# Effects of Pantethine on Action Potential of Canine Papillary Muscle during Hypoxic Perfusion

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SUMMARY

Pantethine, which is known to be converted to coenzyme A, has been reported to have antiarrhythmic action on experimental cardiac arrhythmias. Using standard microelectrode techniques, the electrophysiological effects of pantethine under hypoxic (95% $N_2$+5% $CO_2$) perfusion were studied.

Hypoxia decreased resting membrane potential, action potential amplitude and maximum velocity of phase 0 and shortened action potential duration and effective refractory period. Application of pantethine $5\times10^{-3}$ Gm/ml under hypoxic perfusion prolonged action potential duration and effective refractory period significantly. Prolongation of action potential duration by pantethine might be caused by an increase in intracellular ATP.

The findings in this study could be an explanation of the possible antiarrhythmic effects of pantethine.

**Additional Indexing Words:**

Electrophysiology Antiarrhythmic action Coenzyme A ATP

THE earliest and most striking effect of hypoxia on cardiac muscle is shortening of action potential duration.[1),2)] The effect has been attributed to a decrease in glycolytically derived ATP in the cytoplasm.[3),4)]

Pantethine, a coenzyme A (CoA) precursor, has been reported to have antiarrhythmic action on experimental cardiac arrhythmias,[5)] including ouabain-induced arrhythmias in guinea-pigs, $CaCl_2$-induced and aconitine-induced arrhythmias in rats and halothane+epinephrine-induced arrhythmias

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in dogs. As pantethine is reported to be converted to CoA in the liver,[6),7)] it is possible that pantethine may modify both carbohydrate and lipid metabolism and thereby influence myocardial energy production.

The purpose of this study was to observe the electrophysiological effects of pantethine on transmembrane action potentials under hypoxic perfusion.

## Materials and Methods

Mongrel dogs weighing 8–12 Kg were anesthetized with sodium pentobarbital (30 mg/Kg, iv). The hearts were removed and immediately placed in cooled and oxygenated Tyrode's solution. A small sample of papillary muscle was rapidly excised from the right ventricle and placed in a tissue bath. The bath was perfused with 95% $O_2$ and 5% $CO_2$ at a constant rate of 7 ml/min. Temperature was maintained at $36\pm0.5$°C. The pH of the solution was maintained at 7.4. The composition of the solution was as follows in mM: NaCl, 137; $NaHCO_3$, 12; dextrose, 5.5; KCl, 3.0; $CaCl_2$, 2.7; $NaH_2PO_4$, 1.8; and $MgCl_2$, 0.5. The papillary muscle was stimulated at a basic cycle length of 1,000 msec with rectangular pulses of 2.0 msec duration and twice the diastolic threshold in intensity through a bipolar Teflon-coated silver electrode placed on the papillary muscle at one end of the preparation.

Transmembrane action potentials were recorded from papillary muscle by means of a machine-pulled glass microelectrode filled with 3 M KCl and with tip resistances of 10 to 15 megohms. The following parameters were measured: resting membrane potential (RMP), action potential amplitude (APA) and duration of action potential from the upstroke to 20, 50 and 90% repolarization ($APD_{20}$, $APD_{50}$, $APD_{90}$). The effective refractory period (ERP) was measured while the preparation was stimulated at a basic cycle length of 1,000 msec. Premature stimuli were introduced during every eighth cycle. Premature stimuli were initially placed during the absolute refractory period and were then gradually and progressively delayed until the first impulse that propagated to a microelectrode was obtained. The maximum upstroke velocity of phase 0 ($dV/dt_{max}$) was obtained by electronic differentiation and displayed on an oscilloscope.

After the preparation was superfused for at least 30 min under normal perfusing conditions (saturated with 95% $O_2$+5% $CO_2$, $pO_2$ 600–650 mmHg, pH 7.4), the perfusing condition was switched to either (1) hypoxic perfusion for 60 min (saturated with 95% $N_2$+5% $CO_2$, $pO_2$ 60–65 mmHg, pH 7.4) or (2) hypoxic perfusion for 30 min and then hypoxic perfusion containing pantethine $5\times10^{-3}$ Gm/ml for 30 min.

Values are expressed as mean±SD. Statistical analysis was performed

using non-paired Student's t-test and the significance was established at $p < 0.05$.

## Results

### (1) *Effects of hypoxic perfusion*

The sequential changes in various parameters of transmembrane action potentials under hypoxic perfusion are summarized in Fig. 1. RMP was decreased from $-81 \pm 2.0$ to $-78 \pm 2.1$ mV after 30 min and to $-77 \pm 3.2$ mV after 60 min. APA was decreased from $100 \pm 3.5$ to $96 \pm 5.2$ mV after 30 min and to $92 \pm 5.2$ mV after 60 min. $dV/dt_{max}$ was also decreased from

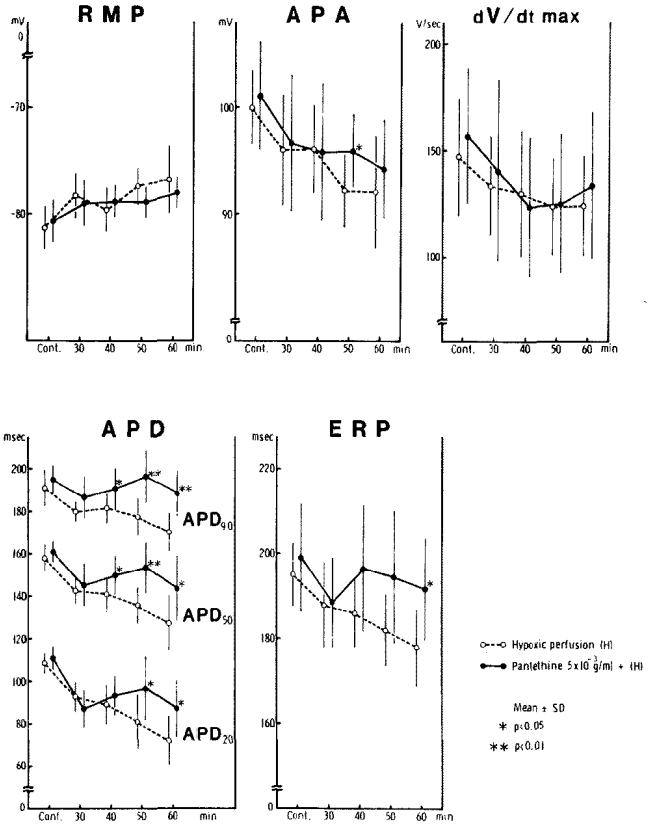


Fig. 1. Sequential changes in various parameters of the transmembrane action potentials of canine papillary muscle produced by hypoxic perfusion and application of pantethine $5 \times 10^{-8}$ Gm/ml under hypoxic perfusion. Abbreviations: RMP=resting membrane potential; APA=action potential amplitude; $dV/dt_{max}$=maximum upstroke velocity of phase 0; APD=action potential duration; ERP=effective refractory period. Asterisks indicate the significant differences by a non-paired t-test.

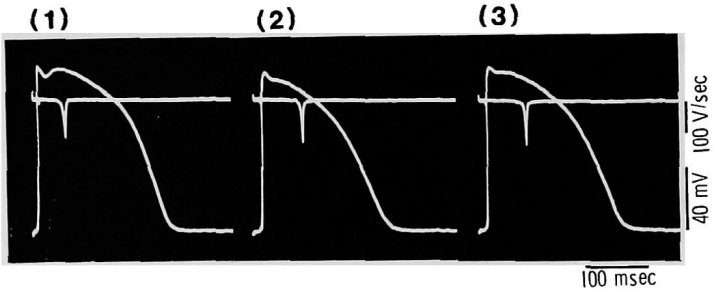


Fig. 2. Effects of hypoxic perfusion on action potential. (1) control (2) 30 min after exposure to hypoxia (3) 50 min after exposure to hypoxia.

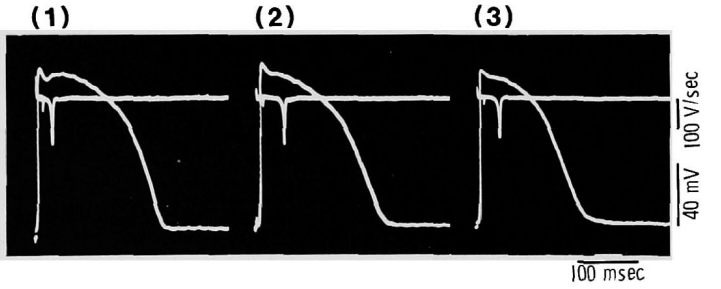


Fig. 3. Effects of pantethine $5\times10^{-3}$ Gm/ml on hypoxia-induced changes in action potential. (1) control (2) 30 min after exposure to hypoxia (before drug application) (3) 20 min after application of pantethine $5\times10^{-3}$ Gm/ml under hypoxia (50 min after exposure to hypoxia).

147±27.4 to 133±23.2 V/sec after 30 min and to 124±23.8 V/sec after 60 min. $APD_{20}$ was shortened from 109±4.9 to 93±7.1, 89±9.0, 81±12.8 and 72±11.9 msec after 30, 40, 50 and 60 min, respectively. $APD_{50}$ was shortened from 158±6.3 to 143±6.2, 141±7.2, 136±8.8 and 127±13.1 msec after 30, 40, 50 and 60 min. $APD_{90}$ was also shortened from 191±8.8 to 180±4.9, 181±6.9, 177±8.8 and 170±9.1 msec after 30, 40, 50 and 60 min. ERP, which had a control value of 195±7.6 msec, was progressively shortened to 188±10.0, 186±8.0, 182±8.4 and 178±9.0 msec after 30, 40, 50 and 60 min, respectively.

Action potentials in a representative case are shown in Fig. 2.

*(2) Effects of pantethine under hypoxic perfusion*

The sequential changes after application of pantethine $5\times10^{-3}$ Gm/ml under hypoxic perfusion are also summarized in Fig. 1. Parameters during the control period and hypoxic perfusion for 30 min did not change from those of hypoxic perfusion.

RMP and $dV/dt_{max}$ did not change significantly with application of

pantethine. Application of pantethine under hypoxic perfusion increased APA significantly to 96±3.4 mV ($p<0.05$) after 20 min (hypoxic perfusion for 50 min). $APD_{20}$ was lengthened to 97±14.7 ($p<0.05$) and 87±13.5 msec ($p<0.05$) after 20 and 30 min, respectively. $APD_{50}$ was lengthened to 150±9.2 ($p<0.05$), 153±12.2 ($p<0.01$) and 143±15.5 msec ($p<0.05$), after 10, 20 and 30 min. $APD_{90}$ was also lengthened to 191±9.3 ($p<0.05$), 197±12.4 ($p<0.01$) and 188±10.7 msec ($p<0.01$), after 10, 20 and 30 min. ERP was lengthened to 192±12.0 msec ($p<0.05$) after 30 min.

Action potentials in a representative case are shown in Fig. 3. Application of pantethine under continued hypoxia partially restored the shortening of APD and ERP caused by hypoxic perfusion.

## Discussion

Pantethine is known to be converted to CoA in the rat liver. CoA is an essential cofactor for many synthetic and catabolic reactions, but its primary importance in cardiac muscle is in energy production. Although the factors[8)–10)] that regulate the biosynthesis of CoA are not clearly understood, the rate of CoA synthesis by the liver appears to be responsive to changes in pantothenic acid concentration under some conditions.[11)] When various amounts of pantothenic acid or pantethine were added to a pantothenic acid deficient diet in the rat, the amounts of CoA were correspondingly increased in the liver.[12)] It has also been suggested that the synthetic pathway from pantethine to CoA is shorter and more effective than from pantothenic acid to CoA. Activation of free fatty acids by CoA occurs in the cytosol, resulting in the formation of fatty acyl CoA esters[13)] which can be used either for $\beta$-oxidation in the mitochondria or for triglyceride and phospholipid synthesis in the cytosol.

During the early phase of myocardial ischemia or hypoxia,[14),15)] it has been reported that levels of free CoA, ATP and creatine phosphate decline. Shibano and Abiko[16)] have reported that partial occlusion of the canine coronary artery reduced the endo- and epicardial ATP and creatine phosphate levels significantly and that the pantethine injection minimized the decrease in the ATP and creatine phosphate levels produced by partial occlusion. They concluded that pantethine increases myocardial pH that had been reduced by partial occlusion of the coronary artery, probably by lowering consumption of myocardial ATP and hence creatine phosphate.

Minami et al[17)] have reported that a pantethine concentration of $5\times 10^{-3}$ Gm/ml induced a significant increase in the isometric tension of the papillary muscles of guinea-pigs and the positive inotropic action of pantethine

might be attributed to the increased biosynthesis of CoA in the myocardium.

In the present study, hypoxia caused appreciable changes in the transmembrane action potential of canine papillary muscle. RMP, APA and $dV/dt_{max}$ were decreased and APD and ERP were shortened. The shortening of APD and ERP in this study was less than in previous reports,[1),2)] as oxygen content of hypoxic perfusion ($pO_2$ 60–65 mmHg) was higher and thus hypoxia in this study was milder than that in previous reports. Application of pantethine under hypoxic perfusion prolonged APD and ERP significantly.

The shortening of the action potential during hypoxia has been attributed to an increase in potassium outward current[18)] or a decrease in slow inward current.[19)] The decrease in slow inward current may be the consequence of a decrease in $g_{ca}$ and/or a decrease in equilibrium potential for $Ca^{2+}$ ions. On the other hand, the APD of the hypoxic guinea-pig was related to ATP generated by glycolysis[4)] and intracellular injection of ATP has been shown to elevate the plateau potential and prolong APD.[20)] A close relationship between ATP and slow inward current may be due to two mechanisms. First, a decrease in ATP, which is a strong chelator of $Ca^{2+}$, would increase the concentration of free $Ca^{2+}$ near the inner surface of the membrane, decreasing the driving force for $Ca^{2+}$ ions.[21)] Secondly, ATP might control $g_{ca}$ directly via intracellular cyclic AMP.[22)] An increase in potassium outward current caused by hypoxia may be mediated by a rise in intracellular $Ca^{2+}$ activity.[23)] Recently, an ATP-regulated $K^+$ channel[24)] has been shown in cardiac muscle by use of the patch-clamp technique. Therefore, it may be reasonable to speculate that prolongation of APD by pantethine during hypoxia is caused by an increase in intracellular ATP content through an increase in slow inward current or a decrease in potassium outward current.

A decrease in high energy phosphates such as ATP and creatine phosphate results in a depression of the active transport system and in an increase in the intracellular concentration of $Na^+$.[25)] An increase in the intracellular $Na^+$ content has been found to cause shortening of ADP.[26)] Intracellular acidification might decrease slow inward current,[27)] though the fall in intracellular pH in hypoxia is much less than in ischemia.[28)] The mechanism of the effects of pantethine during hypoxia demands further investigation.

In this study, it was shown that external application of pantethine could protect and partially reverse the deterioration in action potential produced by hypoxic perfusion. These findings could be an explanation of the possible antiarrhythmic effects of pantethine.

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