EFFECT OF SOME SURFACTANTS ON NEUROMUSCULAR TRANSMISSION OF EXCITATION

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The effect of the sodium salts of bile acids (BA), saponin, and Tween 80 on end-plate potentials (EPPs) of the myoneural junction of the frog sartorius muscle was investigated by a microelectrode method. Salts of BA in a concentration of 10^{-5} g/ml had no appreciable effect on these potentials, but with an increase in their concentration to 10^{-4} g/ml the amplitude of the EPPs was increased by 1.5-3 times. During the action of BA in a concentration of 10^{-3} g/ml the same effect was observed, but there was a much more rapid increase in amplitude of the EPPs followed by a decrease. Saponin and Tween 80 were less effective as regards the EPPs, but they did affect the contractile activity of the muscle. The increase in amplitude of EPPs under the influence of the substances tested is due principally, it is suggested, to stimulation of the liberation of acetylcholine by nerve terminals.

KEY WORDS: microelectrode method; surfactants; end-plate potential.

Because of the asymmetrical structure of their molecules and their well-developed water-repellent radicals, surfactants can actively affect the hydrophobic bonds of lipoprotein complexes of membranes as well as the conformation of enzyme proteins. There have been several investigations into the effect of surfactants on the membrane of various cells [5, 7] and also on the activity of enzymes located on the membrane [6, 8]. Most investigations of the effect of these substances have been carried out on separate subcellular fractions. No electrophysiological data on the effect of surfactants on neuromuscular transmission, on which they may be considered to be particularly effective, have yet appeared in the literature.

An investigation of changes in the end-plate potential (EPP) during the action of various surfactants and, in particular, those found in the body (salts of bile acids – BA), is described below.

EXPERIMENTAL METHOD

A nerve-muscle preparation of the frog (Rana ridibunda) sartorius muscle was used. The preparation was kept in a chamber with two compartments. The muscle was placed in a compartment containing running Ringer's solution. All experiments were carried out at room temperature. The sciatic nerve was placed on nonpolarizing electrodes in the other part of the chamber, which was filled with mineral oil and stimulated by square pulses 0.5 msec in duration. EPPs were recorded intracellularly by glass electrodes with a resistance of 10-15 MΩ. The microelectrode was connected to the grid of a cathode follower which, in turn, was connected to a type UBP-01 dc amplifier. After amplification, the signal was led to the vertical deflecting plates of a type S1-16 cathode-ray oscilloscope. Sweeps of the beam, synchronized with the stimulation, were recorded on photographic film on the oscilloscope screen by means of the FOR-2 camera.

Ringer's solution of the following composition was used: NaCl 118 mM, KCl 2.5 mM, $CaCl_2$ 1.9 mM. Neuromuscular transmission was blocked by D-tubocurarine chloride in concentrations of $0.9 \cdot 10^{-6}$ to

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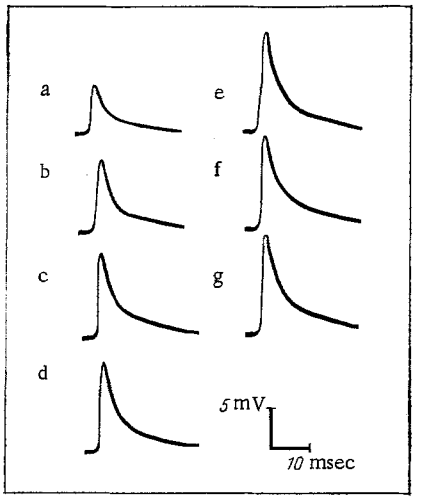

Fig. 1

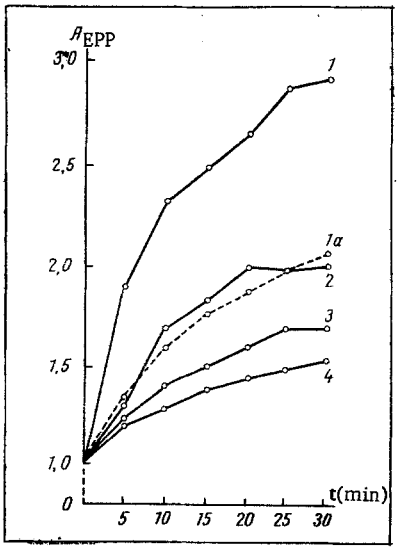

Fig. 2

Fig. 1. Effect of sodium glycocholate on EPP amplitude: a) EPP in Ringer's solution; b, c, d, e, f, g) EPPs after exposure to sodium glycocholate in a concentration of $1 \cdot 10^{-4}$ g/ml for 5, 10, 15, 20, 25, and 30 min, respectively.

Fig. 2. Changes in EPP amplitude under the influence of salts of BA: 1) sodium choleate; 2) sodium glycocholate; 3) sodium cholate; 4) sodium deoxycholate; 1a) repeated action of sodium choleate. Abscissa, time of action of substance (in min); ordinate, amplitude of EPPs (in relative units).

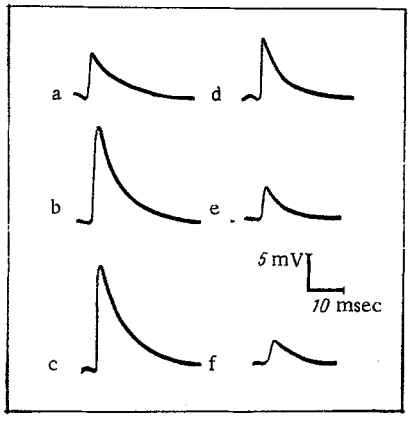

Fig. 3. Action of sodium choleate on EPPs: 1) EPP in Ringer's solution; b, c, d, e, f) EPPs after exposure to sodium choleate in a concentration of $1 \cdot 10^{-3}$ g/ml for 2, 5, 8, 10, and 13 min, respectively.

$1.3 \cdot 10^{-6}$ g/ml. Anionic surfactants – the sodium salts of BA (deoxycholic, glycocholic, cholic, and choleic acids) – and also nonionogenic surfactants (saponin and Tween 80) were used in concentrations of between 10^{-5} and 10^{-3} g/ml.

EXPERIMENTAL RESULTS

The experiments showed that sodium salts of BA in a concentration of 10^{-5} g/ml had no appreciable effect in most experiments on EPPs, but with an increase in their concentration to 10^{-4} g/ml, a marked increase in amplitude of the EPPs was observed. As Fig. 1 shows, during the action of sodium glycocholate the amplitude of the EPPs increased by 30% over a period of 5 min. This rapid increase in amplitude continued for 20 min (Fig. 1a, b, c, d, e), but then its pace slowed somewhat (Fig. 1f, g) and, by the 30th minute of action of sodium glycocholate, the amplitude became stabilized at a level twice as high as initially, or in some cases it decreased a little.

Salts of other BA had a similar action on the amplitude of the EPPs. However, a substantial quantitative difference in the action of these substances must be noted (Fig. 2). Deoxycholate, for instance, increased the EPP amplitude by 1.5 times, cholate by 1.6 times, and glycocholate by 2.0 times. Sodium choleate, which increased the EPP amplitude by almost three times, was the most effective. Although the absolute increase in amplitude of EPPs thus varied depending both on the actual value of the EPP and the nature of the salt, on the whole the sodium salts of all BA tested in a concentration of 10^{-4} g/ml were found to have a similar action on the synaptic potential, which was increased relative to its initial value by 1.5-3 times. The resting membrane potential (RP) and the time constant of the membrane showed no significant change. If exposure of the specimen to a given concentration was preceded by exposure to a lower concentration (10^{-5} g/ml), the effect was much

weaker (Fig. 2, 1a). To restore the initial value of the EPP, the preparation had to be rinsed for a long time, for rinsing for only 30 min was followed by only partial recovery.

Sodium salts of BA in a concentration of 10^{-3} g/ml sharply increased the EPP amplitude in the first 5-7 min. However, this increase did not exceed the maximal increase in amplitude following exposure to the corresponding salt in a concentration of 10^{-4} g/ml; it merely developed much faster. The amplitude of the EPPs then fell, until their total disappearance after 10-15 min (Fig. 3). In some experiments as the amplitudes of the EPPs were reduced, contractile activity of the muscles appeared. Rinsing restored the EPPs only very slightly.

These results point to the particularly effective influence of salts of BA on neuromuscular transmission of excitation. They support the observations of Ganitkevich [2, 3], who found that mediator systems are highly sensitive to BA. The amplitude and duration of EPPs are known to depend on the state of the pre-and postsynaptic membranes and also on acetylcholinesterase (ACE) activity. Contradictory data on changes in ACE activity under the influence of surfactants are given in the literature. Koelle and Hossaini [8], for instance, found a decrease in ACE activity in cholinergic structures of the stellate and ciliary ganglia of the cat under the influence of Triton X-100. Aprison and Jackson [6] found that nonionogenic surfactants (Renex 690, Tween 80) potentiate the activity of this enzyme by about 1.5 times, whereas anionogenic and cationogenic surfactants inhibit the enzyme. However, substances inhibiting the action of ACE and potentiating the action of acetylcholine are known to increase the duration of EPPs considerably as well as to increase their amplitudes, [4] and this was not observed in the present experiments. The changes in EPP amplitude under the influence of surfactants thus cannot be explained by inhibition of ACE activity.

On the other hand, during the action of salts of BA in the concentrations tested, like other workers [5, 7], the present writers observed no marked changes in RP and, consequently, the increase in EPP amplitude could not be attributed to hyperpolarization of the membrane. Presumably the observed increase in EPP amplitude arises through increased secretion of acetylcholine. Preliminary experiments with miniature end-plate potentials (MEPPs) confirmed this hypothesis, for the frequency of the MEPPs was appreciably increased by surfactants.

A high concentration (10^{-3} g/ml) of salts of BA led to an initial increase in EPP amplitude followed by a decrease, for the surfactant in that concentration acts directly on nerve terminals and impairs impulse conduction [7].

It can be concluded from these results that correlation exists between the size of the hydrophobic part of the molecule of the substances of the homologous series (salts of BA) and their physiological activities, for with an increase in molecular weight in a series of salts of BA their ability to increase the amplitude of EPPs increases. This hypothesis was confirmed by Vavilova's observations on the activation of Mg-dependent ATPase by various detergents [1].

The nonionogenic surfactants saponin and Tween 80 differ considerably in their effects on neuromuscular transmission from the sodium salts of BA, which are anionogenic compounds. For instance, in a concentration of 10^{-4} g/ml Tween 80 caused no appreciable change in EPP amplitude over a period of 30 min. During the next 30 min, the amplitude increased on the average by 20% and it remained at that level after rinsing. The RP and time constant of the membrane remained unchanged.

During the action of the nonionogenic vegetable surfactant saponin in a concentration of 10^{-4} g/ml the amplitude of the EPPs was unchanged. However, with an increase in the saponin concentration to 10^{-3} g/ml, muscular contractions appeared during the first few minutes of its action and it became impossible to record EPPs.

It can be concluded from these experiments that the physiological effect of a surfactant depends on whether the substance concerned is ionogenic or nonionogenic, and in the case of substances of a homologous series, on the size of the hydrophobic part of the molecule.

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