other fractions. Though the trailed area (a) also showed a high activity of 585 cpm, it can be attributed to its close connection and proximity with the raffinose spot (figure 2). It is of interest to note that only 8.8% of the total recovery activity was in the glucose part, showing that the bulk of the $^{14}C$ has got transferred to raffinose, indicative by its increased activity. However, the radioactivity of 5.5 and 6% detected in the galactose and fructose fractions may also be due to the influence of the glucose fraction which lies in close proximity with either of these sugars. The detection of minor activity in the blank areas (X spaces, figure 2) may be due to some breakdown products, which, however, were not assayed.

Since biosynthesis of raffinose has been confirmed to take place in the insect body, the only probable function which it could perform appears, therefore, to be as a reserve sugar in this bug[9].

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## Sodium-dependence of sustained force in potassium contracture of cat ventricle

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*Summary.* Following exposure to low-Na solution, the initial phase of K-contracture in cat ventricle is prominent while the second (sustained) phase is markedly attenuated. Monensin, a Na-specific ionophore, enhances the second phase of K-contracture following exposure to low-Na solution.

The role of Na in the genesis of KCl-contracture in mammalian ventricular muscle is unclear. Although K-contractures are augmented by Na-poor solutions[3,4], some extracellular Na is apparently necessary to permit the appearance of K-contracture[5]. A reduction of extracellular Na or an elevation of intracellular Na facilitates Ca influx via Na:Ca exchange. The contribution of this process to the development and maintenance of K-contracture in ventricular muscle is unclear[5,6]. Additionally, the interpretation of the importance of intracellular Na for K-contracture is complicated by the recent discovery that the bulk of myocardial intracellular Na is sequestered in non-ionic form[7]. The present research was undertaken to reinvestigate the relation of Na to the development and maintenance of contracture during K-depolarization.

*Methods.* The methods used in this study were similar to those previously published[6]. Small (< 1 mm diameter) cat right ventricular muscles were maintained in control Tyrode's solution (solution I). The composition of all solutions used is shown in the table. Solutions I and II were gassed with 95% $O_2$, 5% $CO_2$; pH was 7.4 at 34 °C. Solutions III and IV were gassed with 100% $O_2$; pH was adjusted to 7.4 at 34 °C with HCl. All solutions contained nadolol ($10^{-4}$ M), a beta-adrenergic blocking agent; this concentration has no effect on isometric contraction of mammalian myocardium[8]. In some experiments, a Na-selective ionophore, monensin[9], was added to solutions III and IV. Monensin (mol. wt 670; Lilly) was dissolved in 50% dimethylsulfoxide and 50% ethanol. The vehicle for monensin did not affect the results. Muscles were stimulated (0.33 Hz) only when exposed to solution I. After each contracture, the muscle was exposed to solution I for 45–60 min before proceeding with the next phase of the experiment.

*Results and discussion.* Exposure of ventricular muscles to low-Na (1.8 mM) solution (solution III) resulted in small 'sodium-lack' contractures[10] of variable duration (~ 10–15 min). K-contracture (using solution IV) evoked after their spontaneous relaxation, i.e., after 30 min in solution III, were higher than K-contractures (using solution II) evoked following exposure to normal Na (solution I); peak contracture force after solution III averaged 161% of that seen after solution I. The major change in K-contracture evoked following exposure to low-Na solution is a marked change in the time course of contracture. Following exposure to normal Na, contracture force is generally well sustained for the duration of exposure to elevated-K[6,11] (figure A). Following exposure to low-Na solution, contracture force is markedly phasic, declining from a prominent initial peak to a very low level for the duration of K-depolarization (figure B). [Following exposure to zero Na solution, K-contracture is similar to that noted following exposure to 1.8 mM Na[12].] As suggested by Gibbons and Fozzard[3], the phasic nature of the 1st phase of K-contracture following exposure to low Na solution indicates that this initial phase may be attributed to a release of cellular stores of Ca brought about by the rapid depolarization. The 2nd phase is Na-sensitive and probably represents a balance between inwardly directed Ca and the continued uptake or removal of Ca by cellular organelles (see below).

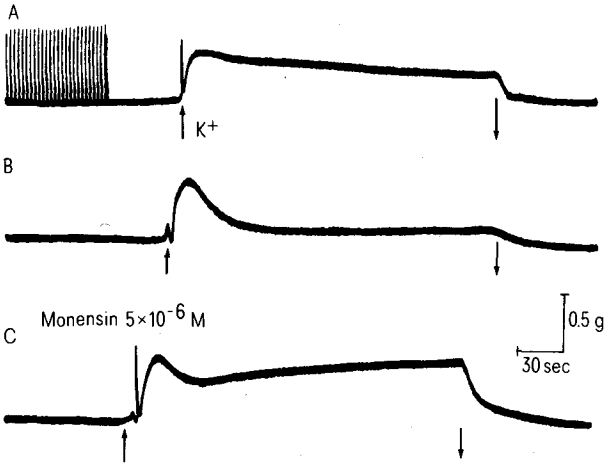
In Na-free solutions, total myocardial tissue Na falls from 60 to less than 5 mmoles/kg tissue water within 20 min[5,13]. This may represent Na 'irreversibly' sequestered in muscle[14] or bound to connective tissue. The free Na activity ($a_{Na}$) within the sarcoplasm is probably much lower than this, since $a_{Na}$ is only 6 mmoles/kg when total intracellular Na is on the order of 40 mmoles/kg[7]. The free Na in the

Composition of saline solutions[a,b]

| | NaCl | KCl | $NaHCO_3$ | $NaH_2PO_4$ | Tris[c] | Sucrose |
|---|---|---|---|---|---|---|
| I | 129 | 4 | 20 | 1.8 | – | – |
| II | – | 133 | 20 | 1.8 | – | – |
| III | – | 4 | – | 1.8 | 5 | 260 |
| IV | – | 133 | – | 1.8 | 5 | – |

[a] All concentrations in mmoles/l. [b] In addition, all solutions contained $CaCl_2$, 2.7; $MgCl_2$, 0.5; and dextrose, 5.5. [c] Tris (hydroxymethyl) aminomethane.

sarcoplasm when the bathing solution contains 1.8 mM Na is more poorly defined, but presumably reflects a similar low activity. Busselen and Carmeliet[5] reported that low concentrations of Na, for example, 14 mM, in the external solution, have a protagonistic effect on K-contracture (i.e., moderate rises in extracellular Na restore K-contracture force in muscles incubated in zero Na solution). The effect runs counter to the expected Na:Ca antagonism and suggests that some extracellular Na is needed to maintain intracellular Na at some specified level[10]. This may potentiate Ca influx via Na:Ca exchange[15] (also, possibly Ca influx across the depolarized cell membrane in the 2nd phase of K-contracture[16]) or interfere with the Ca sequestering system[17]. We investigated this possibility by 'clamping' the intracellular Na activity at an arbitrarily low level, $\leq 1.8$ mM, using the Na-selective ionophore monensin ($5 \times 10^{-6}$ M)[9,18,19]. Although total tissue Na is not controlled by this technique, free $Na^+$ in the sarcoplasm is essentially fixed by the opposing effects of monensin-facilitated Na diffusion and any active Na extrusion systems (e.g., Na, K-ATPase). Monensin did not alter the resting force of the muscle. However, when the muscle is incubated with 1.8 mM Na plus monensin for 30 min and then challenged with K-depolarization, the contracture is markedly altered (figure C). The initial phase of contracture is unchanged from that evoked in the absence of monensin, but this initial phase is now followed by a prominent second phase of force development. In 6 muscles, the force during this 2nd phase was 51% of the initial peak force in the presence of monensin, as compared to less than 10% in the absence of monensin. Thus, intracellular Na activity as low as 1.8 mM can have a pronounced inotropic action on the 2nd phase of K-contracture. In contrast, in zero Na solution, monensin has no effect on either the initial or 2nd phase of the K-contracture[12] while in normal Na solution, it markedly potentiates the initial phase[12,20] and the contracture is often better maintained.




Potassium contractures in cat papillary muscle. Panel *A*. Control K-contracture (solution II) following exposure to normal Na solution (solution I). Muscle stimulation was discontinued 45 sec before the addition of the high-K solution. High-K solution was added at the 1st arrow, replaced by solution I at the 2nd arrow. Panel *B*. K-contracture following exposure to low-Na solution (solution III). At the beginning of superfusion with solution III, the muscle demonstrated a small Na-lack contracture (not shown), which spontaneously relaxed. The muscle was superfused with solution III for 30 min before the K-contracture was elicited. At the 1st arrow, low-Na high-K depolarizing solution (solution IV) was introduced. In marked comparison to the control contracture, the K-contracture now demonstrates 2 phases: a prominent initial phase and a much smaller 2nd phase which is maintained until removal of the high-K solution (2nd arrow). The muscle was then superfused with solution I, and re-stimulated for 45 min, allowing it to regain control force. Panel *C*. K-contracture following exposure to low-Na solution and monensin (5 µM). As in *B*, the muscle was exposed to solution III for 30 min prior to exposure to solution IV. On addition of high-K solution the ionophore-treated muscle demonstrated an initial contracture phase almost identical to that noted in *B*. However, after partial relaxation, there was a very marked 2nd (sustained) phase which lasted until the high-K solution was removed (2nd arrow). Monensin was absent from solution IV, but identical results have been obtained in the maintained presence of the ionophore. Muscle length 3.6 mm, diameter 0.8 mm.

The enhancement of the 2nd phase of contracture by monensin in low Na reflects an increase in Ca around the contractile proteins. The mechanism for this is unclear; it may be related to a Na-dependent Ca influx[10]. Na: Ca exchange[15] is in equilibrium when $[Ca]_o [Na]_i^2 = [Ca]_i [Na]_o^2$. Thus, assuming $[Ca]_o = 2.5$ mM, $[Ca]_i = 1$ µM, and $[Na]_o = 1.8$ mM, Na:Ca exchange will favor Ca influx when $[Na]_i$ is greater than 40 µM. These results suggest that the protagonistic effect of low $[Na]_o$ results from a maintenance of a low, but essential, level of $[Na]_i$, and may indicate that Na:Ca exchange is, in part, voltage-dependent. Alternatively, depolarization may increase Na influx slightly.

Our results do not rule out other effects of monensin on K-contracture. For example, Shlafer and coworkers have shown that monensin has a direct effect on cardiac microsomes to release Ca[19]. However, this effect appears to be limited to isolated microsomes and not to occur in intact muscle[19].

The disparate effects of the ionophore, monensin, on K-contracture following exposure to low Na solutions, leads us to infer that the initial and 2nd phases of K-contracture in mammalian ventricle do, in fact, reflect separate mechanisms.

Note added in proof: An alternative explanation for the inotropic effect of $[Na]_i$ was recently suggested by Einwächter and Brommundt (Pflügers Arch. *375*, 69 (1978)). They proposed that an increase in $[Na]_i$ functions to limit Ca efflux via Ca:Na exchange, thus maintaining $[Ca]_i$ at a higher level, rather than promoting Ca influx via the same mechanism.

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