LITERATURE CITED

1. O. A. Gomazkov, Patol. Fiziol., No. 2, 75 (1980).
2. M. P. Gorizontova, O. V. Alekseev, and A. M. Chernukh, Byull. Eksp. Biol. Med., No. 3, 22 (1975).
3. A. M. Chernukh, P. Oeme, and O. A. Gomazkov, Byull. Éksp. Biol. Med., No. 9, 259 (1980).
4. D. Grisack, D. Cannon, P. Skrabanek, et al., Hormone Metab. Res., 11, 448 (1979).
5. A. Johnson and E. Evdos, Proc. Soc. Exp. Biol. (New York), 142, 1252 (1973).
6. O. Hägevmark, T. Hökfelt, and B. Pernow, J. Invest. Derm., 71, 233 (1978).
7. J. A. Kiernan, Experientia, 28, 653 (1972).
8. J. A. Kiernan, Quart. J. Exp. Physiol., 60, 123 (1975).
9. F. Zemleck et al., Naunyn-Schmiederberg's Arch. Pharmacol., 310, 175 (1979).
10. P. Skrabanek, D. Cannon, J. Kirrane, et al., Irish J. Med. Sci., 145, 399 (1976).

EFFECT OF CALCIUM ANTAGONISTS ON THE ACTION POTENTIAL OF MUSCLE FIBERS OF WARM-BLOODED ANIMALS

B. Yu. Kafengauz, M. A. Poskonova, and L. I. Chudakov

UDC 612.741.15

KEY WORDS: diaphragm; action potential of muscle fiber; denervation; blockers of calcium channels.

Verapamil and its derivative D 600 block the inward calcium current more effectively than polyvalent cations and are used to study calcium channels [6]. These compounds inhibit the electromechanical coupling system, which triggers the inflow of calcium through the outer cell membrane in smooth muscles and the myocardium of mammals [6, 9, 10]. They have been used as calcium antagonists in experiments on the squid axon [4], molluscan neurons [11], and other objects. More recently, however, it has been shown that verapamil and its structural analogs can also inhibit the current formed by sodium ions [4, 5, 13].

The object of this investigation was to study the effect of D 600 on the magnitude, duration, and rate of rise and fall of the action potential (AP) of the diaphragm muscle, when the motor innervation is preserved or absent.

EXPERIMENTAL METHOD

Preparations of mouse diaphragm were used. To compare the results, innervated and denervated halves of the diaphragm from the same animal were used. Denervation was carried out under ether anesthesia by dividing the left phrenic nerve inside the thorax. The experiments were carried out 4 days after operation. The isolated muscle preparation was placed in a constant-temperature chamber (28-30°) with continuously flowing oxygenated Lilly's solution of the following composition: Na^+ 152 mM, K^+ 4 mM, Cl^- 149 mM, Mg^{2+} 1 mM, Ca^{2+} 4 mM, $H_2PO_4^{2-}$ 0.9 mM, HCO_3^- 16.2 mM, glucose 11 mM; pH 7.2-7.4. APs were evoked by intracellular stimulation. The recording electrode and the stimulating electrode filled with potassium citrate were inserted into the muscle fiber 50 μ apart. To compare APs obtained under different experimental conditions, the membrane potential (MP) of the recorded muscle fiber was kept strictly constant, by inducing local hyperpolarization of the membrane by passing a current through the stimulating electrode. To assess the maximal rate of rise of AP, a RC-differential circuit with time constant of 10^{-5} sec was used. In the course of the experiment the original solution was replaced by a solution containing D 600 in a concentration of $5 \cdot 10^{-6}$-$1 \cdot 10^{-4}$ g/ml. The muscle fiber was stimulated either by a single pulse or by a series of pulses with a following frequency of 1 and 2 Hz. Usually the characteristics of the first and 10th responses, when any further change in AP ceased, were compared in a series of reactions.

Department of Physiology of Man and Animals, Biological Faculty, M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 11, pp. 517-520, November, 1981. Original article submitted May 26, 1981.

0007-4888/81/9211-1466$07.50

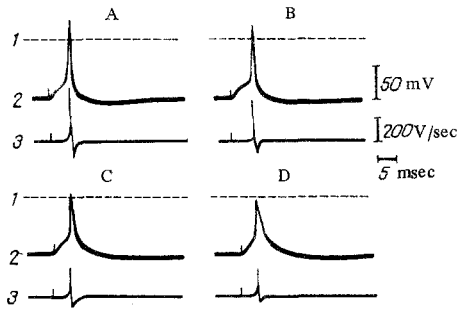

Fig. 1. Intracellular recording of AP and its direct derivative in innervated (A) and denervated (B) muscles under normal conditions and after addition of D 600 in a concentration of $1 \cdot 10^{-4}$ g/ml (C, D). 1) Zero potential level; 2) AP; 3) first derivative of AP.

EXPERIMENTAL RESULTS

The time constant of the membrane did not change significantly during the 4 days after denervation of the muscle fibers [12]. The difference between MP of the innervated and denervated fibers was abolished by local polarization of the membrane to a constant level of between −90 and −100 mV.

APs of innervated and denervated muscle fibers differ appreciably in their rate of rise and decay, in their amplitude, and duration [12]. Changes in AP begin on the 2nd day after denervation and remain constant for 5 days (Fig. 1A, B). The effect of D 600 on AP generation was investigated and the differences between innervated and denervated fibers were taken into account. In normal Lilly's solution (Fig. 1A) the maximal rate of depolarization (V_{max}) of the innervated muscle fiber was 470 V/sec. On the addition of D 600 ($1 \cdot 10^{-4}$ g/ml) this value was reduced by about 40% and the maximal rate of repolarization by 60%; the amplitude of AP was reduced and its duration increased under these circumstances (Fig. 1C). The V_{max} of the denervated muscle fiber in the control was 240 V/sec (Fig. 1B). The addition of D 600 in a concentration of $1 \cdot 10^{-4}$ g/ml reduced V_{max} by 30% and the maximal rate of decay by 40%, i.e., rather less than in the innervated muscle (Fig. 1D). The degree of inhibition of AP depended on the concentration of D 600; there was no change in the value of MP or the input resistance of the membrane.

Comparison of the curves in Fig. 2A shows that the blocker of calcium channels D 600 gave rise to the following effects: First, it induced inhibition of the ascending phase of AP, which is formed by the inward sodium current, and also inhibition of the descending phase of AP, which is due to the outward potassium current. Second, the denervated muscle was less sensitive to D 600. Third, with an increase in the concentration of D 600 the final decrease in V_{max} became equal for both muscles, although the degree of decrease from the initial level still remained different.

Dependence of the blocking action of D 600 on the frequency of AP generation also was studied. Investigations of this type can reveal the character of interaction between the drug and the ion channel [2]. With an increase in the frequency of AP generation (1.2 Hz) the blocking ability of D 600 was found to increase considerably (Fig. 3). The change in V_{max} was seen more clearly than the change in the maximal rate of decay of AP. The frequency of AP generation did not affect its parameters under normal conditions. Dependence of V_{max} on the frequency of AP generation in the presence of D 600 was observed for both groups of muscles. To restore the phase of rise and decay of AP to the level characteristic of that given concentration of D 600, a pause of 10-15 sec was long enough. D 600 evidently interacts with the open sodium channel, causing blocking of sodium permeability and a decrease in AP. Restoration of the parameters of AP when tested after removal of D 600 took place gradually, depending on the concentration and duration of action of the D 600. Throughout the period of rinsing, stimulation of the muscle fiber by a series of pulses caused an additional decrease in V_{max}.

Two principal problems thus remain to be solved: 1) How does D 600, a blocker of calcium channels, reduce the rate of rise and amplitude of MP of a muscle fiber, which are determined by the inward sodium currents; 2) what determines the difference in sensitivity of the AP parameters investigated in these experiments to D 600 in innervated and denervated fibers?

It can be tentatively suggested that D 600 affects sodium permeability, resulting in a decrease in amplitude of AP and V_{max}. It can be concluded from data showing intensification of the blocking effect of D 600 during repetitive AP generation that the compound affects sodium permeability by blocking the activated sodium channel. Similar effects have

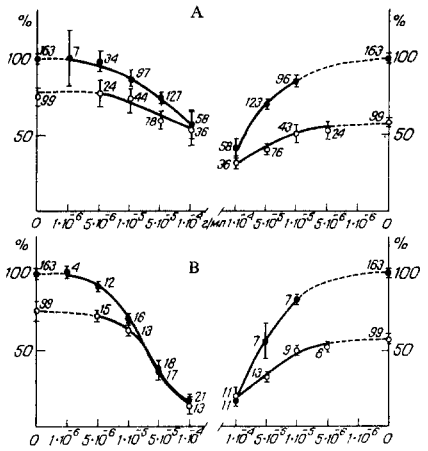

Fig. 2

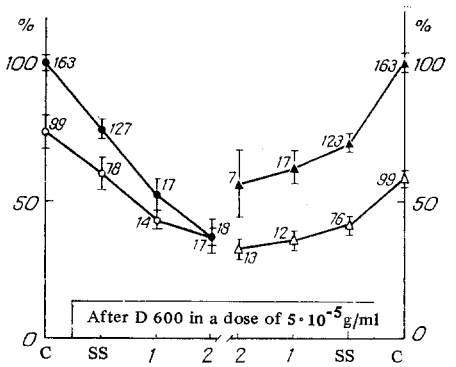

Fig. 3

Fig. 2. Action of D 600 on maximal rates of rise and decay of AP of innervated and denervated muscles during stimulation by a single pulse (A) and series of 10 pulses with frequency of 2 Hz (B). Abscissa, concentration of D 600 (in g/ml), ordinate: on left, maximal rate of rise of AP (in % of mean maximal rate of rise of AP for innervated muscle in normal solution); on right, maximal rate of decay of AP (in % of mean maximal rate of decay of AP for innervated muscle in absence of D 600). Filled circles – innervated fiber, empty circles – denervated fiber.

Fig. 3. Dependence of maximal rates of rise and decay of AP on frequency of AP generation in innervated and denervated fibers in the presence of D 600 ($5 \cdot 10^{-5}$ g/ml). Abscissa: C) control; SS) single stimulation; 1, 2) stimulation with frequencies of 1 and 2 Hz. Remainder of legend as in Fig. 2.

been described for some local anesthetics [2]. There is also evidence that D 600 inhibits the fast veratridine-activated sodium channels of heart muscle and neuroblastoma. This interaction is competitive in type, and the degree of blocking depends on the Ca^{++} concentration [7]. An understanding of these relationships can shed light on the site of interaction of the calcium blocker with the sodium channel and on the nature of the channel itself.

The next problem is that the denervated muscle is less sensitive to D 600 than the innervated muscle. The denervated muscle may acquire resistance not only to D 600, but also to a specific blocker of sodium channels such as tetrodoxin (TT) [12]. In investigations with labeled TT and saxitoxin (ST) the degree of affinity for the toxin of denervated muscle was found to be unchanged, and binding was reduced only by a very small degree [8]. This suggests that the density of the sodium channels is unchanged in denervated muscle and, consequently, this cannot explain the weaker effect of D 600. It has been suggested that after denervation an extrapopulation of slow sodium channels appears [8]. It has been shown on certain objects that such channels have affinity for calcium blockers [1], but in that case the denervated muscle ought to be more susceptible to D 600. Our present experiments showed that this is not so. The resistance of sodium channels of the denervated muscle to TT and D 600 may be connected with changes in the protein environment of the sodium channel during postdenervational structural changes in the membrane and, consequently, with partial screening of the channel by its labile proteins. Treatment of the denervated fiber with proteolytic enzymes (in particular, with papain) increases its sensitivity to TT [3]. It may be that changes in the receptor site for D 600 after denervation do not take place through modification, but through impairment of its accessibility.

LITERATURE CITED

1. N. S. Veselevskii, P. G. Kostyuk, and A. Ya. Tsyndrenko, Dokl. Akad. Nauk SSSR, 250, No. 1, 216 (1980).
2. B. I. Khodorov, in: General Physiology of the Nervous System [in Russian], Leningrad (1979), pp. 44-104.
3. N. K. Chemeris, B. Yu. Kafengauz, and M. A. Poskonova, Studia Biophys. (Berlin), 78, 127 (1980).
4. P. F. Baker, H. Meves, and E. B. Ridgeway, J. Physiol. (London), 231, 511 (1973).
5. P. F. Baker, et al., J. Physiol. (London), 231 527 (1973).
6. A. Fleckenstein and G. Grün, Pflüg. Arch. Ges. Physiol., 307, 26 (1969).
7. J. B. Galper and W. A. Catterall, Mol. Pharmacol., 15, 174 (1979).
8. C. M. Hansen Bay and G. R. Strichartz, J. Physiol. (London), 300, 89 (1980).
9. I. Janke and A. Flechenstein, Pflüg. Arch. Ges. Physiol., 316, 10 (1970).
10. M. Kohlhardi et al., Experienta, 28, 288 (1972).
11. P. G. Kostyuk and O. A. Krishtal, J. Physiol. (London), 270, 545 (1977).
12. P. Redfern and S. Thesleff, Acta Physiol. Scand., 81, 557 (1971).
13. W. van der Kloot and H. Kita, Comp. Biochem. Physiol., 50-C, 121 (1975).