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EFFECT OF VERAPAMIL AND MANGANESE IONS ON ACETYLCHOLINE SENSITIVITY OF THE INTACT AND DENERVATED FROG MUSCLE FIBER MEMBRANE

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One result of interaction between acetylcholine (ACh) and its receptor (AChR) in the muscle fiber is a transient change in ionic permeability of the muscle membrane, followed by a depolarization step of membrane potential (MP). The magnitude of the depolarization response of the denervated rat muscle fiber membrane to microapplication of ACh is reduced by verapamil and its derivative, compound D-600 [3], which can block the inward calcium current in objects such as the cardiomyocyte membrane [9], the molluscan neuron soma [10], or the squid giant axon [5]. Consequently, it can be postulated that the decrease in the ACh potential takes place because of absence of the contribution of Ca^{2+} to its amplitude. However, complete replacement of Ca^{2+} in the solution by their antagonists, Mn^{2+} ions [2], has no significant effect on the value of the ACh potential [3]. All this suggests that the action of verapamil and D-600 on ACh sensitivity of the denervated rat muscle fiber membrane cannot be reduced simply to loss of the contribution of Ca^{2+} to the amplitude of the ACh potential.

Extrasynaptic AChR denervated frog muscle fibers are known to differ from synaptic AChR of the innervated end plate [7].

It was accordingly considered interesting to compare the effect of verapamil on ACh sensitivity of the membrane of denervated and intact frog muscle fibers. The investigation described below was undertaken for this purpose.

EXPERIMENTAL METHODS

Experiments were carried out on the frog sartorius muscle in winter, using the ordinary microelectrode technique. The muscel was denervated under ether anesthesia by extirpation

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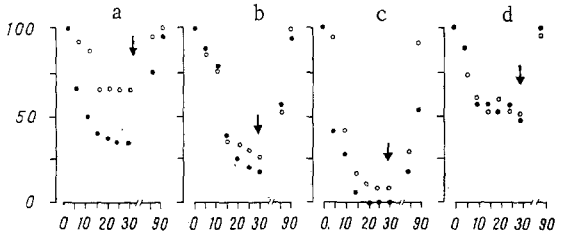

Fig. 1. Effect of verapamil and Mn^{2+} ions on ACh sensitivity of membrane of innervated (empty circles) and denervated (filled circles) frog sartorius muscle fibers. a) Verapamil concentration 10^{-6} g/ml, b) 10^{-5} g/ml, c) 10^{-4} g/ml, d) action of Mn^{2+} ions. Arrow indicates beginning of washing muscle preparation. Abscissa, time (in min); ordinate, ACh sensitivity (in %).

of a segment (2-3 mm) of the nerve at a distance of 1.5-2 cm from its point of entry into the sartorius muscle. The denervated muscles were used in the experiments after 19-21 days, when most of the membrane of the frog muscle fiber becomes sensitive to ACh [1]. All frogs were kept at room temperature. ACh potentials were recorded and the input resistance (R_o) of the muscle membrane measured by the method described previously [1]. The amplitude of the ACh potentials was corrected for the value of MP, namely —90 mV [8]. The equilibrium potential for ACh responses was taken to be —15 mV [6]. During the experiment the muscle was kept in a bath with continuously flowing Ringer's solution of the following ionic composition (in mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.8, in phosphate buffer, pH 7.25 at 20.0 ± 0.5°C.

The experimental scheme was as follows. An area of the muscle fiber membrane which responded to ACh, applied from a micropipet by a pulse of current 0.2-0.5 msec in duration by depolarization with a leading edge of several milliseconds, was found. If the amplitude of the ACh potential remained unchanged for 15-20 min, the solution bathing the muscle was replaced by a solution containing verapamil hydrochloride (from LEK) or by Ringer's solution in which all Ca^{2+} ions were replaced by Mn^{2+} ions (5 mM), and ACh potentials were then recorded every 5 min for 30 min. The muscle preparation was then washed with the original Ringer's solution and ACh potentials were recorded 30 min and 1 h after the beginning of washing.

The effect of verapamil or of replacement of Ca^{2+} in the solution by Mn^{2+} was determined by studying the change in ACh sensitivity of the membrane, measured in millivolts/nanocoulomb as a percentage of its initial value. The data thus obtained were analyzed by Van der Waerden's X criterion [4].

EXPERIMENTAL RESULTS

Addition of verapamil to the solution bathing the muscle reduced the ACh sensitivity of the postsynaptic membrane of intact muscle fibers. The degree of reduction of ACh sensitivity under these circumstances depended on exposure and the concentration of verapamil in the solution (Fig. 1a-c). ACh sensitivity of the postsynaptic membrane 20 min after replacement of the solution, in the presence of 10^{-6} g/ml verapamil, was 63 ± 18% (n = 6). With a similar exposure verapamil in a concentration of 10^{-5} g/ml reduced ACh sensitivity of the postsynaptic membrane to 31 ± 9% (n = 5) and in a concentration of 10^{-4} g/ml it reduced it to 8 ± 4% (n = 5). Values of MP (84.3 ± 0.6 mV; n = 30) and R_o (438 ± 35 kΩ; n = 8) of the membrane were unchanged. The action of verapamil was abolished practically completely by washing the muscle for 1 h (Fig. 1a-c).

Replacement of all the Ca^{2+} ions by Mn^{2+} also reduced the ACh sensitivity of the postsynaptic membrane: 20 min after the change of solution it was 53 ± 14% (n = 6) (Fig. 1d). The ACh sensitivity of the postsynaptic membrane was completely restored 30 min after the beginning of washing. The decrease in ACh sensitivity was accompanied by some decrease in MP to 10 mV; R_o of the membrane remained unchanged under these circumstances (450 ± 40 kΩ; n = 7).

The amplitude of the ACh potential depends on the following factors: the ACh sensitivity of the postsynaptic membrane (properties of the receptor-tubule complex), the magnitudes of MP and R_o of the membrane [8]. Since the last two parameters were unchanged by verapamil, it follows that this drug reduces ACh sensitivity of the postsynaptic membrane through its direct action on the receptor-tubule complex. Verapamil is known to block calcium channels [5, 9, 10]. However, complete replacement of Ca^{2+} in the solution by Mn^{2+} ions, another blocker of calcium channels, did not change the ACh sensitivity of the postsynaptic membrane, just as was the case in the presence of verapamil. Hence it follows that the decrease in the ACh sensitivity of the postsynaptic membrane under the influence of verapamil in frogs is not accounted for entirely by absence of the contribution of the calcium component of the current to the amplitude of the ACh potential.

Verapamil reduced the ACh sensitivity of the membrane in denervated frog muscle also, just as was demonstrated previously in rats [3]. For instance, after 20 min in solution with verapamil the ACh sensitivity of the denervated muscle fiber membrane was 35 ± 7% (n = 6) if the verapamil concentration was 10^{-6} g/ml, 21 ± 9% (n = 6) if the concentration was 10^{-5} g/ml, and sensitivity was completely absent (n = 5) if the concentration was 10^{-4} g/ml (Fig. 1a-c). The last of these observations differs statistically significantly ($P < 0.05$) from data obtained on the innervated end plate. ACh sensitivity of the muscle membrane was almost completely restored after washing for 1 h. The values of MP (81 ± 0.8 mV; n = 30) and R_o (609 ± 40 kΩ; n = 7) of the denervated muscle fiber membrane remained unchanged in the course of the experiment, just as in experiments with innervated muscle fibers.

Removal of Ca^{2+} from the solution and their replacement by Mn^{2+} changed the ACh sensitivity of the denervated muscle fiber membrane almost exactly the same as in the control (Fig. 1). ACh sensitivity 20 min after the change of solution was 60 ± 18% (n = 6). This effect was completely abolished after washing the muscle for 30 min. The decrease in ACh sensitivity was accompanied by a fall in the value of MP on average by 10 mV.

The maximal ACh sensitivity of the denervated muscle fiber membrane, namely 276 ± 85 mV/pC (n = 23), did not differ significantly from the initial values, namely 242 ± 60 mV/pC (n = 22).

Verapamil thus depresses ACh sensitivity of the denervated muscle fiber membrane by a greater degree than that of the innervated end-plate membrane. This difference evidently was not due to unequal values of maximal ACh sensitivity and cannot be attributed entirely to the action of verapamil as an agent limiting the contribution of Ca^{2+} to the amplitude of the ACh potential.

Data in the literature indicating that extrasynaptic and synaptic AChR of the frog muscle membrane are not identical [7] are thus confirmed by the difference in intensity of the effect of verapamil on them, as demonstrated by the present experiments.

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