Denervation Effects on Choline Depolarization of Muscle Membrane

Previous work has established that choline (Ch), or acetylcholine (ACh), in millimolar concentration, brings about a partial depolarization of frog muscle cells[1]. In potassium-Ringer, the depolarization is transitory but in Cesium-Ringer, it is maintained so long as Ch is present. The membrane of muscle fiber pretreated in Cs-Ringer was more sensitive to applied Ch or ACh. In either case, the magnitude of depolarization increases with increasing Ch or ACh concentrations[2]. It was suggested that cholinergic receptors, not necessarily similar to those normally active at the end plates, are present all over the membrane structure and that these extrajunctional receptors are normally kept in a configuration which does not allow the interaction between Ch (or ACh) and its receptor to lead depolarization. The interactions between Ch, or ACh, Cs^+ and K^+ at the membrane were described by assuming a cholinergic receptor system in the membrane[3]. The values for the apparent dissociation constants between Ch or ACh and the receptor show that the effect on the Ch- or ACh-receptor site of replacing K^+ by Cs^+ is to increase the affinity of the receptor for both Ch and ACh[4,5]. In all the work reported from this laboratory so far, the sartorius muscles used have been dissected with the muscle nerve cut as close to the muscle as possible, and data taken from muscle fibers regions free of end-plates within 7 h after dissection. Tests have shown that, under these conditions, the muscle innervation remains functional for at least 15 h. In view of the reported effects of denervation on muscle membrane sensitivity to quaternary ammonium ions[6], the present work was undertaken to study the effects of denervation on the depolarization produced by Ch in the presence of K^+ or Cs^+. 200 frogs (*Leptodactylus ocellatus*) were operated on to denervate the sartorius muscle in one leg only, by the following procedure. The nerve to the sartorius muscle was located through a small incision in the skin of the thigh, and approximately 1 cm of it was excised. After 60 days, the denervated muscle and its paired control from the other leg of the same animal were removed for study by the standard techniques already described[1]. Membrane potentials were measured with microelectrodes along muscle fibers in the presence of 0.5, 1.0 and 2 m*M* of Ch as reported in references[4,5]. Comparable measurements were also made on control muscles from normal non-denervated frogs. The results are shown in Figures 1 and 2. Each point on the Figures represent the average of potential measurements from 200 muscle cells, and each curve represents measurements on 30 muscles. Each of the 3 differently treated muscles reacts differently to the addition of Ch. In K-Ringer, the depolarization response to Ch is transient, with the same general time course in all 3 muscles, and increasing in magnitude with increasing Ch concentration. In any given Ch concentration, the magnitude of the response is least for the normal control muscles from non-denervated frogs, considerably larger for the non-denervated control muscles from the opposite leg of the same frog that the corresponding denervated muscle is taken from, and greatest for the denervated muscles (Figure 1).

In Cs-Ringer, depolarization by Ch is sustained, rather than transient, in both normal muscles from intact frogs and in non-denervated muscles from frogs in which the corresponding muscle of the opposite limb was denervated, but is much larger in the latter case, and much

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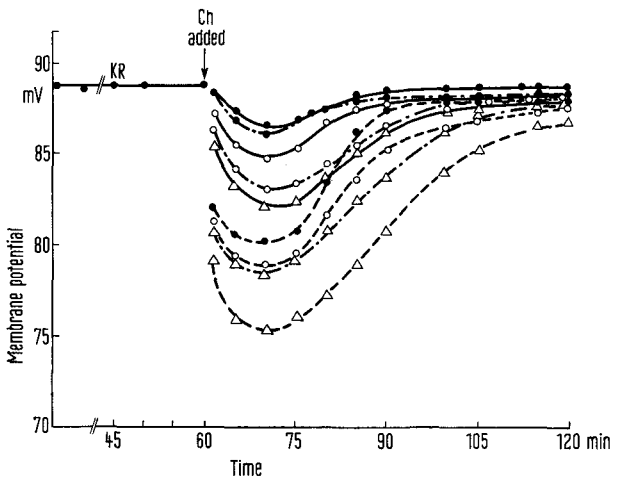

Fig. 1. Resting muscle membrane potential as a function of time, in 2.5 m*M* potassium Ringer. —— control muscle from normal (non-denervated) frogs, ----- denervated muscle, ····· non-denervated control muscles from the opposite leg of the same frog as the denervated muscle. • 0.5 m*M* choline, ○ 1.0 m*M* choline, △ 2.0 m*M* choline.

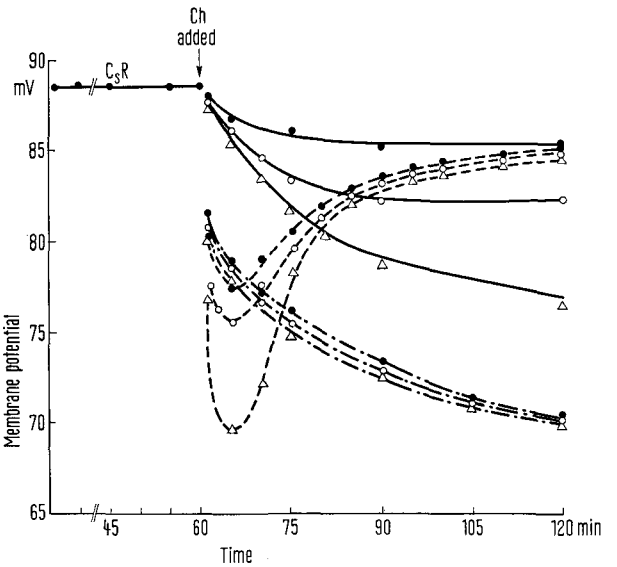

Fig. 2. Same as Figure 1, but in 2.5 m*M* Cesium Ringer.

less sensitive to Ch concentration over the range tested. In denervated muscle, the Ch response is transient, as it is in K-Ringer, but with a faster time course. Its magnitude increases with increasing Ch concentration (Figure 2). Denervation increased the sensitivity of the muscle membrane to choline. It seems likely that this increased sensitivity is ultimately to be explained in terms of changes in the potassium handling machinery in the membrane, in view of the following related phenomena. NICHOLLS[7] demonstrated a fall in total membrane conductance after denervation of muscle, and HARRIS and NICHOLLS[8] found an approximately 20% decrease in potassium uptake of muscle after denervation. HUBBARD[9] has more recently shown a fall in membrane potassium conductance after denervation. Then, in terms of the cholinergic receptor system suggested by PORTELA et al.[3], it is attractive to suppose that denervation somehow affects the molecular conformation of this receptor system present in the muscle membrane, so that its ability to combine with choline, the effect on the potassium handling mechanism of the resulting complex, and the changes of its bioelectric transducer action produced by substituting cesium for potassium are all altered.

Further work to clarify the nature of the striking changes in choline sensitivity in intact muscles from denervated frogs is in progress[10].

Résumé. Dans un muscle non dénervé, la choline provoque une dépolarisation permanente en présence de «Cs-Ringer» et transitoire en présence de «K-Ringer». La dénervation augmente la sensibilité de la membrane musculaire à la choline. Mais dans ce cas, le «Cs-Ringer» donne une réponse transitoire à la choline, semblable à celle obtenue avec le «K-Ringer».

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Effect of Tension Upon Rate of Incorporation of Amino Acids into Proteins of Cross-Striated Muscle

In experimental studies concerning rate of incorporation of amino acids into proteins, generally freely suspended unstretched muscles are used during incubation in the radioactive medium. However, it is well known that heat production[1,2] and oxygen consumption increase in a stretched muscle[3]. Isolated muscles survive longer under the influence of stretch[4] and muscles in organ cultures develop in good condition only when stretched[5]. Thus, stretch applied to the muscle fibre may also be an important factor influencing the rate of incorporation of amino acids into proteins. We have therefore compared rate of incorporation of ^{14}C-leucine into the proteins of 2 muscles, i.e. the levator ani muscle (LA) and the extensor digitorum longus muscle (EDL) of rats using stretched and unstretched preparations. Both muscles are very thin, and therefore well suited for incorporation studies[6,7].

Material and methods. Experiments were performed on (a) the LA muscle of 4-week-old rats and (b) the EDL muscle of-7-days old rats. The muscles were removed with the tendinous insertions; ligatures were applied to both tendinous ends of the muscle with a weight attached to one of the tendons. The resting tension of the muscle giving maximal twitch tension output was determined and the corresponding weight (e.g. 0.2 g for the LA and 0.1 g for the EDL muscle) were used in the experiments. The upper ligature was fixed at the upper circumference of the incubation flask and the muscle, thus vertically stretched, was completely immersed in the incubation medium (Krebs-Ringer bicarbonate buffer, pH 7.4). L-leucine-U-^{14}C was used (0.1 μC/ml, specific activity 32.8 mC/m*M* in the case of EDL muscle and 0.2 μC/ml, specific activity 85.3 mC/m*M* in the case of LA muscle). The muscles were incubated for 2 h (LA) and 90 min (EDL) under continuous shaking at 37 °C. At the end of the experiment, the muscles were homogenized and the proteins precipitated with 5% TCA solution. After removing the nucleic acids and lipids, the precipitated proteins were dried with ether, dissolved in folic acid and number of impulses/min were determined. Number of impulses are referred to mg of noncollageneous proteins determined by the Conway method. In the control experiments, the same muscles immersed freely into the incubation medium were used.

Results and discussion. The Figure shows that incorporation of ^{14}C-leucine into proteins in stretched muscles (LA and EDL) is considerably higher than into the proteins of muscles freely incubated. Expressed in percentage, the incorporation into the stretched muscle increases by 174% in the case of LA muscle and by 50% in the case of EDL muscle. The mechanism of this increase of incorporation is not yet clear. It is possible that the increased oxygen consumption in a stretched muscle might be an important factor. The oxygen consumption in a stretched muscle may increase 2–4 times and is connected with increased ATP hydrolysis and creatine phosphate break-down. With release of stretch, oxygen consumption of the muscle decreases[3]. An auto-

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