 – 235

Biophysical Chemistry

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A study of the perturbation effects of the local anesthetic procaine on human erythrocyte and model membranes and of modifications of the sodium transport in toad skin

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Received 14 April 2005; received in revised form 14 April 2005; accepted 15 April 2005
Available online 13 May 2005

Abstract

The interaction of the local anesthetic procaine with human erythrocytes, isolated unsealed human erythrocyte membranes (IUM), isolated toad skins, and molecular models is described. The latter consisted of phospholipid multilayers built-up of dimyristoylphosphatidylcholine (DMPC) and of dimyristoylphosphatidylethanolamine (DMPE), representatives of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively. Optical and scanning electron microscopy of human erythrocytes revealed that procaine induced the formation of stomatocytes. Experiments performed on IUM at 37 °C by fluorescence spectroscopy showed that procaine interacted with the phospholipid bilayer polar groups but not with the hydrophobic acyl chains. X-ray diffraction indicated that procaine perturbed DMPC structure to a higher extent when compared with DMPE, its polar head region being more affected. Electrophysiological measurements disclosed a significant decrease in the potential difference (PD) and in the short-circuit current (Isc) after the application of procaine to isolated toad skin, reflecting inhibition of active ion transport.

Keywords: Procaine; Local anesthetic; Erythrocyte membrane; Phospholipid bilayer; Toad skin

1. Introduction

The aminoester procaine (Fig. 1) was the first synthetic local anesthetic (LA). Its use is confined to infiltration anesthesia and diagnostic nerve blocks because of its low potency, slow onset and short duration of action [1]. The inhibitory action of local anesthetics (LA) on nerve conduction is primarily due to the interaction of the drug with voltage-gated Na^+ channels [2], responsible for the generation of action potential in excitable tissues [3]. The molecular mechanism by which LA block the Na^+ channels has not been completely established; however, different hypothesis have been proposed such as binding to Na^+ voltage-gated channels [4,5], interacting with multiple sites at the lipid–protein interface [6], and changes in the lipid phase of the membrane [7,8]. For these reasons we thought it is of interest to study the binding affinity of the local anesthetic procaine with cell membranes, its perturbing effect upon membrane structures and the possible consequent alteration of ionic channel functions. With this aim we used (a) human erythrocytes, which were observed by phase contrast and scanning electron microscopy (SEM), (b)

Abbreviations: PD, potential difference; Isc, short-circuit current; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; SEM, scanning electron microscopy; IUM, isolated unsealed human erythrocyte membranes; GP, general polarization; *r*, fluorescence anisotropy.

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doi:10.1016/j.bpc.2005.04.007

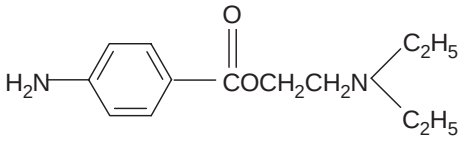

Fig. 1. Structural formula of procaine.

isolated unsealed human erythrocyte membranes (IUM), studied by fluorescence spectroscopy, and (c) molecular models of the erythrocyte membrane consisting in bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), phospholipid classes located in the outer and inner monolayers of cell membranes, respectively [9], particularly of the human erythrocyte membrane [10], which were analyzed by X-ray diffraction. Erythrocytes were chosen because although less specialized than many other cell membranes they carry on enough functions in common with them such as active and passive transport, and the production of ionic and electric gradients, to be considered representative of the plasma membrane in general. In an attempt to further elucidate the effect of procaine on Na^+ transport, the present work examined its influence on the isolated toad skin, taking into account that the toad skin epithelium is a model for transepithelial Na^+ transport, where Na^+ entering the cell across the apical membrane is actively extruded across the basolateral membrane by Na^+–K^+-ATPases [11]. Several reports of procaine effects on frog skin show an increase in transepithelial Na^+ transport and increased Cl^- conductance when procaine was applied in the outside perfusion solution [12,13]; however, the response to this anesthetic is considerably variable in different species of frogs. The procedure used in the current study of procaine effects on the toad skin consisted in the measurement of the electric parameters transepithelial potential difference (PD) and short-circuit current (Isc), which is the amount of current necessary to bring the PD across the skin down to zero, and measures the net transepithelial Na^+ transport across the epithelium [14]. All these systems have been used to determine the interaction with, and perturbing effects on, membranes by the LA dibucaine [15], proparacaine [16], bupivacaine [17] and benzocaine [18].

2. Materials and methods

2.1. SEM studies on human erythrocytes

In vitro interaction of procaine with erythrocytes was achieved by incubating human blood samples taken from healthy male adult donors not currently receiving treatment with any pharmacological agent. Blood samples were obtained after puncture of the ear lobule disinfected with 70% ethanol by aspiration into plastic tuberculin syringes without needles, containing 50 U/ml heparin in saline solution (0.9% NaCl). Red blood cells were centrifuged, washed twice in saline, resuspended in buffer (7.5 mM phosphate, 145 mM NaCl, 5 mM glucose, 1 mM $MgSO_4$, pH 7.4) containing procaine at a final 3 mM concentration and incubated 1 h at 37 °C. Controls were erythrocytes resuspended in incubation buffer without procaine. Red blood cells were fixed overnight at 5 °C by adding one drop of each sample to plastic tubes containing 1 ml 2.5% glutaraldehyde in saline, washed twice with saline, placed on siliconized Al stubs, air-dried at 37 °C for 30 min, and gold coated for 3 min at 10^{-1} Torr in an S 150 sputter device (Edwards S150, Sussex, England). Resulting specimens were examined in an Etec Autoscan SEM (Etec Corporation, Hayward, CA, USA).

2.2. Fluorescence measurements of IUM

The influence of procaine on the physical properties of IUM was examined by fluorescence spectroscopy using DPH and laurdan (Molecular Probe, Eugene, OR, USA) fluorescent probes. DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its fluorescent steady-state anisotropy measurements were used to investigate the structural properties of IUM as it provides a measure of the rotational diffusion of the fluorophor, restricted within a certain region such as a cone, due to the lipid acyl chain packing order. Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra to the physical state of membranes. With the fluorescent moiety within a shallow position in the bilayer, laurdan provides information on the dynamic properties at the level of the phospholipid glycerol backbone. The quantification of laurdan fluorescence shift was effected using the general polarization (GP) concept [19], which is defined by the expression $GP=(I_b-I_r)/(I_b+I_r)$, where I_b and I_r are the emission intensities at the blue and red edges of the emission spectrum, respectively. These intensities have been measured at the emission wavelengths of 440 and 490 nm, which correspond to the emission maxima of laurdan in the gel and liquid crystalline phases, respectively [20]. DPH fluorescence anisotropy (r) was calculated according to the definition: $r=(I_{\parallel}-I_{\perp})/(I_{\parallel}+2I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the corresponding vertical and horizontal polarized emission fluorescence intensities [21]. The emission was measured using a WG-420 Schott high-pass filter (Schott WG-420, Mainz, Germany) with negligible fluorescence. Erythrocytes were separated from heparinized venous blood sample obtained from normal casual donors by centrifugation and washing procedures. IUM were prepared by lysis according to Dodge et al. [22]. DPH and laurdan were incorporated into IUM by the addition of small aliquots of concentrated solutions of the probe in dimethylformamide and ethanol, respectively, and incubated at 37 °C for 1 h. Fluorescence spectra and anisotropy measurements were performed on a Spex Fluorog (Spex Industries Inc., Edison, NJ, USA) and in

Table 1
Effect of procaine on the anisotropy (r) of DPH and the general polarization (GP) of laurdan embedded in isolated unsealed human erythrocyte membrane (IUM) at 37 °C

Procaine (mM)	r (DPH)	PG (laurdan)
0	0.197±0.005	0.200±0.001
1	0.197±0.002	0.195±0.001
2	0.196±0.003	0.186±0.008
4	0.198±0.002	0.172±0.002
6	0.196±0.002	0.170±0.004
10	0.193±0.002	0.156±0.002
16	0.194±0.002	0.153±0.002
20	0.196±0.004	0.148±0.001
30	0.197±0.014	0.146±0.002

a phase shift and modulation Gregg-200 steady-state and time resolved spectrofluorometer (ISS Inc., Champain, IL, USA), respectively, both interfaced to computers. Software from ISS was used for data collection and analysis.

Measurements of IUM suspensions were made at 37 °C using 10-mm path-length square quartz cuvettes. Sample temperature was monitored by an external bath circulator (Cole-Parmer, Chicago, IL, USA) and controlled before and after each measurement using an Omega digital thermometer (Omega Engineering Inc., Stanford, CT, USA). Procaine was incorporated in IUM suspensions by addition of appropriate aliquots in ethanol in order to obtain the different concentrations used in this work. Control experiments to assess the influence of dimethylformamide and ethanol on the anisotropy and GP of samples were performed; the results indicated that at the actual concentrations of 12.9 mM and 17.5 mM, respectively, no effects were apparent. Significant effects were observed only at concentrations above 50 mM. Blank subtraction was performed in all measurements using labeled samples without probes. The data presented in Table 1 represent mean values and standard errors of 10 measurements in two independent samples. Unpaired Student's t-test was used for statistical calculations.

2.3. X-ray diffraction studies of DMPC and DMPE multilayers

Synthetic DMPC (lot 80H8371, A grade, MW 677.9) and DMPE (lot 13H83681, A grade, MW 635.9) and procaine hydrochloride (lot 48H1022, MW 272.8) from Sigma (MO, USA) were used without further purification. About 3.5 mg of each phospholipid were mixed in 2.0 mm diameter glass capillaries (Glas-Technik and Konstruktion, Berlin, Germany) with 200 μl of aqueous procaine solutions (concentration range 10 mM to 360 mM). The specimens were X-ray diffracted in flat-plate cameras with 0.25-mm-diameter glass collimators provided with rotating devices. The blanks consisted of pure samples of each phospholipid with excess water. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from Philips PW 1140 X-ray generator (Eindhoven, Netherlands) was used. The relative reflection intensities on film were measured by peak integration using a Bio-Rad GS-700 densitometer (Hercules, CA, USA) and Molecular Analyst/PC image software; no correction factors were applied. The experiments were performed at 17±2 °C, which is below the main transition temperature of both DMPC and DMPE. Higher temperatures would have induced transitions to more fluid phases, making the detection of structure changes harder.

2.4. Electrophysiological measurements on the isolated toad skin

Skins from toads of either sex (*Pleurodema thaul*, 10–20 g) collected from fresh water ponds were used. The toads were kept at room temperature in bins containing tap water 24 h prior to use and fed on sow bugs (*Oniscus asellus*). The toads were pithed and samples of the abdominal skin were removed, washed in toad Ringer's solution and mounted between two halves of a perspex Ussing-type chamber. A circular area of 1 cm^2 was exposed to 3.0 ml Ringer's solution on each side. The composition of the solution was (in mM): Na^+ 114, K^+ 2.5, Cl^- 117.5, Ca^{+2} 2.0, HCO_3^- 2.3 and glucose 11, and was oxygenated by means of an Elite 800 aerator (R.C. Hagen, W. Yorkshire, U.K.). All experiments were carried out at room temperature (18–22 °C). The Isc was monitored with non-polarizable Ag/AgCl electrodes placed at 15 mm distance from the skin and connected to a voltage-clamp circuit (G. Métraux Electronique, Crissier, Switzerland) set to keep the PD across the skin at 0 mV. The PD was measured with calomel-agar electrodes at intervals of 2 min for 4 s. Both parameters were displayed on a 2-channel Cole-Parmer (Chicago, IL) recorder. 30 min after steady readings had been obtained, procaine was applied in the solution bathing either the outer or the inner surface of the skin in the final concentrations specified in the text. Results are expressed as means±S.E. Student's paired t-test was used for statistical analysis.

3. Results

3.1. SEM Studies on human erythrocytes

SEM observation indicated that 3 mM procaine induced a change in the shape of the erythrocytes. In fact, the erythrocytes underwent a morphological alteration as they changed their discoid shape (Fig. 2A) to stomatocytes (Fig. 2B). According to the bilayer couple hypothesis [23], the shape variations in erythrocytes caused by foreign molecules are due to differential expansion of their two monolayers. Thus, spiculated shapes (echinocytes) are induced when the added compound inserts in the outer monolayer, whereas cup shapes (stomatocytes) arise when the compound accumulates in the inner monolayer. The fact

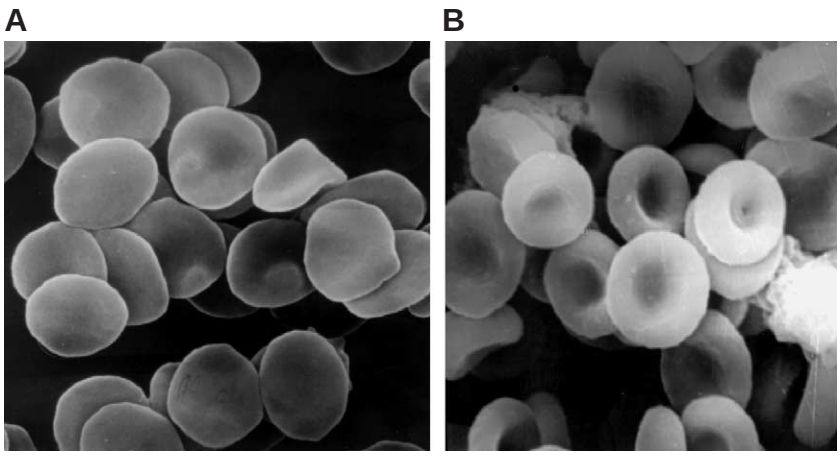

Fig. 2. Effect of procaine on morphology of human erythrocytes. Images (obtained by scanning electron microscopy, 2400×) of (A) untreated erythrocytes and (B) erythrocytes incubated with 3 mM procaine.

that procaine induced the formation of stomatocytes indicates that the anesthetic was located in the inner moiety of the red cell membrane.

3.2. Fluorescence measurements of IUM

The structural effects of procaine on IUM were determined at 37 °C at the acyl chain hydrophobic core and at the hydrophilic/hydrophobic interface regions of the membrane bilayer by evaluation of DPH steady-state fluorescence anisotropy (r) and laurdan GP, respectively. Table 1 shows that the incorporation of procaine in the range 1 to 30 mM produced a 27% decrease in the GP values, while those of the anisotropy (r) remained practically unchanged. These results imply that a disordering effect took place among the polar head groups of the erythrocyte membrane bilayer, whereas the acyl chain groups were not affected.

3.3. X-ray diffraction studies of DMPC and DMPE multilayers

The molecular interactions of procaine with multilayers of the phospholipids DMPC and DMPE in an aqueous medium were determined by X-ray diffraction. Fig. 3A shows a comparison of the diffraction patterns of DMPC alone and of DMPC incubated with procaine in the range of

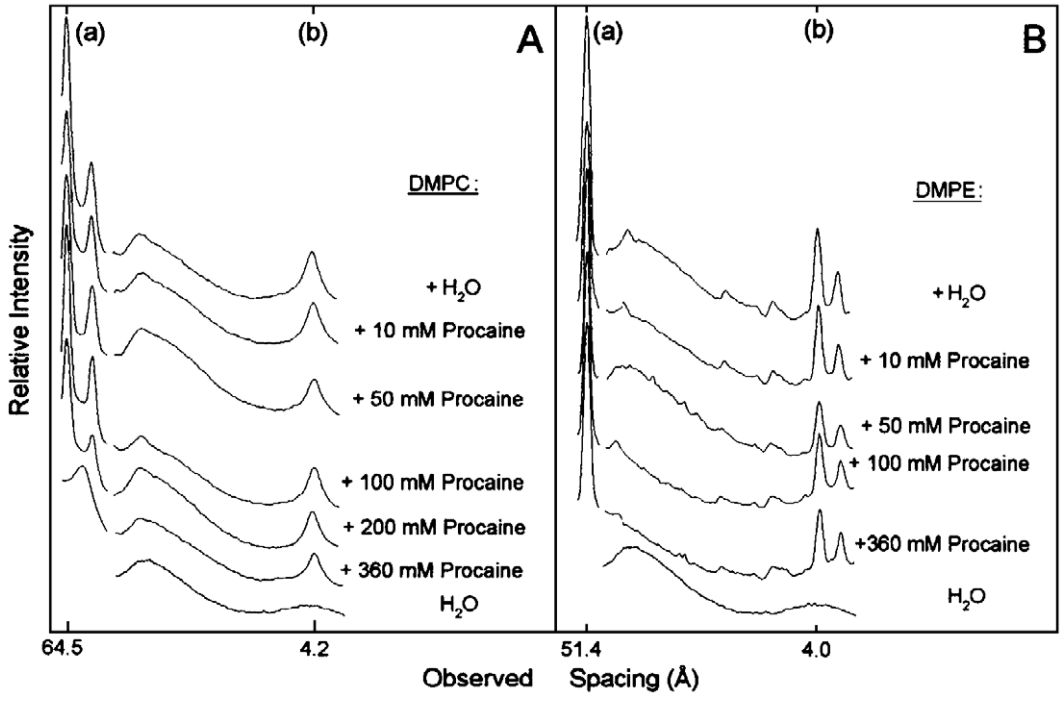

Fig. 3. Microdensitogram from X-ray diffraction diagram of DMPC (A) and DMPE (B) in water and aqueous solutions of procaine; (a) low-angle and (b) high-angle reflections.

10 mM up to 360 mM. As expected, water altered the structure of DMPC: its bilayer repeat increased from about 55 Å in its dry crystalline form [24] to 64 Å when immersed in water, and its reflections were reduced to only the first three orders of the bilayer repeat. On the other hand, a new and strong reflection of 4.2 Å showed up, whose appearance was indicative of the fluid state reached by DMPC bilayers, which corresponded to the average distance between its disordered and hexagonally packed acyl chains. The effects of procaine upon DMPC were observed with the highest assayed concentrations: 200 mM caused a decrease of the low angle reflections (indicated as (a) in the figure), which correspond to the polar head region, and 360 mM procaine induced the total disappearance of these reflections. On the other hand no significant changes were observed in the wide angle region (indicated as (b) in the figure), which corresponds to the acyl region of DMPC. These results imply that procaine induced serious molecular disorder in the polar head groups of DMPC bilayers. Fig. 3B shows the results of the interaction of procaine with DMPE. The perturbing effect of this compound upon the structure of DMPE bilayers was weaker than that induced to DMPC. The two lipids differ only in their terminal amino groups, these being $^{+}N(CH_3)_3$ in DMPC and $^{+}NH_3$ in DMPE. Moreover, both molecular conformations are very similar in their dry crystalline phases [24] with the hydrocarbon chains mostly parallel and extended, and the polar groups lying perpendicular to them. However, the gradual hydration of DMPC results in water filling the highly polar interbilayer spaces. Thus, its bilayer width increases from 54.5 Å when dry up to 64.5 Å when it is fully hydrated. This phenomenon allows the incorporation of procaine into DMPC bilayers, its interaction with the lipid polar groups and the ensuing total perturbation of its polar region at a 360 mM concentration. In contrast, DMPE molecules pack tighter than those of DMPC due to their smaller polar group and inter-lipid hydrogen bonds between amino and phosphate groups, resulting in a very stable bilayer system that is not significantly affected by water [24] nor by a number of compounds [25].

3.4. Electrophysiological measurements on the isolated toad skin

The electrical response of the toad skin to procaine applied in either the outer or the inner bathing solution consisted of a concentration-dependent decrease in PD and in Isc, which may be interpreted as a decrease in ion transport. Fig. 4A and B show that the application of 18 mM procaine was followed by a rapid decline of the electric parameters, which reached a trough in about 25.9±1.3 (n=18) min, and in 85% of the experiments was either irreversible or only partially reversible after repeated washout of the skin, effects which may reflect strong binding to either membrane or cytosol components. Although the duration of the effect was very variable, an average of about 40 min was found; it was followed by a period of spontaneous and partial recovery which lasted

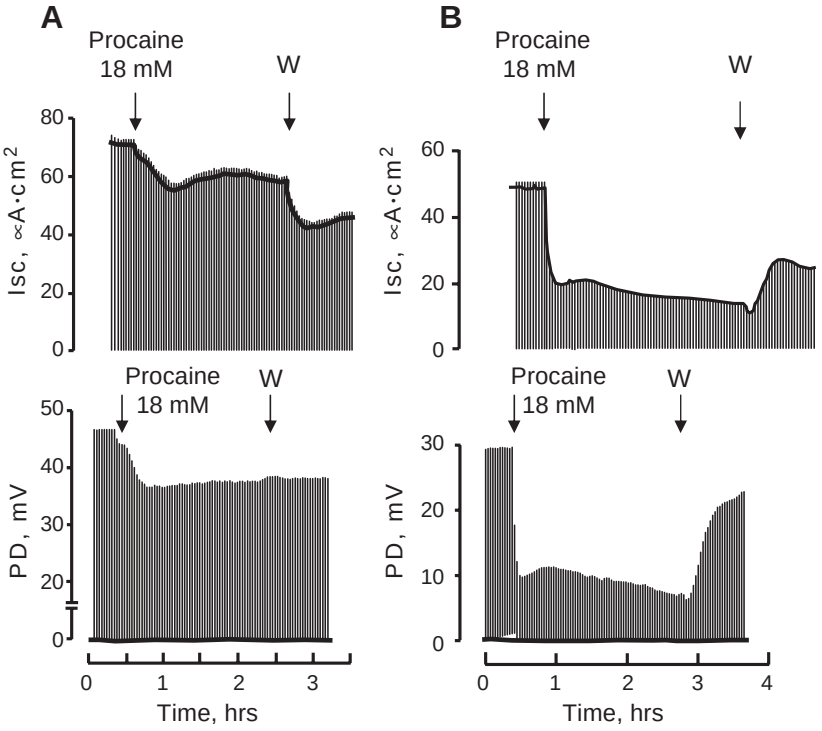

Fig. 4. Single experiment illustrating the time course of the inhibitory effect of 18 mM procaine applied in the outer (A) and in the inner (B) bathing solution, on the electric parameters of the isolated toad skin. Isc=short-circuit current; PD=potential difference; W=washout.

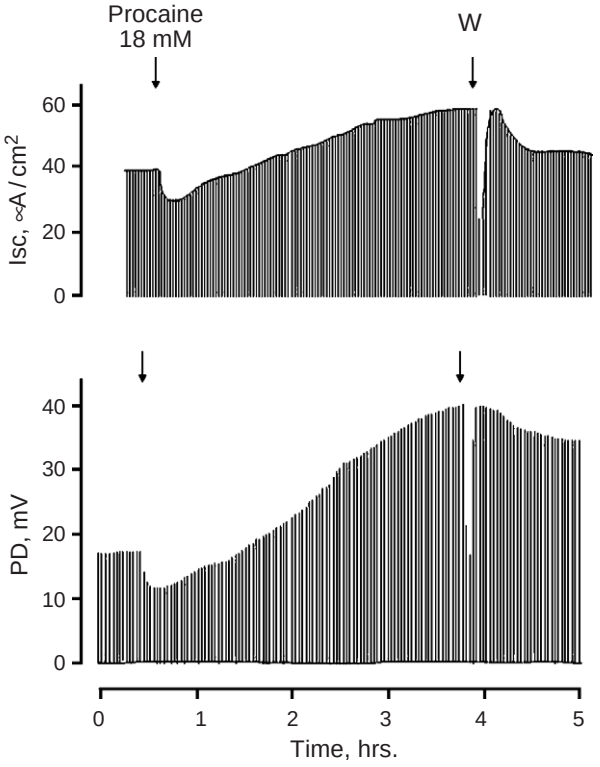

Fig. 5. Single experiment illustrating the time course of a double effect (inhibitory followed by stimulatory) of 18 mM procaine ($n=3$) applied in the outer bathing solution, on the electric parameters of the isolated toad skin. Isc=short-circuit current; PD=potential difference; W=washout.

about 2 h. In most (23) experiments, the decrease induced a decline (12.5±0.1%, $P<0.01$) in resistance, followed by a progressive increase (17.1±0.09%, $P<0.001$) in resistance during the recovery period. These changes in resistance are due to the fact that the decline in PD was usually greater than the decline in Isc, and that during recovery, the PD usually increased to values greater than the values reached by Isc. The slighter reduction in Isc could be due to increased ion transport across paracellular pathways (tight junctions) or to activity of the sodium pump [26]. Fig. 4A also shows that the decline in PD and Isc was only 20% when the anesthetic was applied to the outer surface of the skin, whereas Fig. 4B reveals a far greater effect (a 70% decline) when procaine was applied to the inner surface; the concentration for half-maximal response (EC_{50}) was about 8.5 mM, a value which may be compared with a 50% inhibition of synaptosomal Na^+–K^+-ATPase by 10 mM procaine [27]. Skins isolated from 27 toads were used in order to construct dose–response curves. A noteworthy feature in seven of these experiments was observed: application of either 1.8 mM or 9 mM procaine to the outer surface, after an initial decline lasting less than 40 min, was followed by a gradual increase in Isc lasting over 1 h (20.0±6.9%, $n=4$, $P<0.01$); and 18 mM procaine (Fig. 5), also after an initial decline, induced a 59.0±5.5% increase in Isc lasting about 2.5 h ($n=3$, $P<0.001$). The increment in the PD exceeded that of the Isc. The stimulatory effect was reversed by washout of the anesthetic. The findings encountered with these seven experiments are in accordance with procaine-stimulated transepithelial Na^+ transport across isolated frog skin [28], although the stimulatory effect in the toad skin is far slower than in frog skin. Fig. 6A and B illustrate the concentration-dependent effects of procaine applied to both surfaces of the skin. The greater magnitude of the inhibitory effect of serosally applied procaine could be due to several reasons: there is no cornified layer covering the basolateral membrane and this may contribute to the magnitude of serosal inhibition of ion transport, due to easier procaine access not only to

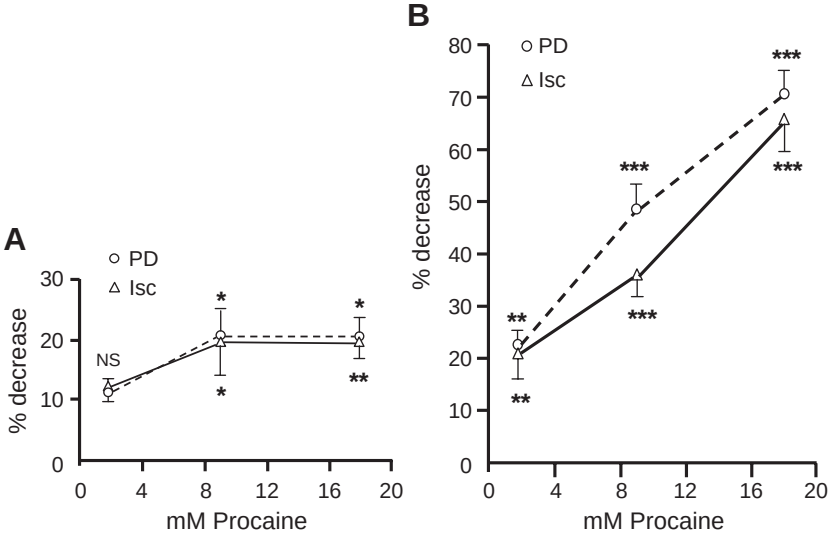

Fig. 6. Effect of procaine in increasing concentrations, on the electric properties of the isolated toad skin. Potential difference (PD) and short-circuit current (Isc) values for untreated skins were 31.7±3.6 mV and 50.3±4.4 μA/cm^2, respectively. Results obtained in the presence of procaine (1.8, 9.0 and 18 mM) are expressed as the percentage decrease of these control values. Each point represents means±S.E.; $n=9$. (A) Procaine applied to the outside surface; (B) procaine applied to the inside surface. Significance by Student's paired t-test: *$P<0.05$; **$P<0.01$; ***$P<0.001$: NS=not significant.

lipid–protein interfaces, but also to ion channels, which are known to be placed in special domains within the apical membrane.

4. Discussion

The foregoing results indicate that procaine, in common with other LA, decreases ion transport across the toad skin. In our work the modest (20%) inhibitory effect of apically applied procaine might be explained considering that only the undissociated molecule of procaine (approximately 6.5% of the total content) is readily lipid soluble and may create finite concentration of the anesthetic in the cytosol of the epithelial cells [13]. During the recovery period, the progressive increase in resistance has been explained because active transport across epithelia is due to: (1) apical membrane permeability; (2) rheogenic activity of Na^+–K^+-ATPase and (3) basolateral K^+ conductance. When resistance increases (or inversely, Na^+ conductance decreases), inwardly rectifying K^+ channels are predominant, and basolateral conductance increases, compensating the decrease in apical membrane permeability, and increasing the transepithelial potential difference which drives ion transport, thus justifying the increase found in many of these experiments after initial inhibition. Especially noteworthy were the seven skins in which outer application of procaine was followed by a notable stimulatory effect; as in other experiments, resistance decreased during initial inhibition and increased during the stimulatory effect (Fig. 5). However, other mechanisms also help to explain this effect. The main hypotheses which attempt to explain the molecular mechanisms of action of procaine in the toad skin are as follows: (a) direct interaction with proteins, particularly epithelial ion channels; (b) induction of structural alterations in their lipid matrix, and (c) action on the lipid–protein interfaces and modulation of enzyme activity. The first mechanism is important for local anesthetics: it should be noted that in most experiments ($n=20$), where procaine was applied to the outer surface of the skin, the results indicating decreased ion transport are not in accordance with the findings of several researchers [12,13] who showed that procaine increased ion transport in three species of frogs, although these results were somewhat variable and a tentative explanation of an unspecific interference with the Na^+ channel was suggested. It is known that the frog skin can both absorb and secrete NaCl: the absorptive system is driven by an active uptake of Na^+ followed passively by Cl^- and the secretory system resides in the glands which actively secrete Cl^- [29]. In accordance with (a), the apical membrane of most epithelia possesses an amiloride-sensitive Na^+ channel (ENaC) [30] which is the rate-limiting step for Na^+ reabsorption in tight epithelia, and which is not homologous with other ion channels such as the voltage-gated Na^+ channels. ENaC is a heteromultimer [31] composed of three subunits (α, β and γ), each with two transmembrane domains (M1 and M2); the α subunit confers ion selectivity [32]. These authors suggest that procaine could bind to heterogeneous subunits; nevertheless, procaine blocked inward Na^+ current in mammalian neuron in a nonspecific manner [33]. This does not rule out the possibility that in the toad skin a minor percentage of the undissociated drug reaches the cytosol from the outer surface and binds to the hydrophobic constriction of the M_1 domain (the gate) at the cytoplasmic end of the channel.

Several papers have shown that the apical Na^+ channel of the frog skin is mainly amiloride sensitive [12,14] and work from our laboratory indicates that *P. thaul* skin also possesses amiloride-sensitive epithelial Na^+ channels [34,35]. The delayed increase of the electrical parameters found in some experiments where procaine was applied to the outer surface of the skin requires further interpretation: although this is speculative, it might be suggested that the increase could be due to mechanisms (b) and (c), leading to an increment in cellular cAMP and activation of protein kinase A, which in turn increases the number of active apical sodium channels [31]. In addition, it should be remembered that the increment in PD exceeded that of the Isc, which might be explained considering the events initiated by the application of mucosal procaine [36]: these authors demonstrated that the increase in Isc is associated with a decrease in sodium conductance and predominance of inwardly rectifying K^+ channels, which thereby increase basolateral conductance and increase the transepithelial PD which drives ion transport and/or ion channels. Procaine inhibited dog kidney Na^+–K^+-ATPase [37], canine renal medulla Na^+–K^+-ATPase ($IC_{50}=17.7$ mM) [38]; the last mentioned study supports the interpretation that the uncharged forms of LA are much more potent inhibitors of Na^+–K^+-ATPase activity than the cationic forms. In addition, procaine has been found to inhibit the Ca^{2+}-dependent ATPase activity of rat brain synaptosomes [39] and of the human erythrocyte membrane [40]. A dose-dependent increase in intracellular calcium $[Ca^{2+}]_i$ is evident after treatment with LA, and the increase in cytosolic Ca^{2+} inhibits apical sodium channels in frog skin epithelium through activation of protein kinase C [41]. The connection between the localization of procaine in the membrane and Na^+ channel blocking is as follows: the anesthetic interacts with the phospholipid bilayer (particularly phosphatidylcholine) surrounding the channel proteins, thus perturbing the functions of these proteins.

The X-ray experiments showed that procaine disordered the polar region of DMPC (the major class of lipid present in the outer monolayer of the erythrocyte membrane). This result agrees with X-ray diffraction experiments made by Coster et al. [42]. They concluded that the polar tertiary amine group of these molecules is almost certainly located in the polar head group region on lecithin/cholesterol multilayers with very high water content and containing procaine. Arias et al. [43] also observed that procaine interacted with the phospholipid polar head group. These

results [42,43] tend to agree with those obtained by our X-ray experiments which showed that procaine was principally located at the DMPC polar group level, and also with our assays performed at 37 °C on IUM, which showed that procaine induced a 27% decrease of the GP, indicative of an enhancement of disorder at the polar head region of the lipid.

Electron microscopy observations of human erythrocytes incubated with procaine indicated that the anesthetic was probably located in the inner moiety of the red cell membrane. However, X-ray diffraction of phospholipid bilayers showed that procaine most likely interacted with the lipids located in the outer monolayer of the erythrocyte membrane. The explanation for this discrepancy with the bilayer couple hypothesis could be based on the "lipid scrambling mechanism" proposed by Schrier et al. [44]. According to them, some cationic amphipaths produce a rapid scrambling of the erythrocyte bilayer with phosphatidylcholine (PC) and sphingomyelins (SM) moving inward while phosphatidylethanolamines (PE) move outward along with phosphatidylserines (PS). Thus, the interaction of procaine with PC in the inner monolayer would lead to stomatocytosis, an effect that can be produced by as little as 0.6% enrichment of the cytoplasmic monolayer [44]. In conclusion, our results showed that procaine interacted with phospholipid bilayers, particularly with PC. Therefore, cell membrane structure and physiological properties such as fluidity, permeability, receptor channel functions may be affected.

Acknowledgements

To FONDECYT (Project 1020476).

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