ductal filling of the mammary gland with lignocaine affected the response pattern.

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Influence of Strontium on the Cardiotropic Actions of Rhodochlorin and Ouabain.* (32528)

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In spite of the early observation by Ringer in 1883 that strontium possesses a positive inotropic action similar to that of calcium, the cardiotropic actions of strontium have not been extensively studied by modern techniques(1-4). We have recently reported the necessity of calcium in the positive inotropic action of ouabain and gamma-methyl rhodochlorin(5). The latter is a porphyrin derivative, which was prepared from chlorophyll *a* and extracted originally from spinach in this laboratory by the method previously described (6). The present investigation concerns the effects upon the cardiotropic actions of ouabain and gamma-methyl rhodochlorin on the frog heart when the calcium in the Ringer's perfusion solution is replaced by equimolar strontium, and with the effects of these cardiotropic agents after perfusing the heart with Ca-free Ringer's solution containing EDTA-Na_2 (ethylene diaminetetraacetate disodium). Evidence is provided to suggest that the positive inotropic action of strontium is calcium dependent and the factors other than the uptake of calcium by the myocardium(7, 8), may be important for the cardiotonic action ouabain.

Methods. The isotonic contractions and electrograms of the isolated spontaneously beating frog heart (*Rana pipiens*, 8-10 cm) were recorded on a standard physiograph as previously described(5). The "normal" Ringer's perfusion solution contained: NaCl, 112.0 mM; KCl, 1.88 mM; $CaCl_2$, 1.08 mM; $NaHCO_3$, 2.38 mM (Ca-Ringer's solution). The pH of the solution was approximately 8 with or without $CaCl_2$, and also with the replacement of $CaCl_2$ with equimolar $SrCl_2$ (Sr-Ringer's solution). The frog heart is relatively insensitive to changes in pH and osmolarity of the perfusion solution(5). All experiments were performed at 25 ± 1°C. Because the heart rate varied from one preparation to another, the number of beats required for the onset of maximal positive inotropic effect after a change in perfusion solution was used to quantitate differences in response(5). At least 8 experiments were performed for each of the observations.

Results. When the calcium in the Ringer's solution was suddenly replaced by equimolar strontium, the force of contractions rapidly diminished to unrecordable levels and after 2 hours perfusion with the Sr-Ringer's solution contractility did not return upon the restoration of calcium to the perfusion solution (Fig. 3-1). However, if ouabain (6.7×10^{-7} M) were present in the Sr-Ringer's solution, a positive inotropic action was observed (Fig. 1), and this cardiotonic action was related to the concentration of strontium in the perfusion solution (Fig. 2). Gamma-methyl rhodochlorin (1.7×10^{-6} M), like ouabain, produced a positive inotropic action upon hypodynamic frog heart deficient in calcium(5), but, unlike ouabain, it was without effect when present in the Sr-Ringer's solution (Fig. 4-3). When additional strontium (0.54-1.08 mM $SrCl_2$) was

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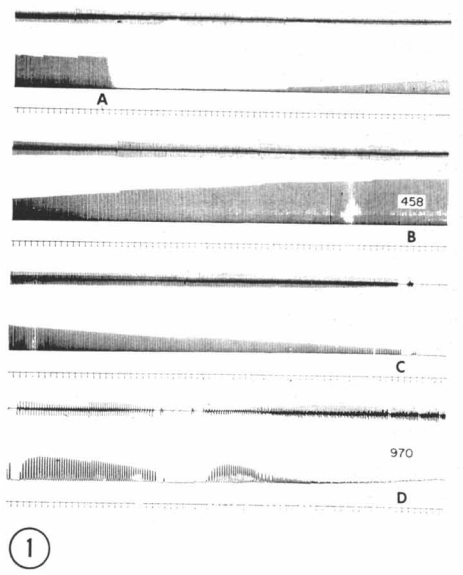
added to the Sr-Ringer's solution early during the perfusion period, increased positive inotropic response was elicited (Fig. 3-2). After 10 minutes of perfusion with a Ca-free Ringer's solution containing 1.34 mM EDTA-Na_2, a positive inotropic response was not observed with a supranormal Sr-Ringer's perfusion solution, but a positive inotropic action was observed with a Ca-Ringer's perfusion solution (Fig. 3-3).

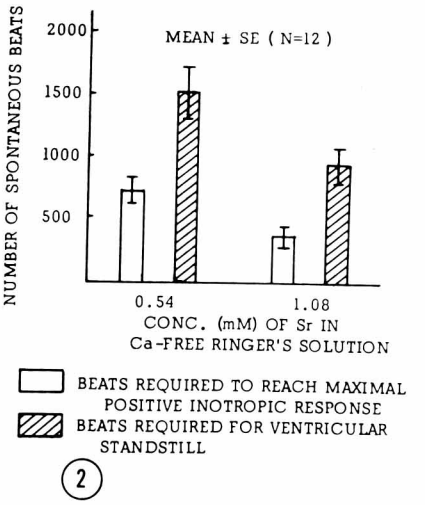
After perfusion with an EDTA-Na_2 (1.34 mM) solution for 10 minutes, ouabain (6.7×10^{-7} M) in the Sr-Ringer's solution was ineffective in eliciting a positive inotropic response (Fig. 4-1), but ouabain in Ca-Ringer's solution was still effective (Fig. 4-2). Perfusion with Sr-Ringer's solution containing gamma-methyl rhodochlorin for 60 minutes elicited no cardiotonic response, but addition of a minimal amount of calcium (0.14 mM) to the perfusion solution produced a weak positive inotropic action (Fig. 4-3).

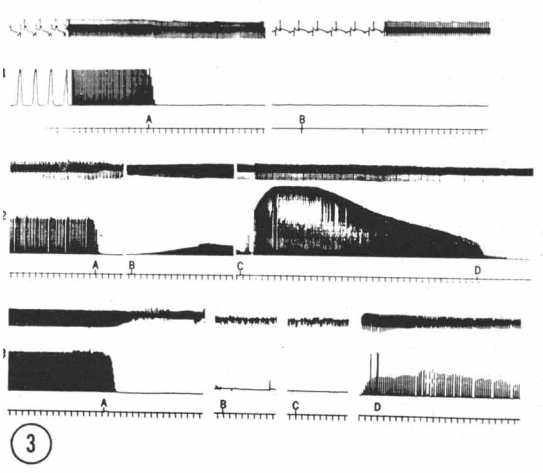
Discussion. It is generally accepted that the increased force of myocardial contractility is associated with an increase in the transmembrane flux of calcium. Similar results have been demonstrated with strontium(3). The latter finding is perhaps not too surprising considering the proximity of calcium and strontium in the periodic table. That strontium may be antagonistic to calcium in the heart has been reported(2) and would be compatible with our results. Perfusion with Sr-Ringer's solution following a *transient* period (1-5 min) of perfusion with an EDTA solution produced contracture and lowered resting transmembrane potential in toad hearts *(Bufo marinus)*(4) and frog hearts *(Rana pipiens)*(9). It is possible that the chelation of membranous calcium by EDTA and/or the formation of an EDTA-membrane complex rendered the membrane more permeable to transmembrane ionic flux, hence strontium was capable of eliciting contractures. In the sequence of experiments presented in Fig. 3, it has been demonstrated that Sr-Ringer's solution was incapable of eliciting a recordable positive inotropic response unless additional strontium was added to the Sr-Ringer's solution, and that the duration of the cardiotonic response thus induced was only short-lived. Prolonged perfusion with the Sr-Ringer's solution produced irreversible ventricular standstill. When there is sufficient chelation of tissue calcium by EDTA, calcium, but not strontium, was still effective in eliciting a positive inotropic response. These observations suggest that strontium has little or no cardiotonic action of itself, but must release residual calcium (membranous or intracellular) for positive inotropic action. Prolonged perfusion with Sr-Ringer's solution resulted in irreversible cardiac standstill which may have been due to replacement of calcium by strontium at the receptor sites.

The positive inotropic action of ouabain has been associated with an increase in transmembrane flux of calcium and a mobilization of intracellular calcium(*e.g.*, 7,8). The cardiotonic action of gamma-methyl rhodochlorin may also be associated with the transmembrane flux of calcium(5). Ouabain was capable of eliciting a positive inotropic action in Sr-Ringer's solution, while rhodochlorin in a similar solution was ineffective. This may indicate that factors in addition to the facilitation of transmembrane flux of strontium and calcium may be important for the cardiotonic action of ouabain. These may include the mobilization of intracellular calcium and an increase in the efficiency of the actomyocin complex in the use of available energy for contraction. The necessity of calcium for strontium-induced cardiotonic action is further supported by the sequence of experiments demonstrated in Fig. 4. After sufficient chelation of tissue calcium by EDTA, ouabain in Sr-Ringer's solution was without cardiotonic effect but ouabain in Ca-Ringer's solution was effective in eliciting a positive inotropic response. Furthermore, the addition of sub-effective concentation of calcium to a Sr-Ringer's solution containing rhodochlorin was capable of eliciting a weak cardiotonic action.

Summary. The effects of calcium, strontium, EDTA, rhodochlorin and ouabain have been evaluated on the isolated spontaneously beating frog heart. Ouabain is capable of eliciting a positive inotropic response in Sr-Ringer's solution, but rhodochlorin is without







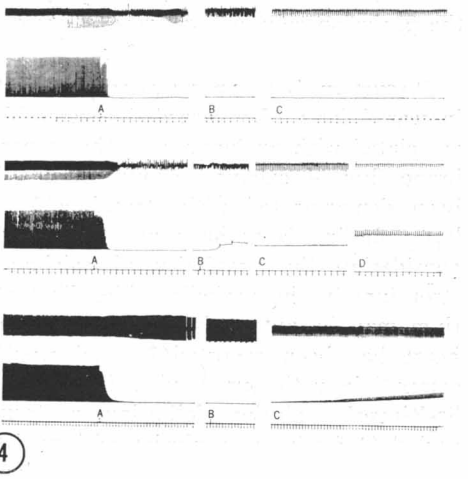


FIG. 1. Cardiotropic actions of ouabain on the isolated spontaneously beating frog heart perfused with 1.08 mM $SrCl_2$ in a Ca-free Ringer's solution. At A, the Ca-Ringer's solution was replaced by a Sr-Ringer's solution containing 6.7×10^{-7} M ouabain. At B, 458 beats after A, maximal positive inotropic effect was observed. At C, cardiac arrhythmia was observed. At D, 970 beats after A, systolic standstill occurred. Top tracing is electrogram. Middle tracing is contractions. Bottom tracing is time scale of 5 seconds.

FIG. 2. Cardiotropic actions of ouabain on the isolated spontaneously beating frog heart perfused with Sc-Ringer's solutions. Comparison of the mean values, in beats required to reach maximal positive inotropic response and in beats required to reach ventricular standstill, indicates significant differences ($P<.05$). Student's test was used to compare the means.

FIG. 3. Cardiotropic effects of calcium, strontium, and EDTA on the isolated spontaneously beating frog heart. Time scale = 5 seconds. 1. At A, the Ca-Ringer's solution was replaced by a Sr-Ringer's solution ($SrCl_2$ = 1.08 mM). At B, 120 min after A, the Sr-Ringer's solution was replaced by a Ca-Ringer's solution. 2. At A, the Ca-Ringer's solution was replaced by a Sr-Ringer's solution. At B, 5 min after A, 0.54 mM $SrCl_2$ was added to the perfusion solution (total $SrCl_2$ = 1.62 mM). At C, 5 min after B, and additional 0.54 mM $SrCl_2$ was added to the perfusion solution (total $SrCl_2$ = 2.16 mM). At D, the supra-normal Sr-Ringer's solution was replaced by Sr-Ringer's solution. 3. At A, the Ca-Ringer's solution was replaced by a Ca-free Ringer's solution containing 1.34 mM EDTA-Na_2. At B, 10 min after A, 2.16 mM Sr-Ringer's solution replaced the EDTA solution. At D, about 30 min after C, a positive inotropic response is elicited.

FIG. 4. Effects of cardiotonic agents on the isolated spontaneously beating frog heart perfused with EDTA. Time scale = 5 seconds. 1. At A, the Ca-Ringer's solution was replaced by a Ca-free Ringer's solution containing 1.34 mM EDTA-Na_2. At B, 10 min after A, a Sr-Ringer's solution containing 6.7×10^{-7} M ouabain replaced the EDTA solution. At C, 120 min after B, no cardiotonic response was observed. 2. At A, the Ca-Ringer's solution was replaced by a Ca-free Ringer's solution containing 1.34 mM EDTA-Na_2. At B, 10 min after A, a Ca-Ringer's solution containing 6.7×10^{-7} M ouabain replaced the EDTA solution. At C, about 15 min after B, weak contractions were recordable. At D, about 30 min after C, maximal positive inotropic action was elicited. 3. At A, the Ca-Ringer's solution was replaced by a Sr-Ringer's solution containing 1.7×10^{-6} M rhodochlorin. At B, 60 min after A, 0.14 mM $CaCl_2$ was added to the perfusion solution. At C, about 60 min after B, a positive inotropic response was elicited.

effect when present in the same solution. Following perfusion with Ca-free Ringer's solution containing EDTA, contractility may be partially restored by perfusing the heart with Ca-Ringer's solution with or without ouabain, but contractility was not restored by supra-normal Sr-Ringer's solution with or without ouabain. Sub-effective concentration of calcium when added to Sr-Ringer's solution containing rhodochlorin also elicited a weak cardiotonic action on the heart. It is suggested that strontium has little or no cardiotonic action of itself but depends upon calcium for its positive inotropic action, and that factors in addition to the facilitation of transmembrane flux of calcium or strontium ions may be important for the cardiotonic action of ouabain.

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Effects of Glucose, Pyruvate and α-Ketoglutarate on Acetate Metabolism In Rat Mammary Gland.* (32529)

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It is well known that lactating mammary gland slices have a high capacity for fatty acid synthesis and that the synthesis of fatty acids from C^{14} labelled substrates such as acetate, pyruvate, lactate, propionate and a number of amino acids is markedly stimulated by addition of glucose to the incubation medium(1-6). Abraham and Chaikoff(7) showed that this pronounced effect of glucose on the fatty acid synthesis of mammary gland slices is limited to the period of lactation. In mammary glands from pregnant or postlactating animals a much smaller effect of glucose on fatty acid synthesis could be detected.

In experiments on lactating rat mammary gland slices Hirsch *et al*(1) found a moderate stimulation of fatty acid synthesis from labelled acetate, when pyruvate was added to the incubation medium. If pyruvate were added in the presence of glucose, the stimulating effect of glucose was reduced.

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