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Mechanism of Cardiac Arrhythmias Induced by Epinephrine in Dogs With Hypokalemia

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To investigate the mechanism of ventricular arrhythmias induced by epinephrine in dogs with hypokalemia, 30 adult mongrel dogs were separated into a control group (n = 13) and a hypokalemia group (n = 17). In the hypokalemia group, sodium polystyrene sulfonate (5 g/kg body weight) was infused into the colon. In both groups, the serum concentrations of sodium, potassium and calcium were measured every 15 minutes for 60 minutes. The mean (± standard deviation) serum potassium level of the hypokalemia group decreased significantly from 3.81 ± 0.21 to 2.92 ± 0.36 mEq/liter; there were no significant changes in other electrolytes. After 60 minutes, epinephrine (10 μg/kg) was injected intravenously in the hypokalemia and control groups, and the arrhythmia ratio (the number of ventricular ectopic beats divided by the total heart rate) was calculated for 5 minutes. Each group was further classified into subgroups of dogs with an arrhythmia ratio higher or lower than 10%. An arrhythmia ratio over 10% was observed in 7.7% of the control group and 53% of the hypokalemia group.

Immediately after 5 minutes of epinephrine injection, myocardial mitochondria and plasma membrane fraction were prepared from each group. Mitochondrial calcium content and phospholipase activity of plasma membrane fraction were determined. Significant increases in both mitochondrial calcium content and phospholipase activity were observed in the dogs with hypokalemia and an arrhythmia ratio greater than 10%. In the hypokalemia group, there was a clear reciprocal correlation (r = −0.79) between serum potassium concentration at 60 minutes and mitochondrial calcium content, and a clear correlation (r = 0.80) between mitochondrial calcium content and phospholipase activity. It was also demonstrated that the dogs with a higher than 10% arrhythmia ratio had a low serum potassium concentration, high mitochondrial calcium content and high phospholipase activity. These results suggest that hypokalemia enhances the calcium influx induced by epinephrine, resulting in activation of phospholipase, which is responsible for the development of ventricular arrhythmias.

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Ventricular arrhythmias often develop in hypokalemia. Holland et al. (1) analyzed 24 hour ambulatory electrocardiograms from 21 hypertensive patients treated with thiazide diuretics and observed that complex ventricular arrhythmias developed in 4 patients with hypokalemia. They reported that these arrhythmias were abolished by potassium repletion. Poole-Wilson (2) estimated that if 1 million hypertensive patients were treated with thiazide diuretics, the life-threatening arrhythmias induced by the associated hypokalemia would give rise to mortality rate of 450 patients a year. Although the development of arrhythmias resulting from hypokalemia is considered to be the major adverse reaction during diuretic therapy (3,4), hypokalemia is not only induced by thiazide therapy, but is one of the common findings in acutely ill patients including those with myocardial infarction. Indeed, malignant arrhythmias frequently occur in hypokalemic patients with myocardial infarction (5–7).

Because ventricular arrhythmias may cause sudden death (8,9), much interest has been attracted to the mechanisms underlying the development of ventricular arrhythmias in hypokalemic conditions. Duke (10) speculated that the hypokalemia itself may contribute to the risk of arrhythmias, whereas Thomas (11) suggested that the hypokalemia was

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related to another factor that was in itself arrhythmogenic. Ventricular arrhythmias are also known to occur in patients with myocardial infarction who show a high circulating level of epinephrine (12), and Johansson and Dziamski (7) emphasized the role of epinephrine in the development of arrhythmias in hypokalemic conditions. It was recently shown (13–15) that arrhythmias may be associated with enhanced activation of phospholipase. Consequently, the present study was designed to clarify the genesis of ventricular arrhythmias evoked in hypokalemic conditions in relation to both the arrhythmogenic role of phospholipase and the relevant effects of epinephrine.

Methods

Animal preparations. Thirty adult mongrel dogs of either sex (weighing from 8 to 15 kg) were anesthetized with sodium pentobarbital (50 mg/kg body weight) given intraperitoneally. After endotracheal intubation, artificial ventilation was instituted with a Harvard ventilator using a mixture of oxygen and room air. Lead II of the electrocardiogram was monitored continuously throughout the experiment by a VC-640G oscillographic recorder (Nihon Koden). The right femoral vein was dissected and cannulated. The dogs were separated into two groups, a control (n = 13) and hypokalemia (n = 17) group. In the control group, physiologic saline solution containing 0.15% (weight per volume) potassium chloride was infused at a rate of 1.0 ml/min from the cannulated right femoral vein. In the hypokalemia group, physiologic saline solution containing 0.02% (weight per volume) calcium chloride was infused at a rate of 1.0 ml/min. A Nelaton catheter was inserted into the colon. To cause hypokalemia, 5 g/kg of sodium polystyrene sulfonate suspended in 200 ml of distilled water was infused through the catheter. The two groups were allowed 60 minutes for stabilization or induction of hypokalemia.

Measurement of serum sodium, potassium and calcium. At the beginning of the experiment and every 15 minutes thereafter for 60 minutes, the serum concentrations of sodium, potassium and calcium were measured. Serum sodium and potassium levels were determined by a Shimadzu CL-12 autoanalyzer, and serum calcium was determined by means of an orthocresolphthalein complexone (16) using an autoanalyzer (Olympus AU550).

Epinephrine injection, measurement of the arrhythmia ratio and total duration of ventricular tachycardia. After the premedication period of 60 minutes, 10 μg/kg of epinephrine was injected intravenously, and the appearance of ventricular arrhythmias was observed for 5 minutes. The severity of arrhythmias was expressed by the arrhythmia ratio, that is, the number of ventricular ectopic beats divided by the total heart rate during 5 minutes after epinephrine injection. Each group was classified into two subgroups depending on the arrhythmia ratio: 1) arrhythmia (+) (dogs with an arrhythmia ratio of more than 10%), and 2) arrhythmia (−) (dogs with an arrhythmia ratio of less than 10%). For the purpose of this study, more than five successive ventricular premature beats were defined as ventricular tachycardia, and the total duration of ventricular tachycardia was also measured for 5 minutes after epinephrine injection.

Figure 1. Time course of changes in serum potassium (**A**), serum sodium (**B**) and serum calcium (**C**) in each group. The serum potassium level decreased significantly in the hypokalemia group, but there was no significant change in serum sodium and calcium throughout the experiment in each group. Values are means ± SD. Control group (**circles**); hypokalemia group (**triangles**). **p < 0.01 versus control.

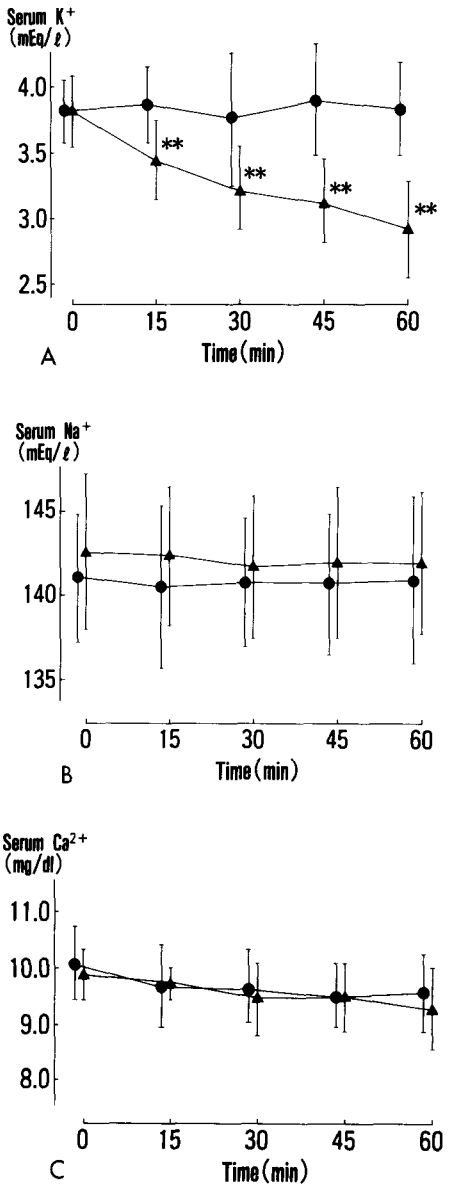


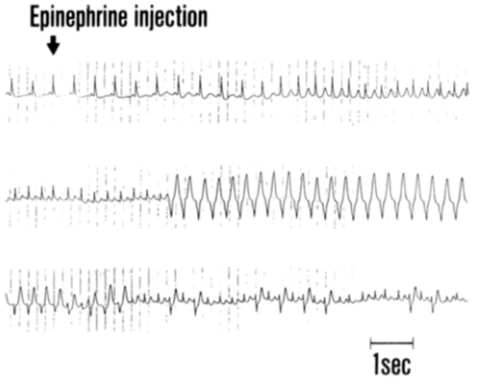
Table 1. The Number of Dogs Classified as Having an Arrhythmia Ratio Greater Than (Arrhythmia [+]) and Less Than (Arrhythmia [−]) 10% and the Incidence of Ventricular Tachycardia in Each Group

	Control Group (n = 13)	Hypokalemia Group (n = 17)
Arrhythmia (+)	1	9‡
Arrhythmia (−)	12	8
Ventricular tachycardia (+)	3 (6 ± 2 s)*	11‡ (32.9 ± 17.6† s)*
Ventricular tachycardia (−)	10	6

*Total duration time of ventricular tachycardia (mean ± SD). †$p < 0.05$ versus control; ‡$p < 0.01$ versus control.

Measurement of phospholipase activity. Five minutes after epinephrine injection, a left thoracotomy was performed and the heart was excised rapidly and washed several times with cold physiologic saline solution. A plasma membrane fraction was prepared according to the method of Williams et al. (17), and phospholipase activity in the plasma membrane fraction was estimated by a method previously described (18). Using di-tridecanoyl phosphatidylcholine as a substrate, the enzyme activity was determined by the amount of tridecanoic acid released from the substrate. The plasma membrane fraction (3 mg of protein) was added to 2 μmol of di-tridecanoyl phosphatidylcholine and incubated at 37°C. After incubation for 30, 60 and 90 minutes, tridecanoic acid was extracted by a modified method of Folch et al. (19). Heptadecanoic acid (100 μmol) was used as an internal standard. The extracted free fatty acids were redissolved with 1.0 ml of 9-anthryldiazomethane (Funakoshi Pharmaceutical) solution (0.5 mg/ml of methanol) and incubated for 3 hours in the dark at room temperature. After conversion to fluorescent derivatives by reaction with 9-anthryldiazomethane, the amount of tridecanoic acid was detected by high performance liquid chromatography with a fluorescent detector (20). The 10 μl solution of the fluorescent derivatives of free fatty acids was injected into a Shimadzu-ODS guard column (0.21 × 5 cm) plus Zolbax-ODS analytical column (0.46 × 15 cm plus 0.46 × 25 cm) at 60°C (back pressure 80 kg/cm^2; flow rate 1 ml/min; solvent methanol: distilled water = 94.7:5.3). The fluorescent derivatives of free fatty acids eluted from the column were detected by a fluorescence spectromonitor (Shimadzu, RF-500LCA) connected to a computerized recorder (Shimadzu, Chromatopac, C-R1A).

Figure 2. Representative electrocardiogram after epinephrine injection in a dog in the hypokalemia group. In most cases, ventricular tachycardia emerged abruptly within seconds after epinephrine injection, usually followed by ventricular bigeminy of short duration.



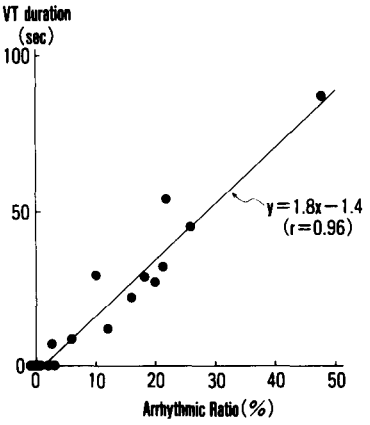
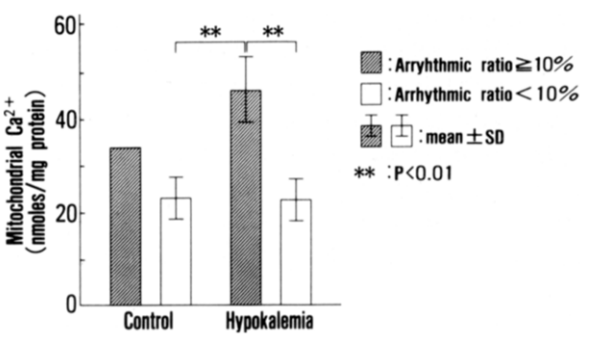


Figure 3. Relation between arrhythmia ratio and total duration of ventricular tachycardia (VT) in the hypokalemia group. There is a close relation between these two variables.

Measurement of mitochondrial calcium content. Heart mitochondria were prepared by the method of Hatefi et al. (21). Calcium content of myocardial mitochondria was measured by the method of Meissner et al. (22). Two milliliters of the mitochondrial suspension (10 mg/ml protein) was added to 2 ml of the solution of 1% $LaCl_3$/10% trichloroacetic acid, and centrifuged at 3,000 rpm for 10 minutes. The supernate was used to determine calcium content by an

Figure 4. Heart mitochondrial calcium content in each group. There was a significant increase in the mitochondrial calcium content in the hypokalemia-arrhythmia (+) group (ratio ≥ 10%) compared with both control and hypokalemia-arrhythmia (−) groups. (ratio < 10%).



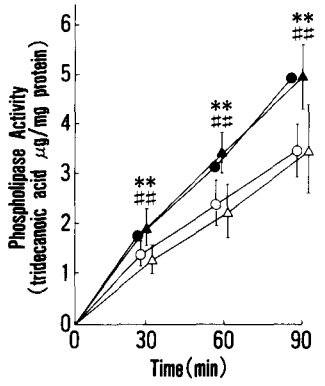


Figure 5. Phospholipase activity in myocardial plasma membrane fraction in each group. A significant elevation of phospholipase activity was observed in the hypokalemia-arrhythmia (+) group compared with both control and hypokalemia-arrhythmia (−) groups. Control-arrhythmia ratio ≥ 10% (**solid circle**), < 10% (**open circle**); hypokalemia-arrhythmia ratio ≥ 10% (**solid triangle**), < 10% (**open triangle**) (mean ± SD). **p < 0.01 versus control-arrhythmia ratio < 10%; ##p < 0.01 versus hypokalemia-arrhythmia ratio < 10%.

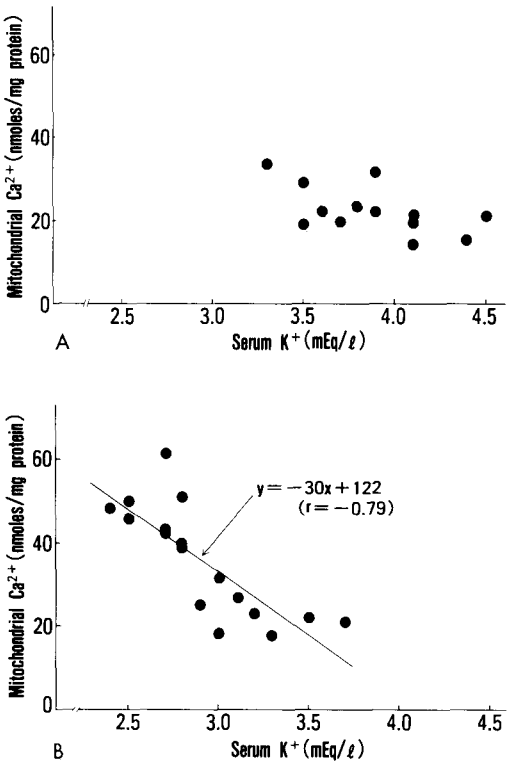


Figure 6. Relation between serum potassium content after 60 minutes and mitochondrial calcium content in the control group (**A**) and in the hypokalemia group (**B**). There is a clear reciprocal correlation (r = −0.79) between serum potassium and mitochondrial calcium content in the hypokalemia group.

atomic absorption spectrometer (Hitachi, 170-10). In the other intact six dogs, heart mitochondrial calcium was also measured for the untreated control condition.

Statistics. Significance of the results was determined by Bonferroni's method except for the data from the dogs classified as arrhythmia (+) or arrhythmia (−) and for the incidence of ventricular arrhythmias, where the chi-square test was used. Probability (p) values of less than 0.05 were considered statistically significant.

Results

Time course of changes in serum electrolytes (Fig. 1). The mean (± standard deviation) concentration of serum potassium (Fig. 1A) significantly decreased in the 17 dogs in the hypokalemia group from 3.81 ± 0.21 to 2.92 ± 0.36 mEq/liter, whereas in the 13 dogs in the control group the serum potassium level did not change significantly throughout the experiment. No significant change was observed in the levels of serum sodium or calcium in either group (Fig. 1B and C).

Arrhythmia ratio and duration time of ventricular tachycardia. Table 1 shows the number of dogs exhibiting arrhythmia (arrhythmia ratio greater than 10%; arrhythmia [+]) and those not exhibiting arrhythmia (arrhythmia ratio less than 10%; arrhythmia [−]) in each group and the incidence of ventricular tachycardia in each group. In the control group, only 1 (7.7%) of the 13 dogs exhibited arrhythmia, and ventricular tachycardia appeared in 3 (23%) of them. In the hypokalemia group, 9 (53%) of the 17 dogs exhibited arrhythmia and ventricular tachycardia appeared in 11 (68%). A representative electrocardiogram after epinephrine injection in a dog in the hypokalemia group is shown in Figure 2. Ventricular tachycardia emerged abruptly within seconds after epinephrine injection, usually followed by ventricular bigeminy. Figure 3 shows a good correlation (r = 0.96) between the arrhythmia ratio and the duration of ventricular tachycardia in the hypokalemia group.

Calcium concentration in heart mitochondria (Fig. 4). In the hypokalemia group, the calcium content of mitochondria prepared from the dogs classified as arrhythmia (+) was significantly greater than that of mitochondria prepared from the dogs classified as arrhythmia (−). In the control group, only one dog showed an arrhythmia ratio of more than 10%, but the mitochondrial calcium content was elevated in this dog. In the untreated group, the mean (± standard deviation) mitochondrial calcium content was 22.8 ± 3.6 nmol/mg protein. In the dogs classified as arrhythmia (−) in both the control and hypokalemia groups, no elevation of the mitochondrial calcium content was observed in comparison with values in the untreated group.

Phospholipase activity (Fig. 5). Phospholipase activity represents the amount of tridecanoic acid released from di-

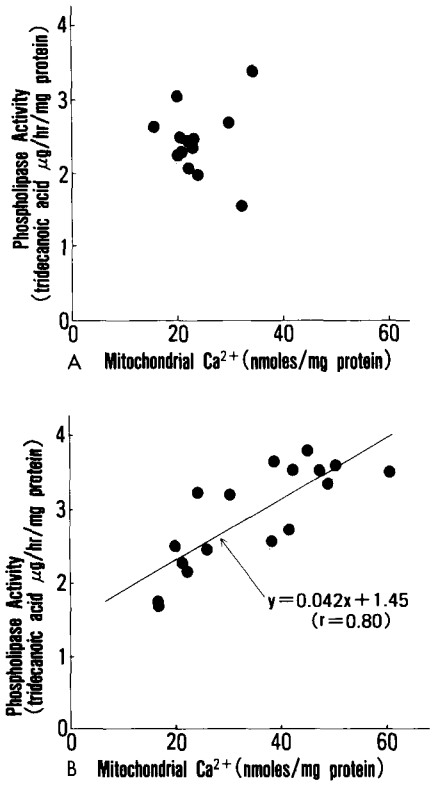


Figure 7. Relation between mitochondrial calcium content and phospholipase activity in the control group (**A**) and the hypokalemia group (**B**). There is a clear correlation (r = 0.80) in the hypokalemia group.

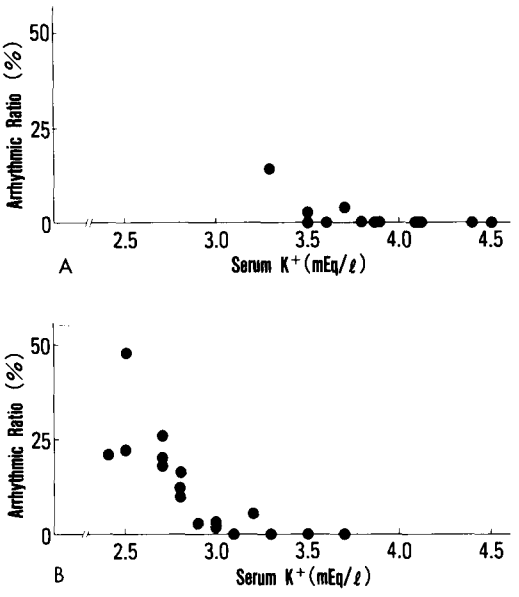


Figure 8. Relation between arrhythmia ratio and serum potassium after 60 minutes in the control group (**A**) and in the hypokalemia group (**B**). These figures reveal that in the dogs classified as arrhythmia (+) (ratio ≥ 10%), there is a low serum potassium concentration.

tridecanoyl phosphatidylcholine by endogenous phospholipase in the myocardial plasma membrane fraction. The release of tridecanoic acid in each group appeared to increase in linear fashion during the incubation period of 90 minutes. A marked elevation was observed in the phospholipase activity of the hypokalemia-arrhythmia (+) group ($p < 0.01$) and in the control-arrhythmia (+) group.

Relation between serum potassium and mitochondrial calcium content (Fig. 6). There was a clear reciprocal correlation (r = −0.79) in the hypokalemia group (Fig. 6B). However, there was no significant correlation in the control group (Fig. 6A).

Relation between the mitochondrial calcium content and phospholipase activity (Fig. 7). There was a good correlation between these variables (r = 0.80) in the hypokalemia group (Fig. 7B). In the control group, there was no significant correlation (Fig. 7A).

Relation among the arrhythmia ratio and serum potassium, mitochondrial calcium and phospholipase activity (Fig. 8 to 10). Results indicated that the dogs classified as arrhythmia (+) possess a low serum potassium concentration, high mitochondrial calcium content and high phospholipase activity.

Discussion

Hypokalemia and ventricular arrhythmias. Sudden death is often observed in patients with hypokalemia and is ascribed to the development of malignant cardiac arrhythmias (8,9). In the present study, we demonstrated that 10 μg/kg of epinephrine, which caused few ventricular arrhythmias in the dogs with normokalemia, evoked severe ventricular arrhythmias in more than half of the dogs with hypokalemia. Thiazide and other potassium-depleting diuretic drugs are widely used to treat hypertension, congestive heart disease and edematous conditions. Along with their potential for benefit, these drugs have significant potential for adverse effects including sudden death (9). The mechanisms of hypokalemia-related cardiac arrhythmias have been studied extensively, particularly from the electrophysiologic viewpoint (23,24). Recently, to elucidate the fundamental mechanisms involved in the genesis of cardiac arrhythmias, the contributions of metabolic and biochemical factors have been emphasized (25).

Arrhythmogenic mechanism of hypokalemia. In the present study, we investigated the arrhythmogenic mechanism of hypokalemia from a biochemical point of view. In the hypokalemia group, the heart mitochondrial calcium content in dogs with a high ventricular arrhythmia ratio was elevated significantly compared with that of dogs with no or only mild arrhythmia. A lower serum potassium level and higher mitochondrial calcium content were observed. It was pointed out (26) that calcium content in mitochondria

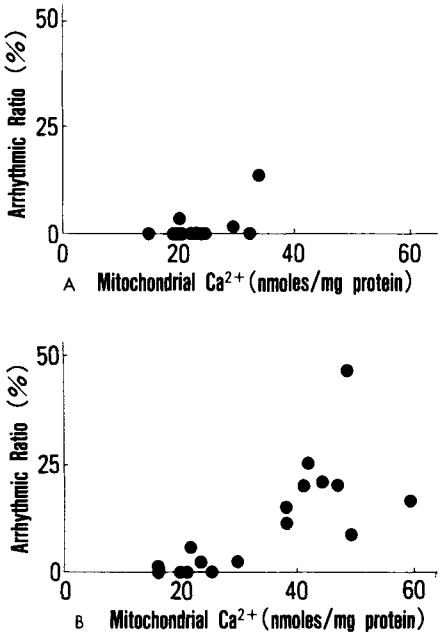


Figure 9. Relation between arrhythmia ratio and mitochondrial calcium content in the control group (**A**) and the hypokalemia group (**B**). These figures show that the dogs classified as arrhythmia (+) (ratio ≥ 10%) possess a high mitochondrial calcium content.

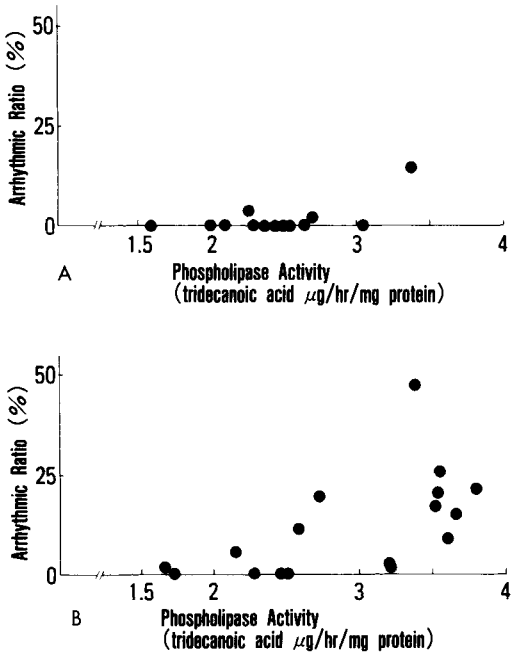


Figure 10. Relation between the arrhythmia ratio and phospholipase activity in the control group (**A**) and in hypokalemia group (**B**). These figures reveal that the dogs classified as arrhythmia (+) (ratio ≥ 10%) have a high phospholipase activity.

is a reflection of increased intracellular calcium concentration. Calcium ion is known to be highly cardiotoxic (27), and is suggested to play an important role in the genesis of cardiac arrhythmias (28,29). In our study, dogs with higher arrhythmia ratios had a higher mitochondrial calcium content. The injurious effect of calcium ion is, at least in part, ascribed to the activation of endogenous phospholipase because the calcium ion is an essential factor for phospholipase activation (30). In our experiment, in the hypokalemia group, there was a good correlation between mitochondrial calcium content and phospholipase activity. Accordingly, intracellular calcium overloading might be a responsible factor in the activation of phospholipase in our model. Epinephrine is well known to evoke arrhythmias (31), and this effect is often used as an experimental model for arrhythmias (32). Our present work now suggests a mechanism for the arrhythmogenic effect of epinephrine.

Role of phospholipase. Phospholipases are known to play various physiologic roles (33,34); however, an enhanced activation of phospholipase may also induce several pathologic conditions associated with the breakdown of membrane phospholipids (35–38). We demonstrated (39) that phospholipase causes a deterioration of the action potential of the cardiac membrane and might induce arrhythmogenic conditions. We also reported (40) that phospholipase activity was accelerated in association with coronary reperfusion, and suggested that the appearance of reperfusion arrhythmias is closely related to the activation of phospholipase. In the present study, the dogs with higher arrhythmia ratios showed higher activity levels of phospholipase. These results suggest that epinephrine-induced calcium influx was accelerated in the hypokalemic condition, resulting in phospholipase activation, which in turn was responsible for the development of cardiac arrhythmias.

Clinical implications. In this study we demonstrated that even relatively small doses of epinephrine, which had little or no effect on normokalemic dogs, evoked severe ventricular arrhythmias in hypokalemic dogs, depending on the degree of potassium depletion. Epinephrine itself induced hypokalemia, but it must be emphasized that the arrhythmogenic effect of epinephrine was enhanced in preexisting hypokalemic conditions. Although there are many causes of hypokalemia, some cases are iatrogenic, caused by diuretic therapy or dietary aberrations. Accordingly, supplementation of serum potassium or the addition of potassium–sparing diuretics should be considered, especially in patients exhibiting an increase in sympathetic tone. We also demonstrated that calcium influx and activation of phospholipase are closely related to the development of ventricular arrhythmias in hypokalemia. Therefore, calcium antagonists or antiphospholipase agents may be expected to prevent these arrhythmias.

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