Trifluoperazine-sensitive activation of the spontaneous transmitter release at the frog motor endplates by low doses of procaine

Institute of Medicine and Pharmacy, Department of Physiology, Universității Str. 16, Iași, R-6600, Romania

Summary. Low concentrations of procaine (10⁻⁶ - 5 × 10⁻⁵ mol/l) induced a significant increase of the spontaneous quantal transmitter release at the neuromuscular junctions of the frog cutaneous pectoris nerve-muscle preparation. The frequency of miniature endplate potentials (mepps) was increased although their size slightly decreased probably on the account of a partial block of Na⁺-channels at the postsynaptic membrane. The activatory effect of procaine was not altered under experimental conditions known to change the Ca²⁺ fluxes across the nerve terminal membrane such as using a Ca²⁺-free Ringer, or a Ca²⁺-channel blocker (D₂₀₀), a high K⁺ Ringer or, finally, a low Na⁺ Ringer. In the presence of caffeine no change of procaine-induced activation appeared. Trifluoperazine (TFP), in a concentration known to specifically block calmodulin, completely blocked the procaine-induced increase of mepp frequency. These data suggest that procaine presumably by way of a calmodulin-dependent mechanism is related to the free cytosolic Ca²⁺ equilibrium. It is possible that procaine increases the free cytosolic Ca²⁺ concentration by blocking an active calmodulin-dependent Ca²⁺ extrusion mechanism.

Key words: Procaine – Transmitter release – Neuromuscular junction – Trifluoperazine – Calmodulin

Introduction

In anaesthetic concentrations (higher than 5 × 10⁻⁵ mol/l) procaine, as well as other local anaesthetics, is known to block synaptic transmission at the neuromuscular junction both by interacting with pre- and postsynaptic sites (Hille 1977; Gage et al. 1983). In the presynaptic nerve terminal these agents block the voltage-dependent Na⁺-channels which results in a decrease of the action potential amplitude, hyperpolarization, decreased excitability and block of nerve impulse conduction. As a consequence, the presynaptic nerve stimulation does not induce a transmitter release anymore, and synaptic transmission is blocked (Straughan 1961; Matthews and Quilliam 1964; Hille 1977). Beside their presynaptic effects the anaesthetic doses of procaine are active also at the postsynaptic membrane. By blocking the end-plate channels, procaine decreases the amplitude of postsynaptic potentials (Kordas 1970; Gage et al. 1983).

In our experiments we investigated the influence of low concentrations of procaine upon the synaptic transmission at the neuromuscular junction of frogs. Under these conditions it was hoped that, by minimizing the pre- and postsynaptic blocking effects of procaine already described, it could be possible to uncover some other action specifically directed toward the mechanisms of spontaneous release of transmitter quanta from the nerve ending.

Methods

The procaine effects upon synaptic transmission were investigated by using conventional microelectrode techniques in the cutaneous pectoris nerve-muscle preparation of both summer and winter Rana ridibunda frogs. The experimental details are the same as described previously (Brănișteanu et al. 1979). Briefly, animals were decapitated, the muscle was rapidly removed and carefully dissected in Ringer solution containing (mM): NaC1, 111; KCl, 2.5; CaCl₂, 1.8; Na₂HPO₄, 0.85; Na₂HPO₄, 2.15. After an equilibration period for at least 2 h in Ringer solution at 1–4°C, the dissected muscle was transferred to the experimental chamber and fixed with miniature pins on the silicon rubber bottom (Sylgard). The perspex bath (capacity 1.5 ml) was perfused with Ringer solution at a rate of 4 ml/min and at room temperature (20°C) by using a constant flow roller pump (Minipuls 2 Gilson). Any change of the Ringer solution was done on an isoosmotic basis by changing the NaCl concentration in order to maintain the osmolarity (256 mOsm).

Glass microelectrodes filled with KCl 3 mol/l (resistance 5–12 MΩ) were inserted into the region of the neuromuscular junction using a micromanipulator. Only the superficial fibers were used (inner surface of the muscle facing upward). The post-synaptic location of the microelectrode tip was confirmed by the recording of miniature endplate potentials (mepps) with rise times shorter than 1.5–2 ms. These potentials were displayed on an oscilloscope and recorded by photographic means.

After continuous recording of mepps throughout the experiments the records were divided into periods of 10 min each, mepps were counted and frequencies (s⁻¹) calculated. Whenever necessary amplitudes were also measured by using optical enlargement.

The resting potential was monitored by using a DC pen recorder (Texas Instr. Dallas, USA). Only experiments during which the resting potential was stable (less than 10% variation) were taken into consideration.

The drugs used were: procaine HCl (Novocain, Hoechst, Frankfurt, FRG), D600-gallopamil (Procorum, Minden...
Results

Postsynaptic effects of procaine

Procaine HCl induced a dose-dependent and reversible reduction of mepp amplitude (Fig. 1) which was significant starting at $5 \times 10^{-5}$ mol/l. Frequency-amplitude histograms of mepps recorded at each procaine concentration indicate that, for procaine concentrations higher than $5 \times 10^{-5}$ mol/l, the lowest range mepps become undetectable (Fig. 1). Therefore, the highest concentration of procaine used in subsequent experiments was $5 \times 10^{-5}$ mol/l and only those experiments were taken into consideration in which the recorded mepps did show a normal frequency-amplitude distribution.

Procaine action upon the spontaneous release of acetylcholine quanta

Low concentrations of procaine HCl ($5 \times 10^{-7}$ to $5 \times 10^{-5}$ mol/l) resulted in significant increases of mepps frequency (Fig. 2a). The threshold concentration of procaine for this effect was $1 \times 10^{-6}$ mol/l. The frequency increase reached a maximum value of 75% at concentration of $5 \times 10^{-5}$ mol/l. The latency for the effect to develop was 15-20 min after starting the perfusion, and normal values were reached after more than 40 min washout (Fig. 2b). The frequency-amplitude histograms of mepps before, during, and after procaine perfusion demonstrated the complete recovery of the spontaneous quantal transmitter release (not shown).

Chem., Minden, FRG, trifluoperazine HCl (Serva, Heidelberg, FRG), caffeine (Serva).

Fig. 1
Upper left: Effects of procaine upon mepp amplitude. Each point represents the mean value ($\pm$ SE) obtained in 5 different preparations (150 mepps each) after 30 min in the mentioned concentration of procaine. Differences in procaine concentrations higher than $5 \times 10^{-5}$ mol/l are significant (Student t-test: $p < 0.05$). Below left: Mepp recordings in the absence and in the presence of procaine in the same neuromuscular junction. Right: Frequency-amplitude histograms of mepps recorded in the same neuromuscular junctions. Concentrations of procaine higher than $5 \times 10^{-5}$ mol/l alter the normal distribution.

Fig. 2. a Procaine induced increase of mepp frequency. Mean values ($\pm$ SE) obtained in 8 different preparations after 30 min of exposure to the mentioned concentration of procaine (150 mepps each). Values were normalized to the reference value obtained in the absence of procaine ($1 = 1.5 \pm 0.42$ mepps x s$^{-1}$). At procaine concentrations higher than $5 \times 10^{-3}$ mol/l the frequency values decrease due to mepps loss (see also Fig. 1). Differences were significant at procaine concentrations between $10^{-6}$ and $10^{-4}$ mol/l ($p < 0.05$). b Time course of procaine action upon mepp frequency. Mean values ($\pm$ SE) obtained in 8 different preparations. Values normalized as shown before (a). Differences were significant ($p < 0.05$).