Tohoku J. exp. Med., 1974, **113**, 187–196

Influence of Calcium and Magnesium Ions on the Sino-atrial Node Pacemaker Activity of the Canine Heart

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HASHIMOTO, K., SUZUKI, Y. and CHIBA, S. *Influence of Calcium and Magnesium Ions on the Sino-atrial Node Pacemaker Activity of the Canine Heart.* Tohoku J. exp. Med., 1974, **113** (2), 187–196 —— Using constant pressure perfusion technique of the canine sinus node artery, effects of changing concentrations of calcium and magnesium ions were investigated on the sino-atrial (SA) pacemaker activity. An increase in calcium concentration produced a diphasic chronotropic action; positive at 5–25 mEq/liter but negative above 30 mEq/liter. The positive chronotropic action of calcium was not prevented by pretreatment with reserpine, but partially depressed by beta-adrenergic blocking agents. On the contrary, an infusion of GEDTA, more specific Ca^{++} chelating agent, slowed the sinus rate and further produced sinus arrhythmia at a higher infusion rate, which was restored to regular sinus rhythm by norepinephrine. Changes in magnesium concentration produced dose-dependently a negative chronotropic effect that was not modified by atropine. Positive chronotropic responses either to calcium or to isoproterenol were significantly depressed by increasing magnesium concentration. Atrial fibrillation induced by acetylcholine was facilitated by increasing calcium concentration but inhibited by administration of either magnesium or GEDTA. Antagonism between calcium and magnesium was demonstrated on the SA node pacemaker activity. It is suggested that calcium and catecholamine facilitate each other the action at the effector sites and that magnesium stabilizes the membrane of the SA node pacemaker against any stimuli of rapid firing. ——— atrial fibrillation; GEDTA; atropine; catecholamine; reserpine

Previously, the present authors reported that the administration of acetylcholine into the sinus node artery of the dog regularly induced atrial fibrillation (Hashimoto et al. 1968). The induction of this atrial fibrillation was prevented by treatment with not only atropine but also anti-adrenergic compounds, guanethidine, bretylium or propranolol and pretreatment with reserpine. Therefore, it was suggested that the participation of adrenergic mechanism may play an important role for the induction of atrial fibrillation by the administration of acetylcholine into the sinus node artery. Recently the present authors (Hashimoto et al. 1970) also described the potassium effect on the sino-atrial (SA) node pacemaker activity

Received for publication, May 1, 1974.

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and demonstrated that hyperpotassemia prevented the induction of atrial fibrillation induced by acetylcholine injection or vagal stimulation. Furthermore, the positive chronotropic effect of catecholamine was significantly suppressed by hyperpotassemia. Thus, it is suggested that the anti-fibrillatory action of excess potassium depends, in part, on its interference with adrenergic mechanism.

In the present experiments, the authors examined the chronotropic effects of calcium and magnesium given into the sinus node artery and also the influence of these ions on effects of autonomic transmitters, with particular references to the mode of atrial fibrillation.

Methods

Twenty-five mongrel dogs of both sexes weighing 9 to 20 kg were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously. Artificial respiration was performed using a "Bird" respirator Mark 8 with room air. The direct perfusion of the sinus node artery used in these experiments was originally devised by James and Nadeau (1962) and modified by the authors for pharmacological research (Hashimoto et al. 1967, 1968, 1970). Constant perfusion pressure was maintained without interrupting the circulation, even when a drug solution was administered. Thus baroceptive response of the SA pacemaker was prevented.

Systemic blood pressure in the carotid artery was measured by an electromanometer (Nihon Kohden RP-2). ECG (lead II and right auricular lead) was recorded with a Nihon Kohden ME-20-TR. The heart rate was recorded by a cardiotachograph (Nihon Kohden RT-2) which was triggered by the R wave of the lead II. Sodium heparin, 500 units/kg, was given at the perfusion and 200 units/kg added at 2-hour intervals. It was necessary to supply additional blood from time to time (50 to 100 ml per hour) intravenously to keep the mean systemic blood pressure above 100 mm Hg.

Bipolar platinum electrodes were placed at the distal end of the cut right vagus nerve for electrical stimulation. Supramaximal stimuli (5V, 0.1 msec, 30 cps) were applied for 6 seconds by use of a square wave stimulation (Nihon Kohden MSE-2).

In order to replace sodium ions by calcium or magnesium ions with negligible change in the other ionic composition, test solutions were prepared by means of the ionic composition listed on Table 1. Test solutions were infused into the perfusion system with an infusion-withdrawal pump (Harvard Apparatus, Model 600–900). The concentrations of sodium and potassium in the plasma were measured by a flame photometer (Hitachi FPF-2) and the concentrations of calcium were determined by the method of Ferro-Ham with an electrophotometer (Akiyama DS 502). The rate of blood flow in the sinus node artery (1.96 ± 0.21 ml/min, mean$\pm$S.E., N=45) was not sufficiently large enough to permit the collection of blood sample to make direct measurement of ionic concentrations. These measurements were, therefore, done on a blood sample to which the test solution was added in an amount calculated from the blood flow and the infusion rate of the test solution. The concentration of magnesium was not measured.

Drugs used were acetylcholine chloride (Daiichi), atropine sulfate (Yamanouchi), *dl*-epinephrine hydrochloride (Sankyo), *dl*-propranolol hydrochloride (Sumitomo), *dl*-LB 46 (Sandoz), *dl*-H 56/28 (Hässle), reserpine (Daiichi), *l*-isoproterenol (Nikken) and glycerol ethylenedianine-tetraacetic acid (GEDTA) (Dojin).

A daily dose of 0.5 mg/kg of reserpine dissolved in 20% ascorbic acid was given subcutaneously to three animals 24 hours and 48 hours prior to the experiment. Iso-osmotic solution of GEDTA (100 mM/liter, pH=7.4) was infused to chelate the plasma calcium. The other drugs were dissolved in the 0.9% physiological saline and 0.01 ml of the solution was injected by a microinjector in a period of 4 seconds into the rubber tube connected closely to the shank of the polyethylene cannula. Two types of microinjectors (Burroughs

TABLE 1. *Ionic composition of the test solutions*

mEq/liter	Standard solution	High-calcium solution	High-magnesium solution
Na	145	40	40
K	4	4	4
Ca	5	150	5
Mg	3	3	150
Cl	157	117	115

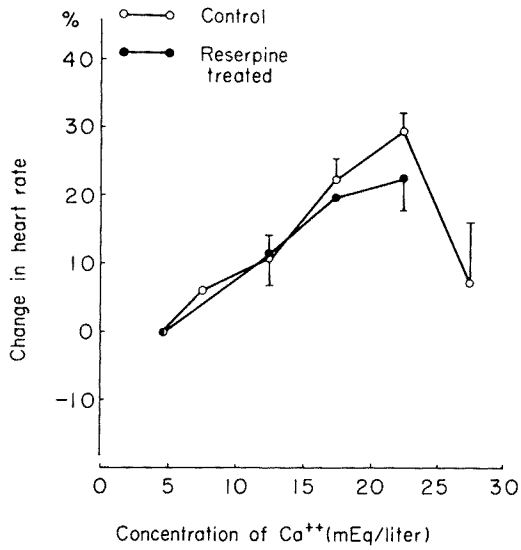

Fig. 1. Relation between the calcium concentration and change in the heart rate (%). Open circle, control animals (13 dogs); closed circle, reserpinized animals (3 dogs). Vertical bar represents standard error.

Wellcome & Co. and Terumo Co.) were used. A separate microinjector was provided for each drug solution in order to avoid contamination.

RESULTS

Chronotropic effect of excess calcium

While the constant perfusion pressure was maintained at 100 mm Hg, the increase in the infusion rate of the standard solution into the sinus node artery up to one third of its blood flow did not result in any change in the sinus rhythm but caused a slight vasodilation of the sinus node artery.

The increase in a calcium concentration caused a positive chronotropic response at the calcium concentration ranging from 5 to about 25 mEq/liter but a negative chronotropic response at the concentration above 30–35 mEq/liter as shown in Fig. 1. The positive chronotropic effect was proportional to the increase in a calcium concentration and the maximum in the sinus rate was observed at about 20–25 mEq/liter. Atrial fibrillation was observed during infusion of a high

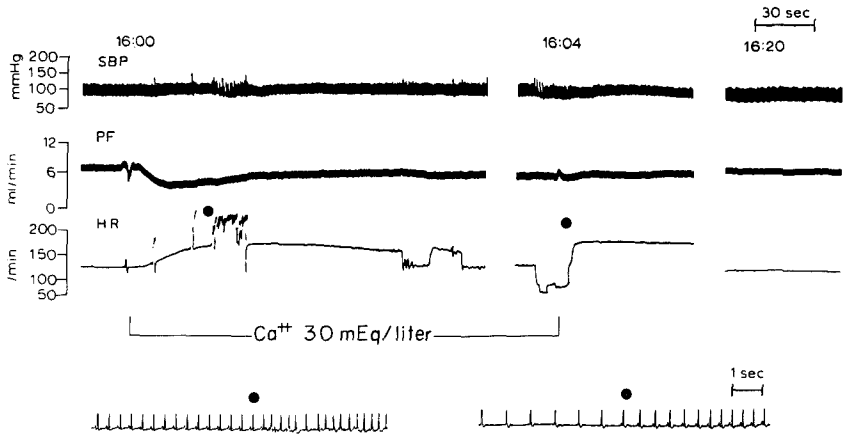

Fig. 2. Effect of infusion of a high calcium solution into the sinus node artery. SBP, systemic blood pressure. ECG in the bottom trace is Lead II.

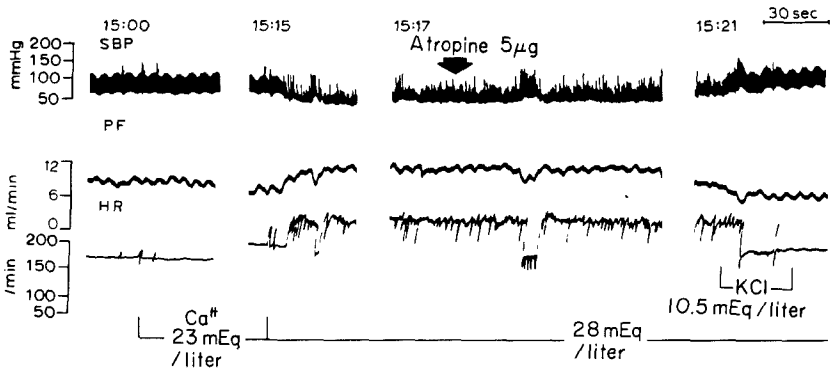

Fig. 3. Potassium-calcium antagonism on the SA node pacemaker activity. Infusion of a high-calcium solution produced sinus tachycardia which was spontaneously changed to sustaining atrial fibrillation. Atropine did not modify the fibrillation but infusion of a high-potassium solution stopped the atrial fibrillation.

calcium solution in 6 out of 14 dogs. The fibrillation was either transient as shown in Fig. 2 or continuing as long as a high calcium solution was infused as shown in Fig. 3. When the calcium concentration exceeded 30–35 mEq/liter, the pacemaker activity of the SA node was inversely depressed and an escape AV nodal rhythm took place. When the escape AV nodal rhythm appeared, infusion of the high calcium solution was stopped, and the sinus tachycardia appeared immediately after the cessation of calcium administration. The rate gradually diminished to the control rate in a few minutes (Fig. 2). Either the positive chronotropic response or the atrial fibrillation was effectively inhibited by infusing a high potassium solution.

A positive chronotropic effect of calcium was also observed at the concentrations from 5 to 25 mEq/liter in reserpinized animals. The concentration-response

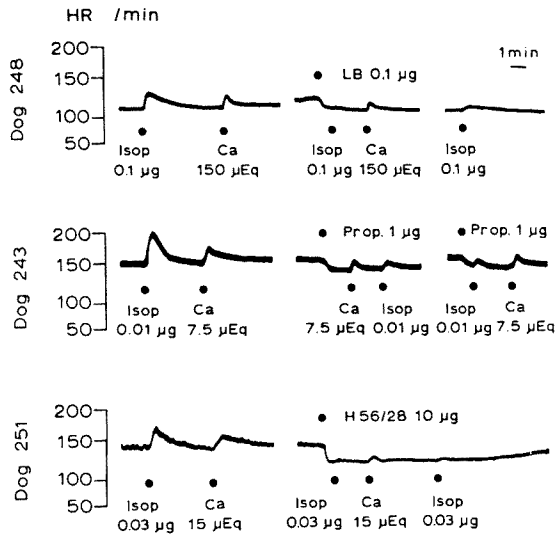

Fig. 4. Effects of beta-adrenergic receptor blocking agents on the positive chronotropic action of isoproterenol and calcium.

curve is illustrated in Fig. 1. The degree of the positive chronotropic response to excess calcium in reserpine-treated animals was identical to that of the control. The negative chronotropic response induced by hypercalcemia above 25 mEq/liter was not studied with the reserpine-treated dog. Effects of beta-adrenergic blocking agents, LB 46, propranolol and H 56/28, on the positive chronotropic response to isoproterenol and calcium were compared on five dogs. While the response to isoproterenol was significantly depressed by all of these blocking agents, that to calcium was less depressed as shown in Fig. 4.

The effect of hypercalcemia on the induction of atrial fibrillation by acetylcholine was investigated in 11 dogs. The threshold dose of acetylcholine for inducing atrial fibrillation was reduced significantly from 5.10 ± 1.41 to 1.35 ± 0.87 μg during tachycardia was observed at 23.7 ± 3.11 mEq/liter of a calcium concentration. Supramaximal electrical stimulation of the right vagus nerve elicited sinus depression, which was followed by a transient atrial fibrillation in eight out of 13 dogs. In the cases that vagal stimulation did not produce fibrillation, the infusion of the high calcium solution facilitated the incidence of transient atrial fibrillation following the bradycardia as shown in Fig. 5.

Effect of glycol ethylenediamine-tetraacetic acid (GEDTA)

An iso-osmotic solution of GEDTA, a highly specific chelating agent for calcium ion, was infused into the sinus node artery to lower the calcium level in the perfused blood. A lower concentration of GEDTA produced sinus bradycardia, while sinus arrhythmia was produced by infusion of a relatively higher dose of GEDTA. When the sinus arrhythmia occurred, a transient normal beat was restored by a

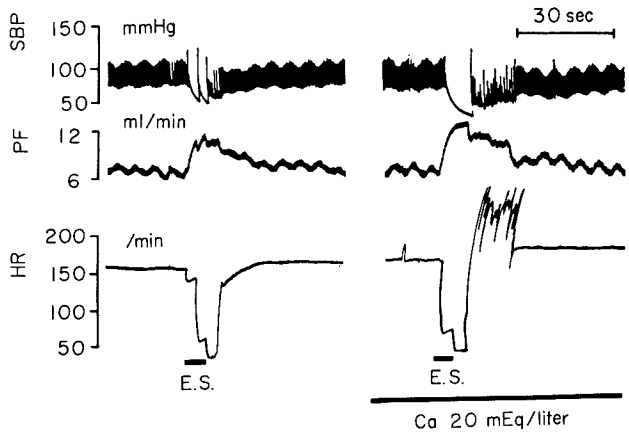

Fig. 5. Facilitatory effect of calcium on the induction of atrial fibrillation by vagal stimulation. E.S.: electrical stimulation of the right vagus (30 cps, 0.1 msec, 5 V, and 6 sec duration..

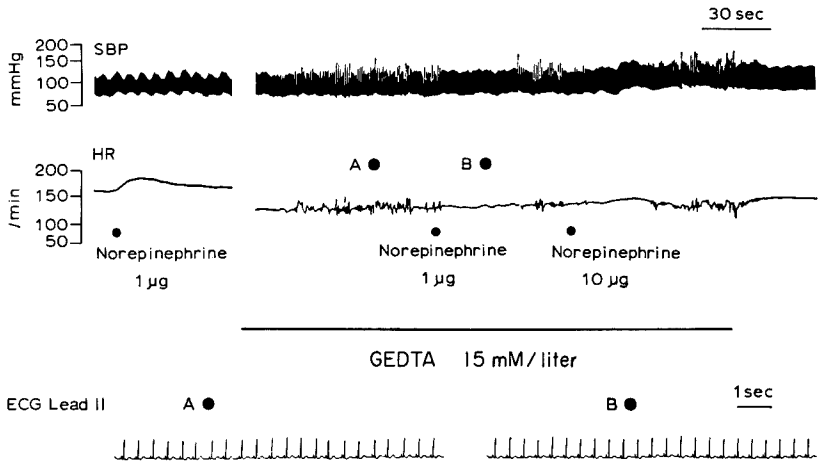

Fig. 6. Transient restoration of normal sinus rhythm from the sinus arrhythmia induced by the infusion of a higher rate of GEDTA. ECG shows sinus arrhythmia (A) and regular sinus rhythm (B).

single dose of catecholamine (Fig. 6), while the positive chronotropic effect of catecholamine was significantly reduced by GEDTA. The induction of atrial fibrillation by a single dose of acetylcholine injected into the sinus node artery was inhibited by infusion of GEDTA at a lower rate, but facilitated at a higher rate enough to produce sinus arrhythmia.

Effect of magnesium ion

When a concentration of magnesium ion in the sinus node artery was raised by infusion of a high magnesium solution, a negative chronotropic effect was dose-dependently induced (Fig. 7), which was not modified by atropine.

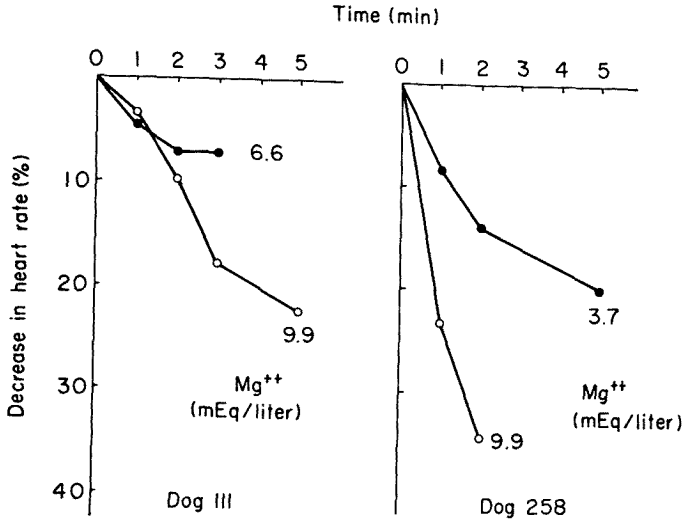

Fig. 7. Negative chronotropic effect of magnesium. Numeral in the graph represents the ratio of infused magnesium/perfused blood flow (liter).

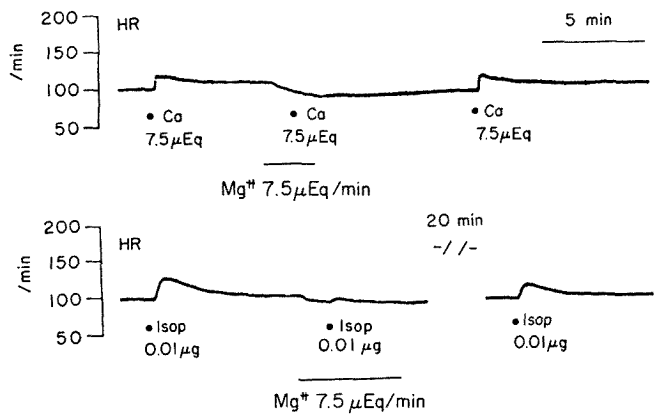

Fig. 8. Effect of magnesium on the positive chronotropic effect of calcium and isoproterenol.

The positive chronotropic responses to calcium as well as to isoproterenol were significantly reduced by the increase in the magnesium concentration (Fig. 8). Induction of transient atrial fibrillation by a single dose of acetylcholine was inhibited during the sinus bradycardia produced by infusion of a high magnesium solution (Fig. 9).

Discussion

In 1930, Mancke observed that an increase in concentration of calcium in a certain range had a rate-increasing effect in the isolated mammalian heart. In recent years, many investigators have studied the chronotropic effect of calcium. Previous studies reported that a rise in calcium concentration caused an increase

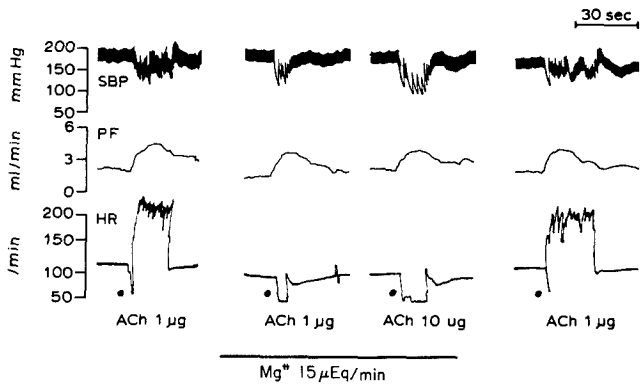

Fig. 9. Blocking effect of magnesium on the induction of atrial fibrillation by acetylcholine.

in rate, reaching the maximum. At higher calcium concentrations, atrial rate declined again (Seifen et al. 1964a, b; Schaer 1964). Midrio and Sperti (1963) reported that the positive chronotropic effect of calcium was mediated by the release of adrenergic transmitter. Vincenti and West (1965) described that the chronotropic action of calcium is ascribed to combination of the direct action on pacemaker fibers and indirect action through the release of autonomic mediator. On the other hand, Seifen et al. (1964a) reported that the effect of calcium on heart rate was not altered by prior administration of atropine, nor was it modified in the depletion of cardiac catecholamines by the treatment with reserpine. Schmidt et al. (1965) obtained a similar result by pretreatment with reserpine. Seifen et al. (1964b) examined effect of calcium on the SA node pacemaker fibers of the rabbit with the intracellular microelectrode technique and concluded as follows: An increase in calcium concentration of the bathing medium increased the rate of slow diastolic depolarization. However, at a higher concentration (10 mM), the threshold potential rose and then the rise in threshold was sufficient to compete with the higher depolarization rate for the generation of the pacemaker potential.

In this study, an infusion of GEDTA produced sinus bradycardia and further increase caused sinus arrhythmia. Reduction of the effective calcium concentration to 1.6 mM by administration of Na-EDTA reduced heart rate (Seifen et al. 1964a). Low calcium resulted in the appearance of multiple pacemakers in the specialized conducting tissue (Hoffman and Suckling 1956). These observations may explain the onset of sinus arrhythmia during severe hypocalcemia in the sinus node artery by the infusion of GEDTA at a relatively higher rate in this study. The positive chronotropic effect of norepinephrine was inhibited during infusion of GEDTA, but the normal sinus rhythm was temporarily restored by a single dose of norepinephrine, which may be ascribed to the effect of calcium mobilized from binding sites by the latter. Thus, catecholamine and calcium may intensify the action each other at the effector sites.

It has been known that magnesium slows the heart rate in the dog heart

(Stanbury and Farah 1950; Schmidt et al. 1965) and in the isolated guinea-pig atria (Schaer 1968). The negative chronotropic effect of magnesium was proportional to the increase in its concentration (Schaer 1968) and was not mediated by cholinergic transmitter (Schmidt et al. 1965; Schaer 1968). Hoffman and Suckling (1956) reported that the change in the concentration of magnesium had little effect on the transmembrane potential of the Purkinje fibers of the dog heart when the calcium concentration was normal. Toda and West (1967) observed that additional magnesium ion (11.0 mM) in the presence of 2.2 mM of calcium changed the transmembrane potential of the SA node pacemaker of the isolated rabbit atria. Magnesium inhibited significantly the positive chronotropic action of not only calcium but also catecholamine. Inhibitory action of magnesium on the positive chronotropic action of catecholamine has not been reported.

In six out of twelve cases, atrial fibrillation occurred when the calcium concentration of the SA node area was elevated. And induction of atrial fibrillation by a single injection of acetylcholine was facilitated by calcium. Briggs and Holland (1960) reported that spontaneous fibrillation appeared in the isolated rabbit atria, when the extracellular calcium concentration was suddenly increased to eight times normal. The SA node is sensitized to a negative chronotropic action of acetylcholine when the calcium concentration of the perfusing fluid is elevated to 11.0 mM in the isolated rabbit atria (Vincenzi and West 1965). The induction of atrial fibrillation by certain interaction with catecholamine is suggested and, thus, excess calcium may facilitate this interaction between acetylcholine and catecholamine.

Somjen and Baskerville (1968) pointed out that the effect of excess magnesium resulted in the increased sensitivity of the cardiac pacemaker to the inhibitory effect of injected acetylcholine. However, in this study, the induction of atrial fibrillation by acetylcholine was effectively inhibited by excess magnesium. The positive chronotropic response to catecholamine was significantly depressed by an increase in magnesium level. The magnesium excess may exert some inhibitory effect on the adrenergic mechanism. The antifibrillatory activity of excess magnesium may, in part, depend on its depressing effect on the action of catecholamines. Stanbury and Farah (1950) described that magnesium lengthened the refractoy period of the atrium in the dog's heart preparation. These facts suggest that magnesium depresses the pacemaker activity and stabilizes the membrane of the fibers of the SA node area against any stimulus of rapid firing.

Acknowledgments

This study was supported partly by grants from the Pharmacological Resarch Foundation, Inc., The Sankyo Central Laboratories, and the Squibb Institute for Medical Research, USA.

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