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THE EFFECT OF IONS UPON THE RESPONSE OF SMOOTH MUSCLE TO COOLING*

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ABSTRACT

The slow tonic responses of the anterior byssus retractor of *Mytilus edulis* to rapid cooling were investigated by simultaneously recording tension and resting potential changes after soaking the muscle in banthine, a powerful neuromuscular blocking agent. The quantitative relations between the amount of cooling and the amount of associated depolarization necessary for contraction at various concentrations of potentiating potassium can be expressed in a family of curves. The plateaus of the curves for sea water and for potassium-free sea water were beneath the depolarization value necessary for contraction, so that it is clear that no amount of cooling with sea water alone or with potassium-free sea water would ever be effective. When the muscle is treated with subthreshold amounts of potassium and rapidly cooled in various concentrations of sodium ion and calcium ion, respectively, the sodium and calcium do not affect the amount of depolarization. Acetylcholine, in subthreshold amounts, has a potentiating effect, but, unlike potassium and cooling, acts through the nervous apparatus. *Mytilus* muscle will respond to cooling with tonic contraction whenever a critical threshold amount of depolarization is achieved. Cooling alone cannot trigger the contraction since it cannot bring about sufficient depolarization. Cooling can result in contraction, however, if used in conjunction with some other subthreshold depolarizing agent. Cooling affects the contractile mechanism by first causing membrane breakdown and depolarization.

INTRODUCTION

It is known that rapid cooling causes membrane depolarization in *Mytilus* smooth muscle and also that if a small concentration of potassium in excess of normal is used, rapid cooling becomes an effective stimulus for this muscle, and contracture results (Guttman and Gross, 1956; Guttman, Dowling, and Ross, 1957). It appears that a critical amount of depolarization is necessary

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for contracture, but that cooling alone produces insufficient depolarization. In order to obtain a critical level of depolarization and contracture on cooling, an excess of potassium is necessary (Guttman and Gross, 1956).

In the present study, the possible mechanism by which cooling acts is further explored. The drug, banthine, is utilized to ascertain whether cooling affects the nerve endings. Also, the possible influence of sodium, calcium and potassium ions, and of acetylcholine is investigated.

Material and Methods

The material studied most extensively was an invertebrate smooth muscle, the anterior byssus retractor of *Mytilus edulis*. The proboscis retractor of *Phascolosoma gouldii* and the lantern retractor of *Thyone briareus* showed the same basic response to cooling and potassium as did *Mytilus* muscle. Because they are more plastic, however, the *Phascolosoma* and *Thyone* preparations are not as favorable material for our purposes.

Mytilus smooth muscle is especially well suited to this type of work for two reasons. In the first place, its fibers are parallel and run the full length of the muscle (van Nieuwenhoven, 1947), and thus it is excellent material for the study of electrical potentials. Second, the muscle possesses both phasic and tonic responses (Hoyle and Lowy, 1956), and the slow, tonic responses were studied in the experiments here reported. Since these tonic contractures are so extremely slow, the latency between the application of a stimulus and the onset of the response can be easily investigated

The muscle was mounted by threading it through holes in two rubber diaphragms in partitions, *D* and *D*, which divided a lucite chamber into three compartments (Fig. 1). The portion of the muscle in compartment A was bathed in 0.26 M KCl, which served to depolarize that end (Curtis and Cole, 1942), but caused no change in tension to be recorded at the experimental end in compartment C. The end of the muscle in compartment C was bathed in sea water or in experimental solution. Solutions bathing the experimental end could be changed quickly by means of inlet and outlet tubes, *E* and *E*, in the floor of this portion of the chamber.

Muscles were cooled by flushing out the experimental compartment C with solutions cooled to 0°C. until the desired temperature was achieved. Temperatures were recorded by means of a mercurial thermometer placed in close proximity to the muscle. It was possible to accomplish a temperature change of 20°C. in approximately 10 seconds. Temperature changes were localized at the experimental end of the muscle and the initial temperature for each change was room temperature.

While no latency between the application of the cooling stimulus and the resting potential change could be demonstrated with our apparatus, the latency in the myogram amounted to approximately 10 seconds. The time to maximum for a typical temperature-induced contracture was about 5 seconds.

The interelectrode stretch of the muscle in B lay in air or in isosmotic glucose. The potential recorded between the injured and normal end of the muscle was between 15 and 20 mv. when the interelectrode length was in air, but when this segment was immersed in isosmotic glucose, potentials were usually between 30 and 40 mv.

The resting potential of the muscle was amplified and led to one channel of a dual

channel rectilinear galvanometric recorder. Tension changes were recorded by means of an RCA mechano-electronic transducer tube in a bridge circuit, amplified, and led to the second channel of the recorder.

Muscles were soaked for an hour, and in some cases longer, in 10^{-4} M banthine (β-diethylaminoethylxanthene-9-carboxylate methobromide), before the start of certain experiments as a method of establishing whether the ions in question were exerting their effects directly on the muscle or through the nerves, since banthine in this

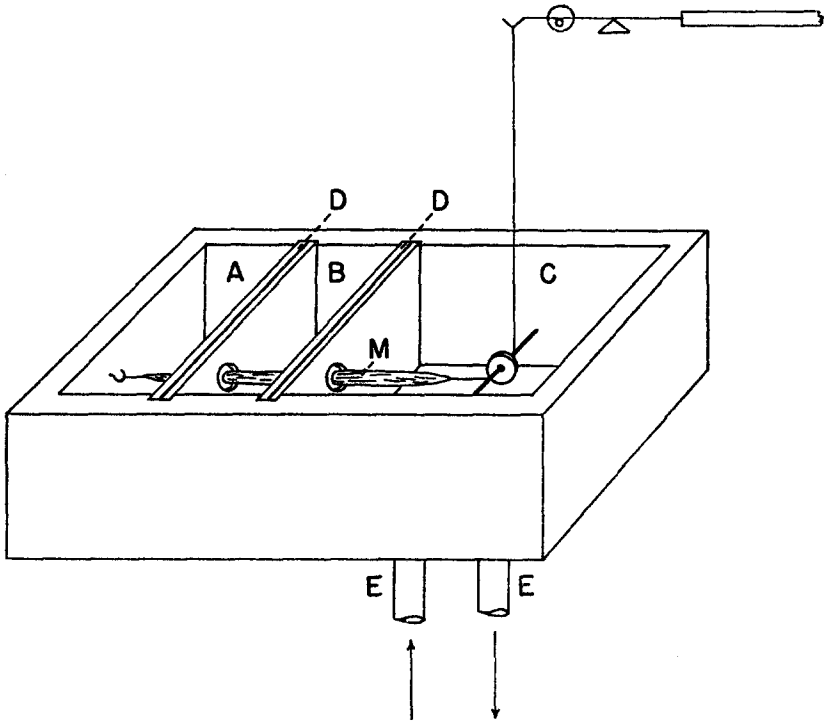

FIG. 1. Mounting chamber for measuring electrical potentials and tension changes in *Mytilus* muscle.

concentration is said to be a powerful neuromuscular blocking agent at acetylcholine sites in *Mytilus* muscle (Twarog, 1954).

In general, solutions were made up daily. Potassium solutions were made up by adding crystals of KCl to Woods Hole sea water (which contains 10 mM KCl per liter). The change in osmotic pressure was offset by adding appropriate amounts of distilled water. However, this adjustment was not accurate and need not be, since the effect of KCl on this preparation is not sensitive to moderate changes in hypertonicity (Guttman, Dowling, and Ross, 1957).

Solutions containing a high and low concentration of sodium were prepared in the manner outlined by Hodgkin and Katz (1949). Three types of solution with a low

sodium content were used: (*a*) 1 part isosmotic dextrose:1 part sea water, (*b*) 4 parts isosmotic dextrose:1 part sea water, or (*c*) 1 part 0.6 M choline chloride:1 part sea water. In the solution high in sodium content, 15 gm. of NaCl crystals was added to a liter of sea water.

The calcium-free sea water had the following composition: NaCl, 25.42 gm.; KCl, 0.72 gm.; $MgCl_2 \cdot 6H_2O$, 6.94 gm.; $MgSO_4 \cdot 7H_2O$, 4.11 gm. per liter of distilled water. 11.5 gm. $CaCl_2 \cdot 2H_2O$ added to a liter of sea water constituted the solution high in calcium content.

In the case of the solutions high in sodium or calcium concentration, it was felt that moderate hypertonicity was preferable to the decrease in potassium concentration that adjustment of tonicity by addition of distilled water would involve.

In each experiment, all data were obtained on the same muscle and the experimental end of the muscle was returned to sea water at room temperature to obtain control values, after each treatment with experimental solution. At the end of each experiment, excitability to electrical stimulation was tested, and in all experiments excitability persisted. All effects were reversible.

When acetylcholine was repeatedly used, excitability was usually so seriously affected, that results as uniform as those presented in Fig. 5 were not the rule. All other figures represent typical results.

RESULTS

Effect of Potassium.—Why cooling is not an effective stimulus for *Mytilus* muscle unless the tissue is treated with subthreshold concentrations of potassium, was investigated by studying the quantitative relations between the amount of cooling and the amount of depolarization necessary for contraction at various concentrations of potentiating potassium (Fig. 2).

The muscle was first equilibrated in high KCl solution at room temperature and then a similar solution at low temperature was applied.

In Fig. 2, the amount of depolarization (ΔP.D.) is plotted against the various amounts of cooling ($-\Delta T$). (The upward trend of this and subsequent figures is probably significant, but the curves drawn in are not.) The solid square indicates contracture. All points are taken on the same muscle.

In this particular muscle, contracture occurs when the resting potential is lowered by about 13 mv. The plateaus of the curves for sea water and for potassium-free sea water are lower than the depolarization value necessary for contracture, therefore it is clear that no amount of cooling with sea water alone or with potassium-free sea water, can ever be effective. Such solutions simply do not result in sufficient depolarization to cause contracture.

These data suggest that the depolarization caused by rapid cooling is a potassium-sensitive phenomenon.

Effect of High and Low Sodium, and of High and Low Calcium Concentrations.—The effect of high and low sodium concentrations is presented in Fig. 3. All solutions used in this experiment contained excess potassium to the extent of four times as much as is normally present in sea water, but still a subthreshold

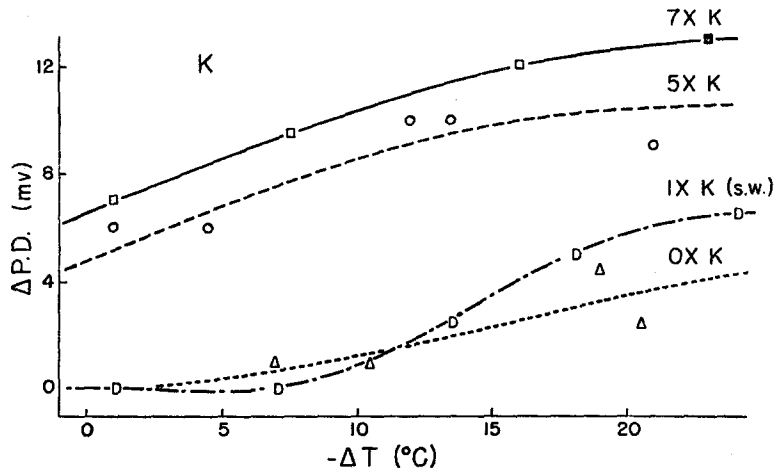

FIG. 2. Amount of potential change (Δ P.D.) obtained on rapidly cooling *Mytilus* smooth muscle various amounts ($-\Delta T$) in potassium-free sea water (0 × K), sea water (1 × K), and sea water containing five (5 × K) and seven (7 × K) times the normal amount of KCl. All readings obtained on same muscle. Contracture occurred at solid square. In this muscle, threshold occurred at a Δ P.D. of 13 mv., corresponding to a $-\Delta T$ of 23°C.

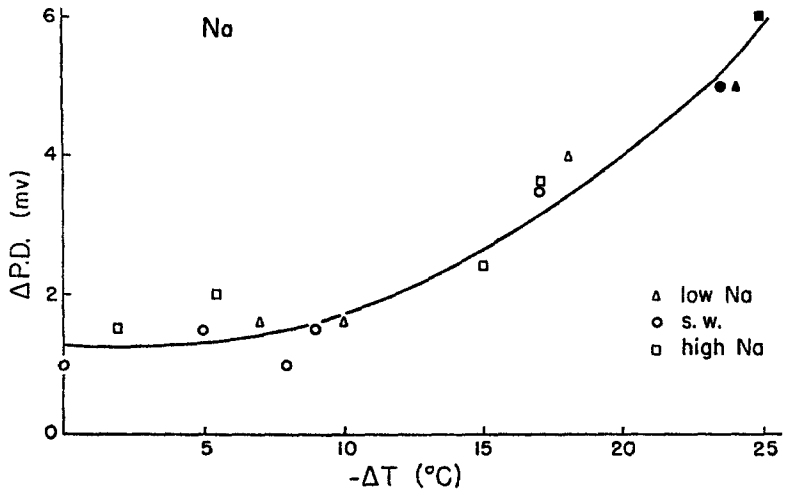

FIG. 3. Amount of potential change (Δ P.D.) obtained on rapidly cooling *Mytilus* smooth muscle various amounts ($-\Delta T$) in sea water (○), low Na (△), and high Na (□) solutions, all containing four times the usual amount of KCl. Note that varying the sodium concentrations does not affect the curve. Contractures occurred at solid points.

quantity. The circles indicate that the excess potassium was contained in a sea water solution. The squares indicate that it was contained in a high sodium solution (sea water containing 15 gm. of added NaCl crystals); and the triangles, that it was present in a solution low in sodium content. In this experiment the solution low in sodium content consisted of one part sea water and one part isosmotic dextrose. Again, ΔP.D. is plotted against $-\Delta T$, and again solid points indicate contracture; open ones, no contracture. It can be seen that varying the sodium content of the solutions had no effect upon the relationship studied, and a single curve can fit all the experimental points. The same holds true when the calcium content of the solutions is varied (Fig. 4). It may be that low cal-

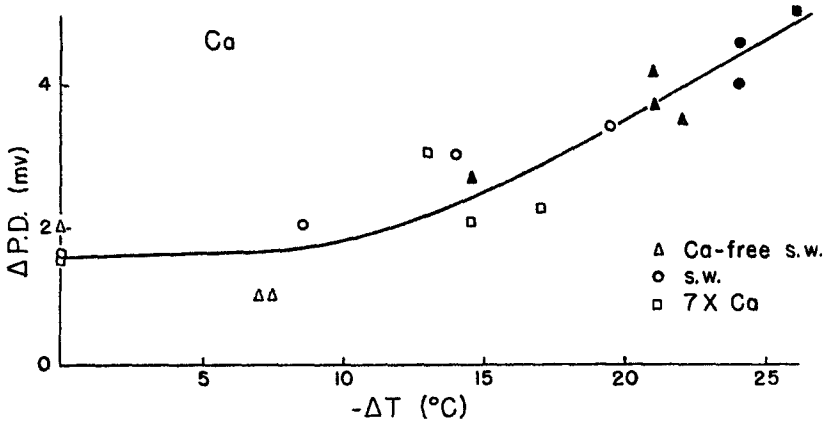

FIG. 4. Amount of potential change (Δ P.D.) obtained on rapidly cooling *Mytilus* smooth muscle various amounts ($-\Delta T$) in sea water (○), in Ca-free sea water (△), and in high Ca sea water (□), all containing seven times the usual amount of KCl. Note that varying the calcium concentration does not affect the curve. Contractures occurred at solid points.

cium concentration effects a change in temperature sensitivity that is not measured by a change in membrane potential (Fig. 4). However, additional experiments are required to prove this conclusively.

Effect of Acetylcholine.—Acetylcholine (Fig. 5) on the other hand, does have a potentiating effect. Acetylcholine is an effective stimulus for *Mytilus* muscle (Twarog, 1954), so concentrations which were subthreshold at room temperature were used. It is apparent that contractures are obtained with lower $-\Delta T$'s when subthreshold amounts of acetylcholine are employed.

Banthine Experiments.—It was found that soaking the muscle in sea water containing 10^{-4} M banthine (which has a curare-like effect on *Mytilus* muscle) for an hour or more, does not interfere with the previously described response of the muscle to rapid cooling and potassium; *i.e.*, does not interfere with de-

polarization or contracture. These experiments with banthine exclude the possibility that the effect of rapid cooling upon muscle is brought about through nerve action, and it seems clear that cooling can stimulate the muscle directly when excess potassium is present.[1]

In conclusion, the present series of experiments suggests that cooling affects the contractile mechanism of the muscle fiber by first causing membrane breakdown and depolarization. Previous experiments (Guttman, Dowling, and Ross, 1957) in which an anodal current, applied to the experimental end of a muscle

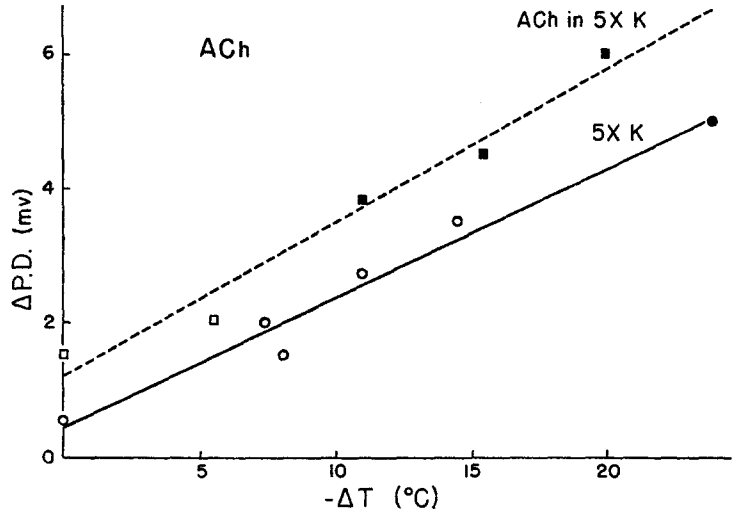

FIG. 5. Amount of potential change (Δ P.D.) obtained on rapidly cooling *Mytilus* muscle various amounts ($-\Delta T$) in sea water containing five times the usual amount of KCl (○), and in sea water containing five times the usual amount of KCl plus 2.5 × 10^{-6} M acetylcholine (□). Contractures occurred at solid points. Note that acetylcholine potentiates the response of the muscle to potassium and cooling.

previously stimulated by cooling and excess potassium, caused repolarization of the membrane and an associated relaxation of the contracture, point to a causal relationship between repolarization and relaxation. These data in general strongly support the view that a change in membrane potential is the connecting link between cooling and the activation of the contractile mechanism.

But what is the nature of the mechanism by which cooling causes membrane depolarization and the associated contracture? It may well involve a simple molecular change, such as a change in the molecular configuration of the muscle fiber membrane. If so, then our data suggest that while the molecular change

[1] Soaking the muscle for about an hour in sea water containing 10^{-5} M banthine, does, however, inhibit the potentiating action of acetylcholine.

in the membrane is insensitive to the sodium ion and to the calcium ion, it is highly sensitive to the potassium ion.

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