Potassium Chloride versus Voltage Clamp Contractures in Ventricular Muscle

Abstract. *In frog ventricle, developed tension was markedly larger in response to depolarization caused by a voltage clamp step than to depolarization induced by high concentrations of potassium chloride. Measurement of extracellular potassium activity at the surface and at the depth of muscle during the development of contractures showed that the diffusion of potassium is much slower than the spread of depolarization through the cross section of muscle. These two observations suggest that competition between the depolarizing and the negative inotropic effects of an increase in the extracellular potassium ion concentration may determine the time course and magnitude of contractile tension in heart muscle.*

Elevation of K^+ suppresses tension and shortens the action potential of ventricular muscle (*1*, *2*). Nevertheless, possible direct effects of K^+ on tension are often ignored when high potassium is used to depolarize cardiac muscle and induce contractures.

We have compared the effects of K^+-induced depolarization and voltage clamp depolarization on the development of tension in frog ventricular muscle strips. Depolarizations with high K^+ concentrations produced one-half to one-fifth as much tension as voltage clamp depolarizations for the same membrane potential. We believe that the depolarizing effect of K^+ dominates early in a potassium contracture, but that K^+ exerts a strong negative inotropic effect as it diffuses slowly into the muscle.

We dissected strips, 0.5 to 0.6 mm in diameter, from the midsection of frog ventricles. When K^+-selective microelectrodes were used (*3*, *4*), we placed strips in a single compartment chamber. We used a single sucrose gap chamber (*5*) for voltage clamp and K^+ contracture experiments. A capacitance transducer (*6*) recorded tension.

Ventricular strips were depolarized with 100 m*M* KCl solution (KCl added in dry weight to Ringer solution containing (in millimoles per liter): NaCl, 116; KCl, 3; $NaHCO_3$, 2; and $CaCl_2$, 0.2 to 1.0. Tension developed rapidly, reaching 50 to 150 percent of twitch tension. Contractures often relaxed spontaneously to about 10 percent of peak tension within 1 to 2 minutes of exposure to KCl solution.

Potassium contracture experiments were carried out in two different ways in order to rule out possible complications arising from variations of tonicity of the experimental solution. In some trials, the osmolarity of the solution was allowed to change when KCl was added. In other trials, an amount of LiCl or sucrose osmotically equivalent to 100 m*M* KCl was added to Ringer solution, so that during the KCl contracture, LiCl or sucrose was withdrawn and replaced by an equivalent amount of KCl; in these experiments, the solutions were kept hypertonic throughout the experiment. All experiments were carried out in both ways. No qualitative differences in the time course of contracture or KCl diffusion profiles were observed regardless of the procedure used. We found that the amplitude of contracture with either procedure depended only on the extracellular calcium concentration, $[Ca]_o$, and the magnitude of membrane depolarization. In solutions containing lower Ca^{2+} concentration (0.2 m*M* or less) we could generate little or no contractile tension, consistent with previously published data (*7*).

The effect of KCl-induced depolarization on development of tension is compared with that of clamp-induced depolarization to −10 mV in Fig. 1. The development of tension accompanying a clamp step to −10 mV is larger than that induced by KCl depolarization to the same potential. We consistently observed large differences between tensions generated with KCl-induced depolarization and those generated by clamp-induced depolarization, even when clamp-induced depolarizations were achieved very slowly (half-time of 1 minute). When twitch tension was altered with variations of $[Ca]_o$ (0.2 and 1.0 m*M*) or $[Na]_o$ (70 to 80 percent of control), the difference in tension generated with clamp- or KCl-induced depolarization was still present. Clamp steps of 30 seconds or longer always produce large local accumulations of K^+ (for example, 13 m*M* in the experiment of Fig. 1), as indicated by the postclamp afterpotential (*8*). Since local K^+ accumulation suppresses tension in frog ventricular muscle (*8*), clamp-induced tensions might have been even larger than those measured had it not been for local K^+ accumulation.

The negative inotropic effect of K^+ was not limited to 100 m*M* KCl solutions. In preparations where we maintained continuous microelectrode impalements throughout various changes of KCl concentrations, the tension generated by each KCl solution tested (20, 40, 60, 80, and 100 m*M*) was considerably lower than that generated by the appropriate depolarization steps when the voltage clamp technique was used. Addition of KCl at the midpoint of the clamp step also suppressed tension. Addition of 100 m*M* KCl relaxed the maintained tension induced by a clamp step to −8 mV (Fig. 2c). This finding was confirmed with all solutions containing K^+ concentrations higher than 30 m*M*. Figure 2 confirms the findings of Fig. 1, with

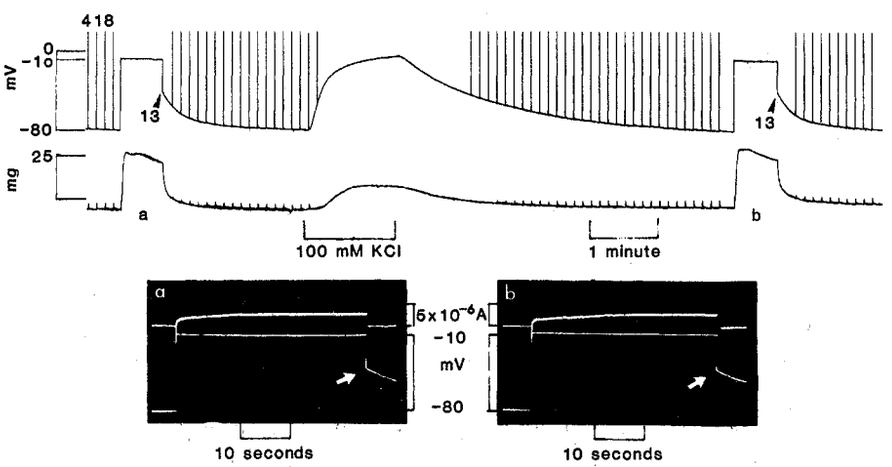

Fig. 1. Comparison of tension developed in response to depolarization caused by KCl and voltage clamp. On continuous chart-recording traces, only tension and membrane potential recordings are indicated. The two inset panels show the membrane currents accompanying clamps to −10 mV. Developed tension induced by voltage clamp depolarization was consistently larger than that produced by equivalent KCl depolarization. Clamp-induced depolarization produced 13 m*M* K^+ accumulation in the extracellular space, as indicated by the depolarizing afterpotentials. Muscle was bathed in hypertonic solution (180 m*M* sucrose plus Ringer solution) throughout the experiment. During KCl contracture trials, sucrose was replaced by 100 m*M* KCl so that contracture solution contained (100 m*M* KCl plus Ringer solution). This procedure was employed to keep $[Na]_o$ constant.

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much longer depolarizations (2 to 4 minutes), that tension generated by clamp-induced depolarization is larger than that generated by addition of KCl (compare Fig. 2a with Fig. 2, b and d). These results suggest that KCl depolarizations do not necessarily represent the maximal tension that ventricular muscle is capable of generating at a particular membrane potential. It may therefore be concluded that K^+ has a rapid and marked negative inotropic effect in addition to its well-known ability to depolarize and generate tension in the frog ventricular muscle.

The reason that large contractures develop when ventricular muscles are depolarized with Ringer solution containing 100 m*M* KCl, despite the negative inotropic effects of KCl solutions, may in part lie in the faster spread of the depolarization wave in comparison with the rate of diffusion of K^+ through the cross section of the muscle. The data regarding the reproducibility of KCl-induced contractures and appropriate conditions for their generation suggest that KCl-induced conctractures in frog heart require a $[Ca^{2+}]_o$ at least 1.0 m*M* or higher, very rapid exchange of the bathing solutions or superfusion media (*7, 9, 10*), and absence of circulating or endogenous catecholamines (*11*). Clamp-maintained tensions are quite easily and reproducibly generated even in preparations bathed in solutions containing 50 μM Ca^{2+} (*12*).

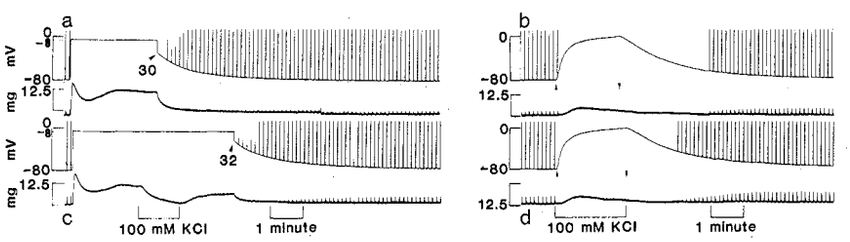

Fig. 2. The effect of 100 m*M* K^+ on a ventricular strip depolarized with a clamp step. Clamp-induced depolarization produces much larger maintained tensions than KCl-induced depolarization for equivalent times and potentials [compare (a) with (b) and (d)]. Addition of KCl after 2 minutes of a depolarizing pulse to −8 mV relaxes the developed tension. Removal of high K^+ solution partially restores tension. Such long depolarizing pulses produce 30 to 32 m*M* K^+ accumulations [(a) and (c)]. Strip diameter is 0.4 mm; length, 0.5 mm; $[Ca]_o$, 0.5 m*M*; and temperature, 23°C. In this experiment 100 m*M* KCl was replaced by 180 m*M* sucrose to maintain osmotic equality during the shift from Ringer to high KCl solution (see legend to Fig. 1 and text for details). The negative inotropic effect of K^+ was independent of its osmotic effects.

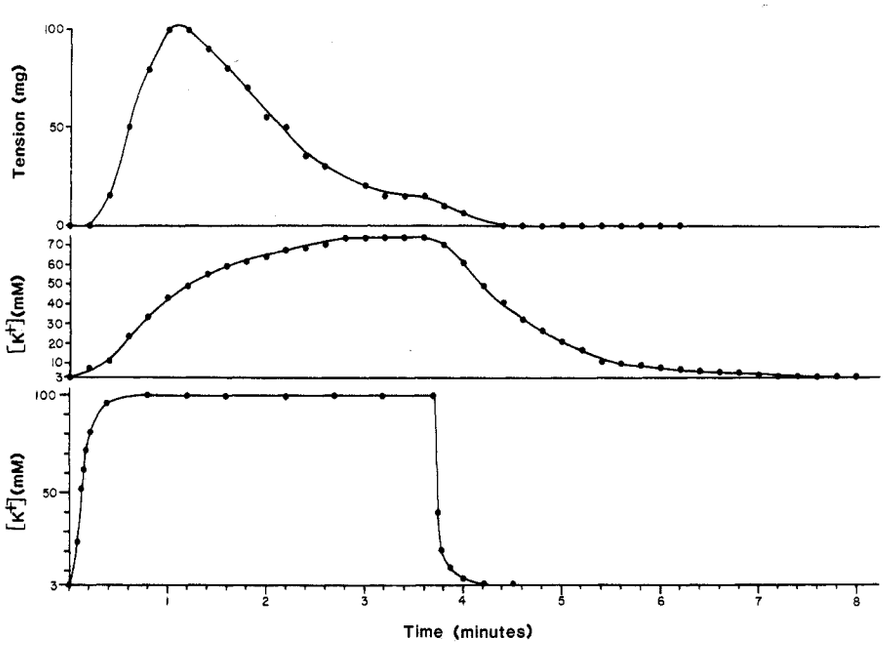

Fig. 3. Comparison of the time course of development of contractile tension with the time course of change in K^+ activity in the extracellular space of the frog ventricular muscle. Contractile tension (upper trace) reaches its peak value about 1 minute after exposure to high KCl solutions, then declines to a lower steady-state value. With the K^+ electrode placed near or at the surface of the muscle (lower trace), K^+ activity changes rapidly, reaching its final value before any appreciable tension is measured. Bathing solutions were exchanged slowly (20 to 30 seconds) so as not to dislodge the K^+-selective microelectrode from its position. The K^+ activity changes more slowly at the core of the muscle (middle trace). Since K^+ diffusion into the deeper extracellular space is a slow process (2 to 3 minutes), it is conceivable that the inner fibers are depolarized by the electrotonic spread resulting from rapid depolarization of surface fibers. Experimental conditions included the following: strip diameter, 1.2 mm; tip of electrode, 2 to 4 μm; $[Ca]_o$, 1.0 m*M*; and temperature, 22°C. In this experiment KCl was replaced by sucrose to maintain osmotic equality during the shift from Ringer to high KCl solution (see text and legend to Fig. 1).

To test directly the rate at which K^+ appears at deeper extracellular compartments of the muscle, we compared the rate at which a K^+-selective microelectrode detects changes in K^+ concentrations at the surface and at the extracellular core of the muscle. Diffusion of K^+ into the extracellular space occurs more slowly than was assumed previously (*13*). In the experiment of Fig. 3, both solutions were exchanged slowly (15 to 25 seconds) to prevent the K^+-selective microelectrode from getting dislodged from its extracellular space. Despite the slow exchange rate of the bathing solution, KCl reached its final concentration on the surface fibers within 25 seconds; in the deeper fibers, K^+ reached its equilibrium value in 2 to 3 minutes. It is likely, therefore, that introduction of high concentrations of KCl at the surface fibers rapidly depolarizes (or clamps) the more centrally located cells of the ventricular strip. Thus, large tensions could develop before KCl reaches equilibrium across the extracellular space of the tissue. The finding that contractures are phasic (that is, tension develops at first and declines later to a much lower steady-state level) is consistent with the dual effect of K^+ on the myocardial cell. In experiments in which we monitored the time course of changes of K^+ activity in the extracellular space of the muscle while maintaining constant osmolarity throughout the various solution changes, K^+ diffused slowly with a time course similar to that illustrated in Fig. 3.

As Ca^{2+} concentrations in the bathing solution are reduced, the suppressant effect of K^+ during depolarization induced by high concentrations of KCl tends to prevent generation of significant contractile tension (*7*). It is, in fact, at these low Ca^{2+} concentrations (0.2 to 0.5 m*M*) that the discrepancy between tension developed with KCl and clamp-induced depolarization was greatest. Little or no direct evidence is available to explain the mechanism of the negative inotropic ef-

fect of KCl on ventricular muscle. Two possibilities have been suggested: One invokes the stimulation of Na^+ pump, reducing the intracellular sodium ion concentration, $[Na^+]_i$, thus suppressing Ca^{2+} influx through the Ca-Na counter exchanger, which in turn leads to reduction of tension (*14, 15*); the other suggests that K^+ competes more directly for the Ca^{2+} transport site on the surface membrane in a manner similar to that already postulated for Na^+(*5, 12*). The rapidity with which K^+ suppresses tension in depolarized ventricular strips (Fig. 2) makes it improbable that $[Na^+]_i$ has sufficient time to change significantly in order to alter tension. Thus the data are more compatible with a direct effect of K^+ on the Ca^{2+} transport site.

Regardless of the mechanism by which the negative inotropic effect of K^+ is mediated, it is clear that in frog ventricular muscle, KCl solutions should not be used solely as depolarizing agents to quantify development of tension. It is, in fact, misleading to assume that K does not interfere at all with the Ca^{2+} transport mechanism, an assumption based on studies in skeletal muscle, in which activator Ca^{2+} is released primarily from an intracellular compartment.

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References and Notes

1. S. Weidmann, *J. Physiol. (London)* **132**, 157 (1956).
2. I. de Burgh Daly and A. J. Clark, *ibid.* **54**, 367 (1921).
3. R. P. Kline and M. Morad, *Biophys. J.* **16**, 367 (1976).
4. ———, *J. Physiol. (London)* **280**, 537 (1978).
5. M. Morad and R. Orkland, *ibid.* **219**, 167 (1971).
6. M. Schilling, *Rev. Sci. Instrum.* **31**, 1215 (1958).
7. R. A. Chapman and J. Tunstall, *J. Physiol. (London)* **215**, 139 (1971).
8. L. Cleeman and M. Morad, *ibid.* **286**, 83 (1979).
9. R. Niedergerke, *ibid.* **134**, 584 (1956).
10. J. F. Lamb and A. J. S. McGuigan, *ibid.* **186**, 261 (1966).
11. M. Morad, *Science* **166**, 505 (1969).
12. ——— and T. Klitzner, in *Biophysical Aspects of Cardiac Muscle*, M. Morad, Ed. (Academic Press, New York, 1978), p. 285.
13. S. G. Page and R. Niedergerke, *J. Cell Sci.* **11**, 179 (1972).
14. D. A. Eisner and W. J. Lederer, *J. Physiol. (London)* **294**, 279 (1979).
15. G. A. Langer, *Annu. Rev. Physiol.* **35**, 55 (1973).

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Therapy of Mouse Lymphoma with Monoclonal Antibodies to Glycolipid: Selection of Low Antigenic Variants in vivo

Abstract. *Growth of mouse lymphoma L5178Y, which contains large quantities of the gangliotriosylceramide ($GgOs_3Cer$), in DBA/2 mice was suppressed by passive immunization with monoclonal immunoglobulin G3 antibodies to $GgOs_3Cer$, but not by immunoglobulin M antibodies with or without added complement. Most groups of mice treated with monoclonal immunoglobulin G3 antibodies did not develop tumors, but the tumor that appeared in a treated animal had a much lower amount of the $GgOs_3Cer$ than the cells used for inoculation. Thus, passive immunization either prevented growth of the lymphoma or caused selection of a variant with a lower quantity of the antigen $GgOs_3Cer$.*

Two general types of changes of glycolipid patterns resulting from malignant transformation are (i) synthesis of new glycolipid determinants as a result activation of normally unexpressed glycosyltransferases and (ii) deletion of more complex glycolipids because of a block in synthesis that frequently leads to accumulation of precursor structures [for a review, see (*1*)]. Although both of these changes can produce tumor-distinctive glycolipids, no studies have demonstrated that such chemically well-characterized glycolipid markers can be used for tumor diagnosis or therapy. We now report that passive immunization of DBA/2 mice with monoclonal immunoglobulin G3 (IgG3) antibodies specific for the glycolipid ganglio-*N*-triosylceramide ($GgOs_3Cer$; GalNAcβ1→4Galβ1→4Glcβ1→1ceramide) (*2*) dramatically suppressed the growth of tumor cells bearing that glycolipid marker. In addition, the tumor cells that eventually appeared in one treated mouse contained decreased amounts of $GgOs_3Cer$.

We used variants of the DBA/2 lymphoma cell line L5178Y that differed in their display of the neutral glycolipid $GgOs_3Cer$ (*3*). One of these variants, designated 1A1, was chosen as an optimal target cell for immunotherapy because it contained a large amount of $GgOs_3Cer$ and was sensitive to lysis in vitro by monoclonal immunoglobulin M (IgM) antibody to $GgOs_3Cer$ plus complement (*3*). Another variant, clone 27AV, lacked detectable $GgOs_3Cer$ and thus provided an appropriate negative control cell line.

For immunotherapy we used two Balb/c monoclonal antibodies that are specific for distinct portions of the nonreducing terminus of $GgOs_3Cer$ (*4*). Pooled ascites fluids from mice bearing each of these hybridomas contained, per milliliter, 15 mg of the IgM antibody produced by clone 2D4 (*4*) and 4 mg per milliliter, of the IgG3 antibody produced by clone D11G10 (*4*). A nonspecific control ascites fluid contained, per milliliter, 10 mg of the monoclonal IgG3 antibody produced by clone 3. Because mice of the DBA/2 strain are known to have low concentrations of complement component C5 (*5*), certain groups of mice received injections of native guinea pig serum as a complement source.

In the initial experiment mice received on day 0 intraperitoneal inoculations of 10^6 subclone 1A1 cells (a dose at least 2 logs greater than the 100 percent lethal dose). Therapy began on day 1 and consisted of intraperitoneal injections of hybridoma ascites fluids with or without guinea pig serum (Fig. 1A). Whereas the median survival time of untreated mice was 34 days, treatment with guinea pig serum alone or with IgM antibody to $GgOs_3Cer$ alone did not significantly improve survival. Only one of three mice treated with the IgM antibody to $GgOs_3Cer$ plus complement was a long-term survivor. In contrast, the mice treated with the IgG3 antibody to $GgOs_3Cer$, either with or without complement, showed prolonged survival. In fact, five of these seven mice were apparently cured as judged by their survival 120 days after they were inoculated with the tumor cells.

In the second experiment this protective effect of the IgG3 antibody to $GgOs_3Cer$ was reproduced (Fig. 1B). To rule out nonspecific effects of IgG3 antibodies, we treated one group of mice with ascites fluid containing the nonspecific monoclonal IgG3 antibodies produced by clone 3, which did not show reactivity with the subclone 1A1 lymphoma cells by immunofluorescence (data not shown). This antibody failed to protect mice against challenge with the subclone 1A1 cells; the median survival for this group was 31 days compared to 30 days for the untreated group. In this experiment the IgM antibody to $GgOs_3Cer$ had a slight protective effect. However, therapy with the ascites fluid containing IgG3 antibody to $GgOs_3Cer$, either with or without complement, was most effective. Of the 12 mice treated with intraperitoneal injections of the IgG3 antibody, nine survived at least 120 days after they were injected with tumor cells. In addition, intravenous therapy with IgG3 antibody to $GgOs_3Cer$ pro-

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