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FREQUENCY-DEPENDENT CHANGES IN THE CARDIAC SARCOLEMMAL ATPASE

J.C. KHATTER & K. PRASAD

Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO

1 Effects of various frequencies (0.25, 0.5, 1.0, 1.5 or 2.0 Hz) of stimulation for various durations (2, 5, 10 or 15 min) on the contractile force of trabecular or papillary muscles of dog myocardium were investigated.

2 Effects of various frequencies (0, 0.25, 0.5, 1.0, 2.0 Hz) of various stimulus strengths (0.5, 1, 10 V) for various durations (2, 5, 10 or 15 min) on the Mg^{2+}-dependent Na^+-K^+-adenosinetriphosphatase (ATPase) of isolated sarcolemmal fraction of dog myocardium were determined.

3 There was a frequency-dependent increase in the contractility and inhibition of the Na^+-K^+-ATPase within 2 minutes.

4 Frequency-dependent increase in the contractility and inhibition of Na^+-K^+-ATPase decreased as the duration of stimulation was increased.

5 The diminution in the inhibition of ATPase was associated with a decrease in the contractility with prolonged stimulation.

6 These results suggest that the frequency-dependent increase in the myocardial contractility might be mediated through an inhibition of the sarcolemmal ATPase.

Introduction

Frequency-dependent changes in the force of contraction have been reported both *in situ* (Lendrum, Boyd & Katz, 1960) and in isolated tissue preparations (Blinks & Koch-Weser, 1961; Katzung & Scheider, 1957). Striking similarities between the positive inotropic effect produced by cardiac glycosides and that produced by changes in the frequency of contraction have also been demonstrated. Thus, as with cardiac glycosides, an increase in frequency of stimulation has been reported to produce an increase in the rate of tension development and a reduction in time to peak tension (Abbot & Mommerts, 1959; Blinks & Koch-Weser, 1961; Koch-Weser, 1963; Reiter & Strickel, 1968). Since it has been suggested that cardiac glycosides produce their positive inotropic effect by inhibiting Mg^{2+}-dependent Na^+-K^+-stimulated membrane adenosinetriphosphatase (ATPase) (Prasad & Callaghan, 1969; Akera, Larsen & Brody, 1970; Besch, Allen, Glick & Schwartz, 1970; Prasad, 1970; 1972; 1974; 1975), it is possible that the positive inotropic effect of increasing the frequency of stimulation might be mediated through an inhibition of the membrane ATPase of the myocardium. If the increase in the force of contraction is related to the inhibition of Na^+-K^+-ATPase, the possibility exists that this inhibition of Na^+-K^+-ATPase is frequency-dependent. Indeed preliminary data of Prasad & Kidwai (1974) do indicate that this may be so.

It was therefore decided to study in detail, the effects of frequency of stimulation on Mg^{2+}-dependent Na^+-K^+-stimulated sarcolemmal ATPase of isolated membrane fraction from the dog ventricle.

Methods

Dogs of either sex weighing between 16–20 kg were anaesthetized with pentobarbitone sodium 35 mg/kg intravenously. The chest was opened through the left fifth intercostal space and the heart was exposed. The pericardium was removed and 5 to 10 g of the left or right ventricle was removed and placed in a cold (–4°C) 0.25 M sucrose solution prepared in glass distilled water. Relatively pure sarcolemma from cardiac muscle was prepared by the method of Kidwai, Radcliffe, Duchon & Daniel (1971) and the Mg^{2+}-dependent Na^+-K^+stimulated ATPase was determined. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Protein determinations were performed before ATPase activity measurements so that equivalent amounts of protein could be used for study. The total volume of enzyme assay system was 1.0 ml and contained

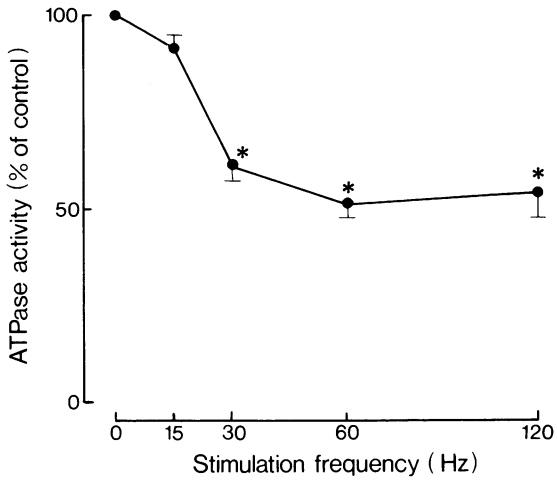

Figure 1 Effects of various frequencies of sarcolemmal stimulation on sarcolemmal Na^+-K^+-ATPase in dog heart; stimulus strengths 0.5 V, duration 2 minutes. The results are expressed as percentage of control (without stimulation but incubated for 2 minutes). Each point on the curve is the mean value ± s.e. (bar) from 6 experiments. * Indicates significant difference from control ($P < 0.05$).

(mmol/l): $MgCl_2$ 4, NaCl 50, KCl 4, Tris ATP 4, L-histidine 50 and 50 µg of sarcolemmal protein. The reaction mixture (the enzyme assay system minus Tris ATP) was placed in special tubes, fitted at the base with platinum electrodes such that both the electrodes were submerged in the mixture medium. The electrodes were connected to the Grass stimulator (Model SD9) for stimulation of the mixture media. The tubes containing the reaction mixture was placed in a constant temperature water bath (Dubnoff Metabolic Shaking Incubator, Precision Scientific Corporation) at 37°C. The temperature was maintained at 37°C. The reaction was started by addition of ATP and terminated by addition of 1.0 ml of 10% trichloracetic acid. Inorganic phosphate was determined by the method of Fiske & SubbaRow (1925). The results are expressed as µmol of inorganic phosphate liberated mg^{-1} protein h^{-1}. Adenosinetriphosphatase activity assayed in the presence of ouabain 1 mmol/l was subtracted from total activity assayed in the absence of ouabain. This value represented the Mg^{2+}-dependent Na^+-K^+-stimulated ouabain-sensitive portion of ATPase activity. Similarly Na^+-K^+-ATPase in the presence of various frequencies of stimulation was determined.

For electrical stimulation of the isolated sarcolemmal fraction, the reaction mixture without ATP was placed in a special tube, fitted at the base with platinum electrodes such that both the electrodes were submerged in the mixture medium. The electrodes were connected to the Grass Model SD9 stimulator for stimulation of the mixture media at different frequencies. The tubes containing reaction mixture were placed in the constant temperature water bath (Dubnoff Metabolic Shaking Incubator) at 37°C. The mixtures were incubated at 37°C and were stimulated at various frequencies and voltages for various times. Addition of the ATP to and stimulation of the mixtures were carried out simultaneously. The stimulation of the mixture was carried out at four different frequencies (0.25, 0.5, 1.0 and 2.0 Hz) and stimulus strength of 0.5, 1 and 10 V each for a period of 2, 5, 10 and 15 minutes. The duration of each impulse was 5 ms in all cases. At the end of the experiment the reaction was terminated by addition of 1 ml of 10% trichloracetic acid (TCA).

Trabeculae carnae or papillary muscles were removed from the right or left ventricle of the same dog from which the muscles were removed for isolation of membrane fraction. The set-up for recording the contractility of the muscle was the same as used by Prasad (1975). The thickness of the muscles used in this experiment was such that there was no central core of hypoxia.

Results

Stimulation frequency and ATPase

Experiments were conducted in which reaction mixtures containing sarcolemmal fraction incubated at 37°C were stimulated at frequencies of 0, 0.25, 0.5, 1.0 or 2.0 Hz with a stimulus strength of 0.5 V for a duration of 2 min and the sarcolemmal ATPase was determined for each frequency of stimulation. The results are summarized in Figure 1. The sarcolemmal ATPase for the unstimulated fraction incubated for 2 min was found to be 13.39 ± 0.9 µmol mg^{-1} protein h^{-1}. The sarcolemmal ATPase was found to be inhibited at all frequencies of stimulation, the inhibition being directly proportional to the rate of stimulation from 0 to 1.0 Hz. A further increase in the frequency of stimulation to 2.0 Hz did not produce any further inhibition of sarcolemmal ATPase, on the other hand there was a tendency for a decrease in the inhibition.

Stimulation frequency and contractile force

In five experiments the trabecular muscles were stimulated at various frequencies (0.5, 1.0, 1.5 or 2.0 Hz) for various lengths of time (2, 5, 10 or 15 min) and the changes in the contractile force were recorded. Figure 2 is a representative recording from one trabecular muscle. It is apparent from the tracing that the increase in the contractile force was frequency-

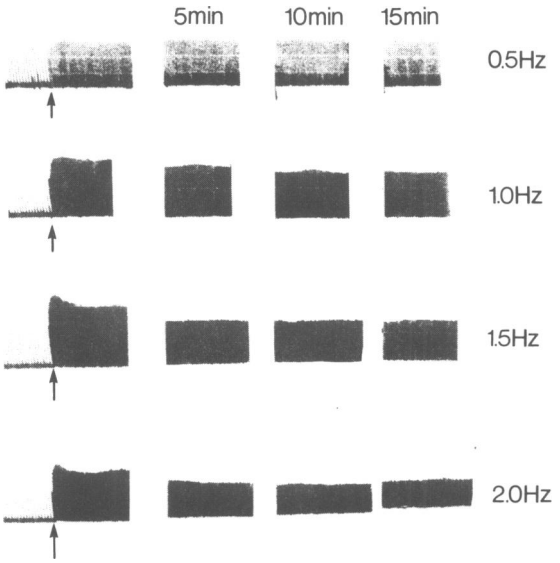

Figure 2 Effects of different rates of stimulation for different periods on the contractility of trabecular muscle of dog heart. The muscles were stimulated in the control period at the rate of 0.25 Hz. Arrows mark the beginning of the stimulation of the muscles at different frequencies given at the end of each strip of tracing. The time at the top of the tracings indicate the time when that strip was recorded.

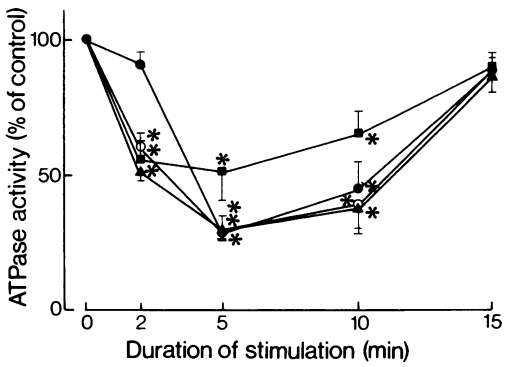

Figure 3 Effects of different frequencies of stimulation for various periods (2, 5, 10 or 15 min) on sarcolemmal ATPase of dog heart; the results are expressed as percentage of control (without stimulation but incubated for 2, 5, 10 or 15 minutes). Stimulation frequency: (●) 0.25, (○) 0.5, (▲) 1.0 and (■) 2.0 Hz, stimulus strength 0.5 V. Each point on the curve is the mean value from 6 experiments. Vertical lines show s.e. mean. The value at 0 along the duration axis denotes the value of ATPase for different incubation periods without stimulation (control). * Indicates that the changes are significant ($P<0.05$).

dependent initially but with stimulation for 5 min and longer the increase in the contractility was inversely related to the rate of stimulation. At 15 min the contractility was even less than control when stimulated at a rate of 2.0 Hz. These results indicate that high rates of stimulation for prolonged periods did not increase the contractility as they did initially.

Stimulation duration and ATPase

The preceding experiment showed that long periods of stimulation at different frequencies affected the contractility adversely. This decline in the increase of contractility with increased duration might be due to less inhibition of the ATPase and/or decrease in the amount of available energy for contraction. To see if a decrease in the inhibition of ATPase with increased duration for different frequencies might be a factor for reduction in the increase in contractility, experiments were conducted in which the mixtures containing sarcolemmal fraction were stimulated at different frequencies with stimulus strength of 0.5 V for various periods (2, 5, 10, or 15 min) and the sarcolemmal ATPase was determined. The results are summarized in Figure 3. The sarcolemmal ATPase activity without stimulation was found to be 13.4 ± 0.9, 12.15 ± 0.28, 11.1 ± 0.46, 11.43 ± 0.79 μmol mg^{-1} protein h^{-1} respectively for 2, 5, 10 and 15 min duration of incubation without stimulation. An increase in the duration of stimulation from 2 to 5 min caused an increased inhibition of ATPase at all frequencies. A further increase in the duration of stimulation caused less inhibition of ATPase than with a shorter duration. At 15 min, the ATPase activity tended to approach the control value. At a frequency of 2 Hz the inhibition of ATPase was less than that with lower frequencies. These results indicate that higher frequencies and longer duration, produced less inhibition of the sarcolemmal ATPase.

Stimulus strength and ATPase

The above results suggested that the heat produced by the increase in the frequency or duration of stimulation might produce less inhibition. If this is the case then an increase in the strength of stimulation at those frequencies and duration might produce more heat and hence would produce still less inhibition of ATPase. To test this assumption eight experiments were carried out in which reaction mixtures containing sarcolemmal fraction were stimulated at various frequencies with a stimulus strength of 1 or 10 V for various durations and the sarcolemmal ATPase was determined in each case. The results are summarized

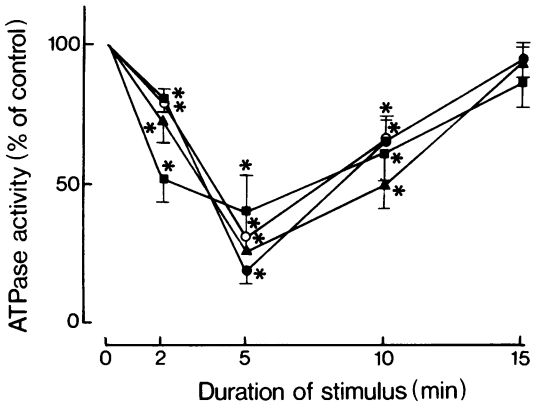

Figure 4 Effect of stimulation of mixture containing sarcolemmal fraction on sarcolemmal ATPase of dog heart at various frequencies and durations with a stimulus strength of 1 Volt. Frequencies: (●) 0.25; (○) 0.5; (▲) 1.0 and (■) 2.0 Hz. Each point on the curves is mean from 8 experiments. Vertical lines show s.e. mean. * Indicates the changes are significant ($P < 0.05$).

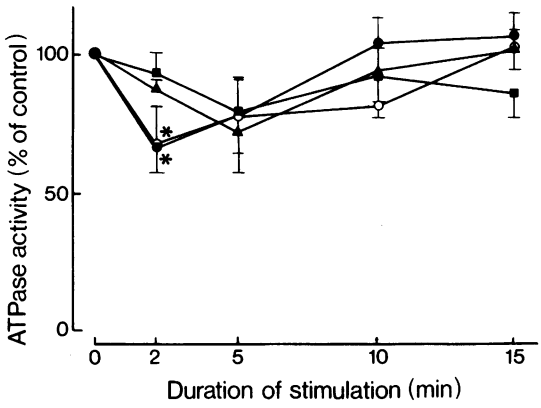

Figure 5 Effects of stimulation of reaction mixtures containing sarcolemmal fraction on the sarcolemmal ATPase of dog heart at various frequencies and durations with a stimulus strength of 10 Volts. Frequencies: (●) 0.25; (○) 0.5; (▲) 1.0 and (■) 2.0 Hz. Each point on the curves is the mean from 8 experiments. Vertical lines show s.e. mean. * Indicates the changes are significant ($P < 0.05$).

in Figures 3, 4 and 5. An increase in the stimulus strength from 0.5 to 1.0 V caused slightly more inhibition of ATPase at all frequencies of stimulation. However, when stimulus strength was increased to 10 V there was no significant inhibition of the ATPase at any frequencies and durations except at 2 min with frequencies of 0.25 and 0.5 Hz. At 10 and 15 min duration of stimulation the ATPase was almost normal or more than control value.

Effects of stimulation on temperature changes in the reaction mixture

Since the sarcolemmal Na^+-K^+-ATPase is affected by changes in temperature (Charnock & Post, 1963) and since the electrical stimulation of the reaction mixture might raise the temperature of the reaction mixture, it was decided to see if there was a change in the temperature in the reaction mixture due to electrical stimulation.

Effects of different frequencies (0.25, 0.5, 1.0 and 2.0 Hz) of stimulus strength of 0.5, 1 and 10 V for duration of 2, 5, 10 and 15 min were studied on the temperature changes in the reaction mixture incubated in the constant temperature water bath (Dubnoff Metabolic Shaking Incubator) at 37°C. The probe of a Thermistor Bridge (E & M Instrument Co. Inc., Houston, Texas) was placed in the reaction mixture and the temperature was continuously recorded on the Physiograph MKIII. The temperature changes were recorded with and without stimulation of the reaction mixture. There were phasic changes in the temperature, the maximum change being 0.44°C (Figure 4). This phasic change was associated with the off and on of the heater of the Dufnoff metabolic shaker. Stimulation of the reaction mixture at all frequencies and stimulus strength for all durations did not change the temperature. The phasic change in the temperature associated with the 'off' and 'on' of the heater of the Dufnoff Metabolic Shaker was present during the stimulation of the reaction mixture and this phasic change was never more than 0.44°C. A typical tracing with maximum voltage (10 V) and frequency (2.0 Hz) for 15 min is shown in Figure 6. It is thus apparent that the electrical stimulation of the reaction mixture in a temperature-controlled water bath did not produce any significant change in the temperature of the reaction mixture.

Discussion

The results indicate that there is a frequency-dependent increase in the contractility and an inhibition of sarcolemmal Na^+-K^+-ATPase. However, an increase in the duration of stimulation or an increase in the strength of stimulus at different frequencies produced a decrease in the inhibition of sarcolemmal ATPase. Also the frequency-dependent increase in the contractility decreased when the duration of stimulation was increased.

The increase in cardiac contractility with an increase in the frequency of stimulation is well known (Katzung, Rasin & Scheider, 1957; Lendrum, Feinberg, Boyd & Katz, 1960; Blinks & Koch-Weser, 1961). The present results confirm our (Prasad &

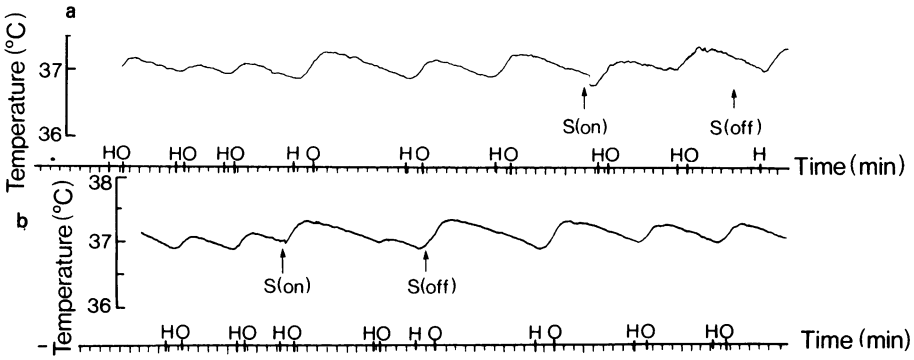

Figure 6 Effects of electrical stimulation on the temperature changes in reaction mixture. In (a) temperature was recorded for 50 min before electrical stimulation started. In (b) temperature was recorded before and after electrical stimulation. H, pilot light of Dubnoff Metabolic Shaker is on when the temperature starts falling; O, pilot light of the heater off when the temperature starts rising; S, stimulation of reaction mixture at the rate of 2.0 Hz with a stimulus strength of 10 V of 5 ms duration. At the arrow S(On) the stimulation of the reaction mixture begins and at arrow S(Off) the stimulation ends. The rest of the tracing is of the temperature changes in reaction mixture without stimulation. Each mark on the time scale represents one minute. Note that the electrical stimulation of the mixture did not produce any significant change in the temperature of the reaction mixture.

Kidwai, 1974) preliminary observation that electrical stimulation of isolated sarcolemmal fraction produces an inhibition of the sarcolemmal ATPase. The increase in the frequency from 0.25 to 0.5, 1.0, 1.5 or 2.0 produced an increase in the contractility that was inversely related to the rate of stimulation when the duration of stimulation was increased. Similarly the increase in the rate of stimulation produced an inhibition of the sarcolemmal Na^+-K^+-ATPase which was inversely related to the rate of stimulation when the duration of stimulation was increased. Thus it appears that the frequency-dependent increase in contractility might be related to an inhibition of the sarcolemmal Na^+-K^+-ATPase. It has been observed, in our laboratory (unpublished results), that agents which inhibit membrane ATPase either reduce or completely abolish the rate-dependent increase in contractility. However, the frequency-dependent increase in contractility decreased at very high frequency (2.0 Hz) and when the duration of stimulation was increased. This could be possible if there were a decrease in the inhibition of the sarcolemmal ATPase and/or a decrease in the available energy at higher frequencies and prolonged stimulation. Certainly these results show that there was a decrease in the inhibition of the sarcolemmal Na^+-K^+-ATPase at higher frequencies and prolonged stimulation. The question of available energy is not considered in the present paper. The question arises why there should be a decrease in the inhibition of Na^+-K^+-ATPase at very high frequency or prolonged stimulation. The decrease in the inhibition of sarcolemmal Na^+-K^+-ATPase with prolonged stimulation at various frequencies might be due to the fact that electrical stimulation of the reaction mixture containing sarcolemmal fraction might be producing enough heat to raise the temperature of the reaction media which in turn might stimulate the sarcolemmal Na^+-K^+-ATPase. An increase in temperature has been reported to increase the Mg^{2+}-dependent, Na^+-K^+-stimulated microsomal ATPase (Charnock & Post, 1963; Emmelot & Bos, 1966). However, the present results show that there was no significant change in temperature of the reaction mixture due to electrical stimulation possibly because of the controlled temperature water bath. At present no plausible explanation for less inhibition of Na^+-K^+-ATPase at higher frequencies or with prolonged stimulation or at higher voltages of stimulation can be offered.

The question now arises how this inhibition of Na^+-K^+-ATPase would increase myocardial contractility. Several models have been proposed for ionic movement in cardiac muscle (Langer, 1971; Prasad, 1974). These models suggest that sarcolemmal Na^+-K^+-ATPase is not only involved in the efflux and influx of Na^+ and K^+ but also in influx and efflux of Ca^{2+}. An inhibition of sarcolemmal Na^+-K^+-ATPase has been suggested to increase Ca^{2+} influx (Prasad, 1970; 1974). The present results indicate that there is a frequency-dependent inhibition of the sarcolemmal ATPase. This inhibition of ATPase would increase the Ca^{2+} influx and hence the contractility. Thus, the results suggest that frequency-dependent increase in contractility might be mediated through an inhibition of the sarcolemmal Na^+-K^+-ATPase.

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References

ABBOTT, B.C. & MOMMAERTS, W.F.H.M. (1959). A study of inotropic mechanisms in papillary muscle preparation. *J. gen. Physiol.*, **42**, 533–551.

AKERA, T., LARSEN, F.S. & BRODY, T.M. (1970). Correlation of cardiac sodium- and potassium-activated adenosine triphosphatase activity with ouabain-induced inotropic stimulation. *J. Pharmac. exp. Ther.*, **173**, 145–151.

BESCH, H.R. Jr., ALLEN, J.C., GLICK, G. & SCHWARTZ, A. (1970). Correlation between the inotropic action of ouabain and its effects on subcellular enzyme systems from canine myocardium. *J. Pharmac. exp. Ther.*, **171**, 1–12.

BLINKS, J.R. & KOCH-WESER, J. (1961). Analysis of the effects of changes in rate and rhythm upon myocardial contractility. *J. Pharmac. exp. Ther.*, **134**, 373–389.

CHARNOCK, J.C. & POST, R.L. (1963). Studies of the mechanism of cation transport. I. The preparation and properties of cation stimulated ATPase from guinea pig kidney cortex. *Austral. J. exp. Biol.*, **41**, 547–560.

EMMELOT, P. & BOS, C.J. (1966). Studies on plasma membrane Mg^{++} ATPase, Na^{+}-K^{+}-Mg^{++} ATPase and 5′-nucleotidase activity of plasma membrane in rat liver. *Biochimica biophysica Acta*, **120**, 369–382.

FISKE, C.H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem.*, **66**, 375–400.

KATZUNG, B., RASIN, H. & SCHEIDER, F. (1957). Frequency-force relationship in the rabbit auricle and its modification by some metabolic inhibitors. *J. Pharmac. exp. Ther.*, **120**, 324–333.

KIDWAI, A.M., RADCLIFFE, M.A., DUCHON, G. & DANIEL, E.E. (1971). Isolation of plasma membrane from cardiac muscle. *Biochemic. Biophys. Res. Communic.*, **45**, 901–910.

KOCH-WESER, J. (1963). Effect of rate change on strength and time course of contraction of papillary muscle. *Am. J. Physiol.*, **204**, 451–457.

LANGER, G.A. (1971). The intrinsic control of myocardial contraction-ionic factors. *New Eng. J. Med.*, **285**, 1065–1071.

LENDRUM, B., FEINBERG, H., BOYD, E. & KATZ, L.N. (1960). Rhythm effects on contractility of the beating isovolumic left ventricle. *Am. J. Physiol.*, **199**, 1115–1120.

LOWRY, O.H., ROSEBROUGH, J.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

PRASAD, K. (1970). Influence of energy supply and Ca^{++} on the low Na^{+}-induced changes in the transmembrane potential and contraction of guinea pig papillary muscle. *Can. J. Physiol. Pharmac.*, **48**, 241–253.

PRASAD, K. (1972). Transmembrane potential, contraction and ATPase activity of human heart in relation to ouabain. *Jap. Heart J.*, **13**, 59–72.

PRASAD, K. (1974). Membrane Na^{+}-K^{+}-ATPase and electromechanics of human heart. In *Recent Advances in Studies on Cardiac Structure and Metabolism*, Vol. 4, Myocardial Biology, ed. Dhalla, N.S. pp. 91–105. Baltimore: University Park Press.

PRASAD, K. (1975). Glucagon-induced changes in the action potential, contraction and Na^{+}-K^{+} ATPase of cardiac muscle. *Cardiovascular Res.*, **9**, 355–365.

PRASAD, K. & CALLAGHAN, J.C. (1969). Effect of replacement of potassium with rubidium on the transmembrane action potential and contractility of human papillary muscle. *Circulation Res.*, **24**, 157–166.

PRASAD, K. & KIDWAI, A.M. (1974). Frequency-dependent changes in contractility, action potential duration and membrane Na^{+}-K^{+}-ATPase in cardiac muscle. *Proc. Canad. Fed. Biol. Soc.*, **17**, 16.

REITER, M. & STRICKEL, F.J. (1968). Der Einfluss der Kontraktionsfrequenz auf das Aktionspotential des Meerschweinchen-Papillarmuskels. *Naunyn Schmiedebergs Arch. Pharmak. exp. Path.*, **260**, 342–365.

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