Effect of L-Carnitine Chloride and Its Acetyl Derivative on the Electrophysiological Derangement Induced by Palmityl-L-Carnitine in Isolated Canine Ventricular Muscle

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Abstract—Using the microelectrode technique, the effects of L-carnitine (LC) and acetyl-L-carnitine (ALC) on the changes in the transmembrane action potential of the canine ventricular muscle induced by palmityl-L-carnitine (PLC) were studied in comparison with those of disopyramide (D). LC (5×10⁻³ M) itself had no effect on the electrophysiological parameters of the ventricular muscle. ALC (5×10⁻³ M) increased the maximum rate of rise (dV/dt max) slightly and decreased the action potential duration (APD), although these changes were not statistically significant. D (1.5×10⁻³ M) decreased dV/dt max and prolonged APD and the absolute refractory period (ARP). PLC (3×10⁻⁴ M) decreased the resting membrane potential, action potential amplitude and dV/dt max, and it shortened APD and ARP. LC and ALC (5×10⁻³ M) improved the electrophysiological derangement produced by PLC to the same degree. On the other hand, application of D (1.5×10⁻⁵ M) resulted in no improvement of the electrophysiological derangement produced by PLC.

It is well documented that carnitine plays an important role in free fatty acid (FFA) metabolism as a carrier for transmembrane movements of activated long chain acyl groups (1). Recently, much attention has been focused on the loss of carnitine and the accumulation of FFA and related intermediates such as long chain acyl CoA and long chain acylcarnitine (2) as the factor predisposing to malignant dysrhythmias in the ischemic heart. Indeed, during episodes of ischemia, acyl CoA increases 2-fold and acylcarnitine increases from 4- to 6-fold above the level in the non-ischemic zone (3, 4). It has been demonstrated that acyl CoA inhibits adenine nucleotide translocase activity and disturbs the mitochondrial oxidative phosphorylation (1, 5, 6). Furthermore, Pande and Blanchaer (7) reported that addition of L-carnitine led to a restoration of ADP-coupled mitochondrial oxidations, and experimental reports indicated that carnitine could exhibit antiarrhythmic effects on cardiac arrhythmias following ischemia or excess FFA (8–10). In a previous study, we also demonstrated that L-carnitine and acetyl-L-carnitine could suppress the ventricular arrhythmia induced by two-stage coronary ligation and ascribed the antiarrhythmic effect to the improvement of mitochondrial oxidative phosphorylation (11). The electrophysiological alterations induced by acylcarnitines (the most used one is palmitylcarnitine) in normoxic Purkinje fibers in vitro experiments closely resembled the ones observed in the ischemic tissue in vivo (12–14).

The purpose of the present work was to investigate the effects of L-carnitine and its acetyl derivative on the electrophysiological derangements induced by palmityl-L-carnitine in the transmembrane action potential of the canine ventricular muscle in comparison with those of disopyramide.
Materials and Methods

Tissue preparations: Adult mongrel dogs of either sex weighing 6 to 20 kg were anesthetized with sodium thiopental (25 mg/kg, i.v.) and ventilated artificially. The heart was quickly isolated and placed in oxygenated Tyrode's solution at 4°C. Papillary muscle and trabeculae were dissected from the right ventricular free wall, pinned to the bottom of a 5.0 ml tissue bath and superfused with modified Tyrode's solution equilibrated with a 95% O2+5% CO2 gas mixture at a constant rate of 3.0 ml/min. The composition of the Tyrode's solution (in mM) was: NaCl, 124; NaHCO3, 24; NaH2PO4, 0.4; MgCl2, 0.5; CaCl2, 1.9; KCl, 4.0 and glucose, 5.6. The temperature of the tissue bath was maintained at 37±0.5°C.

Preparations were stimulated with a field stimulation technique to ensure synchronous excitation of the whole muscle fiber. Two silver plate electrodes (5 X 15 mm) separated by 6 mm were placed parallel to the muscle. Stimuli were rectangular pulses (1 Hz) of 1.2–1.5 times the threshold intensity and 1 msec duration delivered by an electronic stimulator (Nihon Kohden SEN-6100) and passed through an isolator (Nihon Kohden SS-302J). The electronic stimulator was triggered by a digitimer (Devices 3290).

Action potentials were recorded with glass microelectrodes filled with 3 M KCl. Electrode resistances ranged from 5 to 20 MΩ. The electrodes were coupled to silver-silver chloride wires leading to the input stage of a high-impedance microelectrode amplifier (Nihon Kohden MZ-4). The maximum rate of rise (dV/dt max) was obtained by electronic differentiation. Transmembrane action potential and dV/dt max were displayed on an oscilloscope (Nihon Kohden VC7) and recorded on a film with a long-recording camera (Nihon Kohden RLC-6101). Resting membrane potential (RMP), action potential amplitude (APA), action potential duration at 20, 50 and 90% repolarization (APD20, APD50, APD90) and dV/dt max were measured as the electrophysiological parameters. The absolute refractory period (ARP) was determined by delivering a premature stimulus progressively earlier every ten drive stimuli. The intensities of the drive stimuli (1 Hz, 1 msec duration) used were 1.5 times the threshold. ARP was defined as the longest stimulus interval at which a premature stimulus does not elicit a action potential.

Experimental protocols: In all experiments, the preparation was superfused for at least 60 min under control perfusing conditions (equilibrated with 95% O2+5% CO2, PO2 >300 mmHg). After the stability of all the transmembrane potential parameters was ascertained during this control period, drugs were added to the perfusion solution. The preparation was exposed to the drug for 20–30 min. The drugs used were l-carnitine chloride, acetyl-l-carnitine chloride, palmitoyl-l-carnitine chloride (Earth Chemical Co., Ltd.) and disopyramide phosphate (Roussel Laboratories). L-Carnitine, acetyl-l-carnitine and palmitoyl-l-carnitine were dissolved in distilled water, and the pH was adjusted to pH 7.0 with 1N NaOH solution. The experiments were conducted in the following three groups:

1) Addition of l-carnitine and acetyl-l-carnitine at a concentration of 5x10⁻³ M and disopyramide at a concentration of 1.5x10⁻⁵ M.
2) Addition of palmitoyl-l-carnitine at a concentration of 3x10⁻⁴ M.
3) A combination of the above two procedures.

The concentration (5x10⁻³ M) of l-carnitine was chosen according to the plasma concentration measured at 30 min after injection of l-carnitine (300 mg/kg, i.v.) in Harris' two stage coronary ligation models (11). For comparison, the same concentration of acetyl-l-carnitine was used, although the plasma concentration was only 1/10 that of l-carnitine.

Statistics: All data were expressed as the mean±S.E.M. Statistical comparisons were made using the paired t-test. A P-value of 0.05 or less was considered significant.

Results

The effects of l-carnitine and acetyl-l-carnitine on the action potential characteristics and on the absolute refractory period are summarized in Table 1. Figure 1 shows the results from a typical experiment. No
significant changes were observed in any variables measured during 30 minutes' superfusion with 1-carnitine (5 × 10⁻³ M). With acetyl-l-carnitine (5 × 10⁻³ M), there was a slight prolongation of APD and increases in dV/dt max. These changes were, however, not statistically significant.

Figure 2 shows the effects of disopyramide (1.5 × 10⁻⁵ M) on the action potential characteristics of ventricular muscle. ARP: absolute refractory period. Other abbreviations are the same as in Table 1. Symbols represent mean values ± S.E.M. (n = 3-4). *Significantly different from the values before drug application (*P < 0.05).

The effects of palmityl-l-carnitine, a member of the long chain acylcarnitines, on the transmembrane action potential were studied in fifteen preparations. As shown in Fig. 3, at a concentration of 3 × 10⁻⁴ M, this
compound caused a significant decrease in \(dV/dt\) max, APA, RMP, APD and ARP, the percent decreases in \(dV/dt\) max, APA, RMP, APD\(_{20}\), APD\(_{50}\), APD\(_{90}\) and ARP at 30 min after superfusion being 50, 23, 21, 44, 34, 22 and 11, respectively.

To elucidate the electrophysiological interaction of l-carnitine and palmityl-l-carnitine, we studied the effects of l-carnitine on the derangements of the transmembrane action potential induced by palmityl-l-carnitine. Figure 4 shows a typical example, and Fig. 5 summarizes the data. At 30 min after the addition of palmityl-l-carnitine (3\(\times\)10\(^{-4}\) M), the ventricular muscles were additionally superfused with l-carnitine (5\(\times\)10\(^{-3}\) M). Improvements of the action potential parameters were observed. At 20 min after the start of superfusion with l-carnitine, \(dV/dt\) max, APA, RMP, APD\(_{50}\) and APD\(_{90}\) were restored up to about 86, 94, 93, 77 and 87%, respectively, of the values

![Fig. 3. Effect of palmityl-l-carnitine (3\(\times\)10\(^{-4}\) M) on the action potential characteristics of ventricular muscle. Abbreviations are the same as in Fig. 2. Symbols represent mean values±S.E.M. (n=14–15). *Significantly different from the values before drug application (*P<0.05, **P<0.01, ***P<0.001).](image)

Fig. 4. Effect of l-carnitine and acetyl-l-carnitine on the action potential derangements induced by palmityl-l-carnitine (3\(\times\)10\(^{-4}\) M). Top left: Control action potential. Top center: Potential obtained following exposure to palmityl-l-carnitine. Top right: Potential obtained following addition of l-carnitine (5\(\times\)10\(^{-3}\) M) in the presence of palmityl-l-carnitine. Bottom left: Control action potential. Bottom center: Potential obtained following exposure to palmityl-l-carnitine. Bottom right: Potential obtained following addition of acetyl-l-carnitine (5\(\times\)10\(^{-3}\) M) in the presence of palmityl-l-carnitine.
before application of palmityl-l-carnitine. ARP was significantly prolonged in accordance with the prolongation of APD.

A typical example of the effects of acetyl-l-carnitine (5×10⁻³ M) is also shown in Fig. 4. Figure 6 summarizes the data. Acetyl-l-carnitine improved the decrease in dV/dt max, APA and RMP, and shortening of APD and ARP caused by palmityl-l-carnitine. The percent recovery of dV/dt max, APA, RMP, APD₅₀, APD₉₀ and ARP produced by this compound were about 99, 92, 95, 85, 96 and 97% of the values before palmityl-l-carnitine application, respectively, indicating

Fig. 5. Effect of l-carnitine (l-Car.) (5×10⁻³ M) on the action potential derangement induced by palmityl-l-carnitine (3×10⁻⁴ M). Abbreviations are the same as in Fig. 2. Symbols represent mean values±S.E.M. (n=4-5). *Significantly different from the values before palmityl-l-carnitine application (P<0.05, **P<0.01). *Significantly different from the values immediately before 1-carnitine application (*P<0.05, **P<0.01).

Fig. 6. Effect of acetyl-l-carnitine (Acetyl-l-car.) (5×10⁻³ M) on the action potential derangement induced by palmityl-l-carnitine (3×10⁻⁴ M). Abbreviations are the same as in Fig. 2. Symbols represent mean values±S.E.M. (n=5). *Significantly different from the values before palmityl-l-carnitine application (P<0.05, **P<0.01). *Significantly different from the values immediately before acetyl-l-carnitine application (*P<0.05, **P<0.01).
that the improvement by acetyl-l-carnitine was nearly of the same order as that of carnitine.

Figure 7 shows the effects of disopyramide (1.5 x 10^{-5} M) on the derangements of the action potential induced by palmityl-l-carnitine (3 x 10^{-4} M). APA and RMP were not altered by disopyramide as shown in Fig. 7. A further decrease in dV/dt max of 25% was seen. However, there was a significant prolongation of 16% of APD_{90}. Although there were prolongations of APD_{20} and APD_{50}, these were not statistically significant. These effects of disopyramide were similar to the action of this substance on the preparation without pretreatment with palmityl-l-carnitine.

**Discussion**

The present study conducted with the electrophysiological method clearly demonstrated that I-carnitine and acetyl-l-carnitine had no effect on the normal electrophysiological activities of the canine ventricular myocardium. In other words, these two compounds had no direct membrane action. A representative antiarrhythmic agent, disopyramide decreased dV/dt max and prolonged APD and ARP, in agreement with the results of other investigators ascribing the "quinidine-like" property to this compound. The word quinidine-like denotes the depression of the dV/dt max in the absence of changes in RMP, the depression of the conduction velocity, prolongation of APD, and associated prolongation of the refractory period (15, 16).

In this study, palmityl-l-carnitine, a member of the long chain acylcarnitines produced definite electrophysiological derangements in the canine ventricular myocardium: RMP, APA and dV/dt max were decreased, and APD and ARP were shortened. This is in agreement with the suggestion of other investigators (13, 14). Furthermore, the electrophysiological alternations induced by palmityl-l-carnitine (decreased RMP and APA and shortened APD) closely resembled the changes observed in early ischemia (12). The concentration of long chain acylcarnitine has been found to increase in ischemic tissue. According to Shug et al. (3) and Shug (5), the tissue content of acylcarnitine 30 min after ligation of the coronary artery in the dog is 221 nmole/g. It increased to as much as 300 nmole/g after 20 min of ischemia in the isolated working rat hearts. These values
correspond closely to $3 \times 10^{-4}$ M used in this study. Thus it seems that the accumulation of long chain acylcarnitine has something to do with the induction of dysrhythmias during early ischemia. As the reason of electrophysiological derangements induced by palmitoyl-l-carnitine, an accumulation of acyl CoA may be invoked, the accumulation of which was shown to produce an inhibition of the adenine nucleotide translocase and a depletion of the cytoplasmic ATP. The reduction of APD produced by palmitate was ascribed to a depletion of the cytoplasmic ATP (17). Shrago and Sul (18) have demonstrated an accumulation of acyl CoA in mitochondria following incubation with palmitoylcarnitine in the heart. Wood (19) has suggested the inhibition of the heart mitochondrial oxidative phosphorylation by palmitoylcarnitine. Indeed, the inhibition of the adenine nucleotide translocase of liver mitochondria (20) by long chain acylcarnitine have been reported. However, with heart mitochondria, Pande and Blanchaer (7) failed to show such an effect with palmitoylcarnitine. As an alternative explanation, the alteration of the ionic movements responsible for the generation of the cardiac action potential may be taken into consideration, for it has been reported that palmitoylcarnitine inhibits sarcolemmal Na*, K*-ATPase activity and inhibits sarcoplasmic reticulum Ca**-ATPase activity of the dog heart (21, 22).

In the present study, carnitine and its acetyl derivative reversed the arrhythmogenic effect of palmitoyl-l-carnitine, while disopyramide aggravated it by further decreasing the dV/dt max as an extension of the effects of this substance itself. These findings indicate that the antiarrhythmic effect of disopyramide depends mainly on the direct membrane action, while that of L-carnitine and its acetyl derivative may probably be ascribed to the improvement of mitochondrial oxidation of FFA intermediates such as long chain acyl CoA. The prevention of the arrhythmogenic effects of palmitoylcarnitine by carnitine was also reported by Hayashi et al. (14).

The improvement by acetyl-l-carnitine and L-carnitine of the derangements of the membrane excitation induced by palmitoyl-carnitine were nearly of the same order in vitro, although Sugiyama et al. (23) reported that the acetyl derivative, which was thought to be more penetrative into the tissue than l-carnitine, was more effective than l-carnitine. We have at present no adequate explanation for this discrepancy.

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