The Effects of Ethanol on the Dog Purkinje Fiber

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INTRODUCTION

It was reported by MAC GREGOR et al (1) and BIGELOW et al (2) that the administration of ethanol was effective in preventing the ventricular fibrillation in the hypothermia.

On the other hand, Wakim reported that the ventricle often fibrillated when higher concentration of alcohol were used (3).

It has also been reported that in the Purkinje fiber of the dog the fibrillation produced by aconitine was prevented to some extent by ethanol (4).

In the preliminary experiment the author (5) also investigated that the body temperature, when the fibrillation or the stop of the heart beating appeared, was lower in the adult dog treated with ethanol than that in the control.

In the present experiment, the effect of ethanol on the electrophysiological character of dog Purkinje fiber was studied with a microelectrode technique at 37°C or 30°C, and the possible mechanisms of ethanol effect on the fibrillation were discussed.

METHOD

Adult dogs of mixed bleed, weighing 15 to 20kg, were used. Purkinje fibers taken out of the left ventricle were used throughout the experiments.

Tyrode solution had a following composition ; Na ; 149, K ; 4.0, Ca ; 1.8, Mg ; 1.05, Cl ; 145 (in mM/1) and buffered by phosphate and bicarbonate (HCO_3 ; 13.5, H_2PO_4 ; 2.4, in mM/1).

Purkinje fibers were pinned on vinyl plate in a small plastic chamber through which test solutions were introduced. Test solutions, 37°C in temperature, were well gased by the mixture of 95 % of oxygen and 5 % of carbon dioxide (pH 7.4–7.6).

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An intracellular electrode was used to record the membrane potential, and the current was passed into the fiber through a second nitracellular electrode. Recording electrode was conventionaly 3 M–KCl filled microcapillary electrode (6), having the resistance of which was between 10 and 30 megohm. The current passing electrode was filled with 2M–K–citrate (7) and the resistance was between 8 and 12 megohm.

To investigate the changes of threshold potential, single square pulse was applied through the second electrode, as shown in Fig. 1. A train of 50 msec rectangular pulses was passed through the second electrode in order to examine the change of membrane conductance. Twenty megohm resistance was inserted between the pulse generator and the second electrode to avoid the effect of change of electrode resistance.

Certified pure ethanol (99.4 % nonhydro-Ethyl Alcohol. Katayama chemical) was diluted with Tyrode solution by the concentration of 0.1%–0.2% (v/v). It was introduced into the small plastic chamber after stable configuration of action potential was observed.

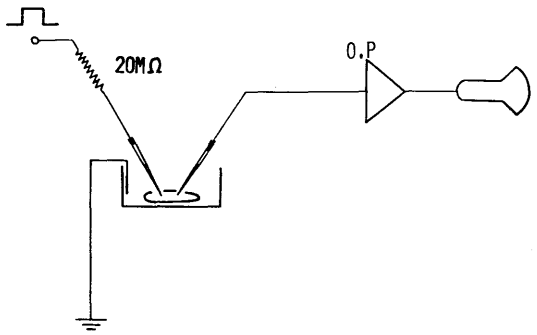

Fig. 1. Shows the pattern that two microelectrodes, an intracellular electrode which recorded the membrane potential and a second intracellular electrode through which current was passed into the fiber, were very nearly inserted into purkinje fiber. 20 mΩ resistance was intervented between a pulse generator and the second electrode to avoid the effect of electrode resistance. Recording electrodes are convential 3M-KCl filled microcapillaries which have resistance between 10 and 30 megohm, and current passing electrodes were filled with 2M-K-Citrate and they had resistance between 8 and 12 megohm.

RESULTS

1. Effect of ethanol on the slope of the diastolic depolarization

The effect of ethanol on spontaneously firing action potential was observed when its concentration was 0.1% or 0.15%. On the other hand, when higher concent ration of ethanol (more than 0.2 %) was used, the microelectrode tended to be

slipped out of the cell.

Figure 2 illustrates the effect of 0.1 % ethanol on spontaneously firing action potential of dog Purkinje fiber. When the preparation was kept in good condition and concentration of ethanol was 0.1 %, the beating of the preparation was continued for more than one hour, although decreased in frequency from 45 to 32 per min. However, no remarable changes in the configuration of the action potential could be observed.

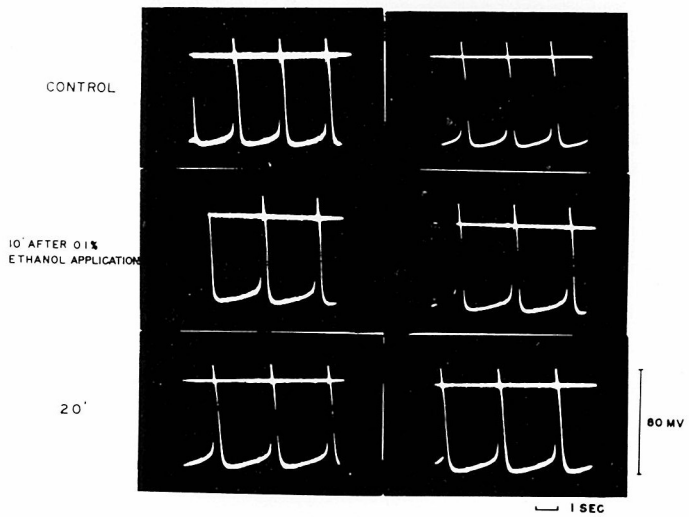

Fig. 2. Shows one example of 0.15 % ethanol effects on spontaneously firing action potential. A and B show the records obtained from two different preparationes respectively.

Figure 3 was recorded at one hour after the administration of ethanol. The beating frequency was nearly stable ranging between 28 and 35 per min. It was very interesting that an abortive oscillatory potential in diastolic depolarization was recorded in some preparations, as shown in the record taken at 50 min after the administration of ethanol. However, regarding to the oscillatory potential the explanation was described in the previous report (4).

It is well-known that the changes of beating frequency is caused either by the change of threshold level or by that of the slope in diastolic depolarization (8). Since firing level of this preparation could not be precisely measured from the recording in Fig. 3, the changes of slope in diastolic depolarization were measured. However, it could not be found that the slope was changed by the concentration mentioned above.

These observations may lead to the assumption that ethanol acts mainly on the excitability at the level of threshold potential.

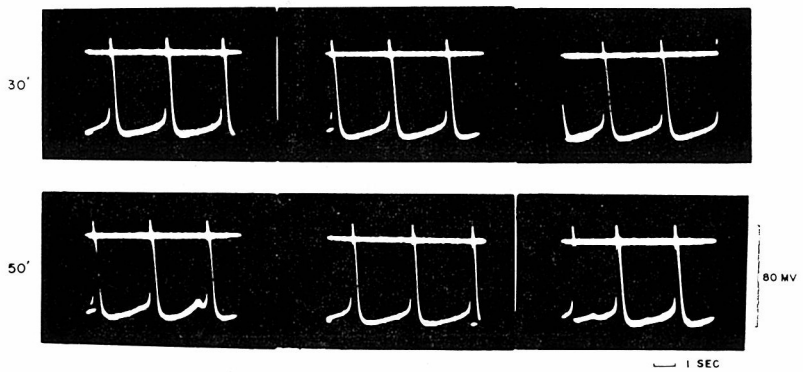

Fig. 3. Shows the spontaneously firing action potential recorded in one hour after ethanolization. The beating frequency decreased from 32 to 28/min by ethanol and this range of the (beating) frequency lasted stably.
A, B and C: Records from three different preparations respectively.

2. Threshold potentiall (firing level)

Changes of threshold potential were measured with the quiescent fiber; single square pulse was applied through the second electrode. The duration of square pulse was 50 msec. When the current of the pulse was increased little by little, the action potential was finally induced. The potential changes at this procedure were superimposed on the same film (Fig. 4).

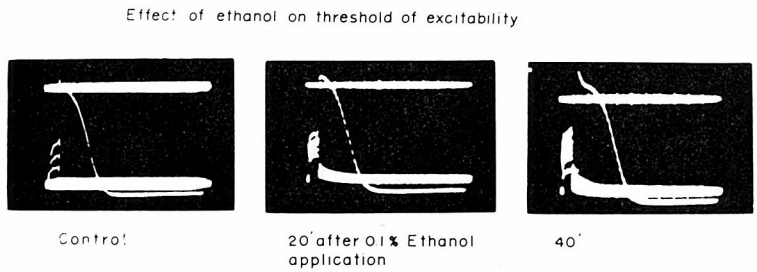

Fig. 4. Shows that threshold potential is increased after 0.1% ethanol application. To investigate the change of threshold potential, single square pulse was applied through the second electrode. The duration of square pulse is 50 msec. When the output of current of the second pulse generator was increaserd little by little, the spike of action potential was at last generated and this observation was photographed continuously.

Fig. 4 shows that the threshold potential was increased when 0.1% ethanol was applied. The spike of the action potential was evoked at the level of −50mV, in the control. When 40 minutes elapsed after 0.1 % ethanol application, the level of the threshold potential was −35mV. These changes could be seen at 20

minutes after the application of ethanol, and further elevation of the threshold could hardly be observed. This observation may imply that the frequency decreased by ethanol was mainly caused by the change of excitability at the level of the threshold potential, because the slope of diastolic depolarization was not influenced by ethanol.

3. Maximum rate of rise (rising time) of the action potential

It was so difficult directly to measure the rising time of the action potential that differential circuit was inserted between the output of high impedance amplifier and the main amplifier.

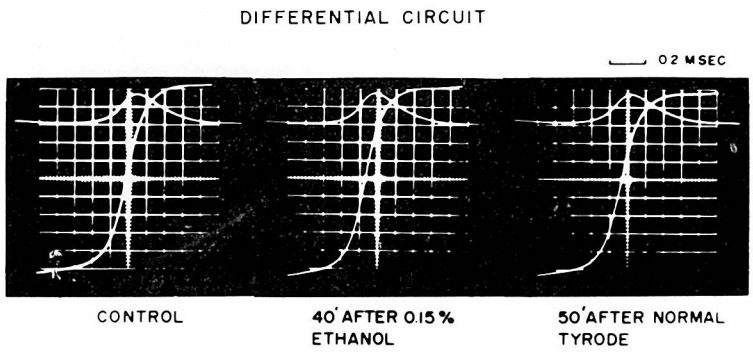

Fig. 5. Shows that no effects of ethanol on the rising time are observed by 0.15 % ethanol. Even when stimulation was intensified up to five fold of supraminimal level, any noticeable influence could not be found on the change of rising time.

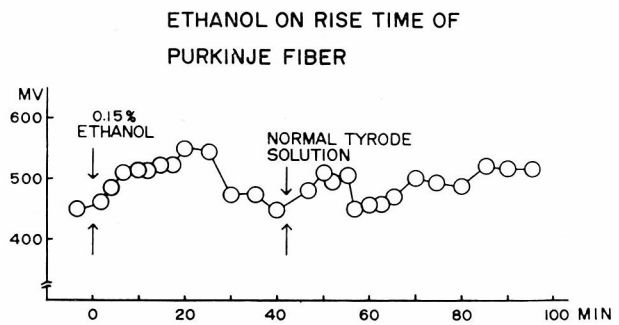

Fig. 6. Shows that any change can be hardly observed both after ethanol application and switching back to normal tyrode solution in many cases. The rising time is usually stable in the range between 450mV and 500mV/sec in one hour after ethanolization.

As represented in Fig. 5, apparent effects could not be observed on the rising time from this figure, after application of both 0.1 % and 0.15 % ethanol. Even when stimulation was intensified up to five hold of threshold, any noticeable influence could not be seen in the rising time of Fig. 5. However evident effects could be proved when the rising time was in practice measured from Fig. 5, in

accordance with lapse of time. The rising time of the action potential, before, during, and after the application of ethanol was successively plotted in Fig. 6. When 0.15 % ethanol was administered to these preparation, the rising time increased little by little, and then decreased after 20 minutes. When the solution was switched back to the normal tyrode solution, there were increasing and decreasing of the rising time after about 10 minutes, and also increasing after about 15 minutes. It was very interesting that descending part of differential recording became more convex in the preparation treated with ethanol than in the control.

As illustrated in Fig. 7, when it passed about 40 minutes after the administration of 0.2% ethanol, the rising time was decreased suddenly. It was frequently observed that the preparation was deteriorated by 0.2 % ethanol. So it seems likely that the higher concentration than 0.15 % ethanol is not suitable to examine the effects of ethanol on the purkinje fiber.

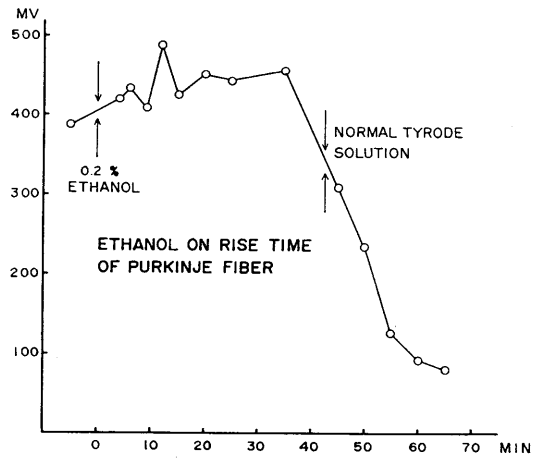

Fig. 7. Shows that the plotted slope of rise time decreases suddenly, when about 40 minutes elapsed after ethanolization by 0.2% ethanol. Regardless of alteration of the external solution, similar findings were obtained.

4. Duration of the action potential and plateau level

Ethanol did not show an evident influence on the duration of the action potential when the bathing fluid was kept in 37°C. On the other hand, it is well known that the duration of action potential is lengthened by the lowering of environmental temperature (9–11). Therefore, the effect of ethanol on the duration of the action potential was investigated in the lower temperature (30°C), as illustrated in Fig. 8. Both the duration and the plateau level of action potenial were always reversible when ethanol was lower than 0.15 % in concentration.

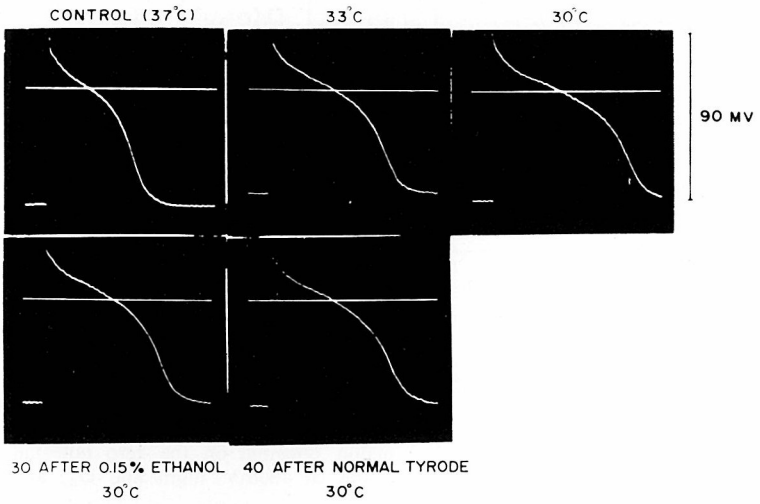

Fig. 8. Shows the effect of lower temperature on the plateau of the action potential in the normal tyrode solution. Temperature was gradually ranged from 37°C. to 30°C. Then, ethanol was added, and afterwards replaced with normal tyrode solution.

Some investigators have reported that, when the bathing fluid was very rapidly cooled, the duration of the action potential is prolonged and the overshoot is transiently increased in the initial stage of cooling (12, 13). It took usually 10 minutes to lower the temperature of bathing fluid from 37°C to 30°C. The plateau was obviously lengthened, the resting potential was edcreased a little (depolarization of 8mV–10mV) and the overshoot was somewhat increased (3mV–5mV). When 0.15% ethanol was administered to these preparation, the plateau was shortened and the overshoot was decreased (1mV–3mV). The depolarization caused by low temperature (30°C) was still more increased after treating with ethanol. In about 40 minutes after switching back to Tyrode solution, the duration of the action potential and plateau level was not changed and a little degree of hyperpolarization (3–5mV) was observed.

In Fig. 9, four kinds of the action potential were recorded in normal tyrode solution (at 37°C and 30°C respectively), during 0.15 % ethanol-administration (30°C) and after switching back to Tyrode (30°C), and they were superimposed. It may be concluded from these results that ethanol certainly influenced on plateau level of the action potential in lower temperature.

In the Table 1, D_1 denotes the duration of the action potential at zero level, D_2 at -30mV level and D_3 at -50mV respectively. It is clearly demonstrated that, in low temperature (30°C), the effect of ethanol on the duration of the action potential can be more evident than that of the same concentration in 37°C.

	RP(mV)	AP(mV)	OS(mV)	D_1(0mV) msec	D_2(30mV) msec	D_3(50mV) msec
CONTROL 37°C	80	100	20	185	330	370
32°C	75	96	21	210	375	417.5
30°C	70	95	25	240	432.5	480
0.15% ETHANOL (30°C)	65.5	87.5	22	222.5	397.5	445
NORMAL TYRODE (30°C)	70	88	18	225	400	445

Table 1. Shows the amplitude of resting potential (RP), action potential (AP) and overshoot (OS).
D_1 indicates the duration of action potential on the zero potential level, D_2, the duration of action potential at -30mV hight and D_3, at -50mV respectively.
Effects of both temperature and ethanol on the Purkinje fiber were examined.

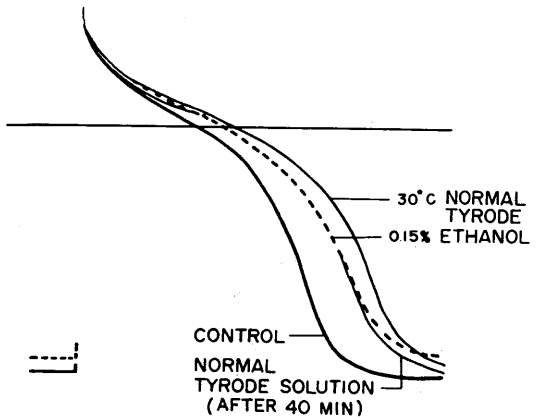

Fig. 9. Shows that the records of four kinds of action potentials were superimposed: in normal tyrode solution (at 37°C and 30°C), in presence of 0.15 % ethanol (at 30°C), and after switching back to tyrode solution respectively.

5. Membrane conductance

Considering the effect of ethanol on the duration of the action potential or on the plateau level, it seems likely that the membrane conductance in the time-course of the action potential might be changed by the administration of it. Therefore, the superimposed records for measuring successive change of conductance during the action potential were obtained following the Weidman's original method (14). The speed of sweep was 50mm/sec. Either 0.1 or 0.15 % ethanol was used in the concentration.

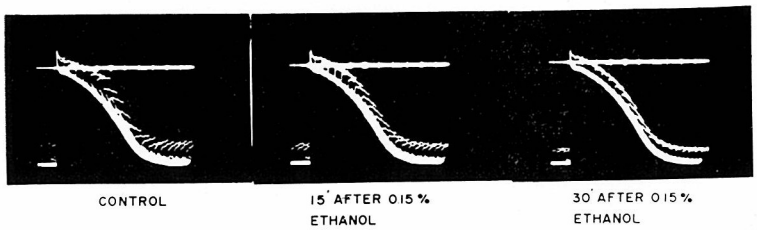

Fig. 10. Shows the changes of the membrane conductance accompanied with generation of the action potential. The first pulse generator was used in order to produce the action potential. At first the sweep of action potential was fixed with rapid speed of 50mm/sec. A train of 50msec rectangular pulses was passed through the second electrode in order to examine the change of membrane ionic conductance in whole duration of action potential. Pulse frequency of the first generator was slipped off from that of the second generator, so square pulses between 15 and 20 in number were superimposed on the patern of action potential and these observations were photographed continuously.

Figure 10 represented the effect of ethanol on the membrane conductance in the time-course of the action potential. The effects were recognized more remarkably, in the records at 30 min than that at 15 minutes after the ethanol application. However, the still more variation of the membrane conductance could not be observed further. It was noteworthy that the changes of the membrane conuctance during the action potential were mainly observed in plateau level (slow repolarization phase).

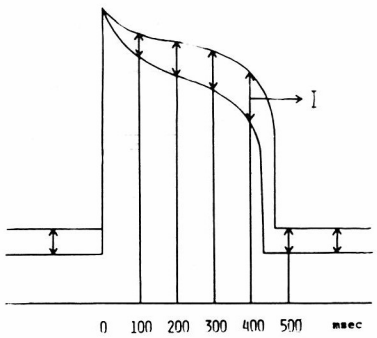

Fig. 11. Shows that the length of I ismeasured at each point of plateau, divided into 100 msec in order to minutely analyze the conductance value. The length of I indicates the value of changes of voltage responses to the constant current pulses. In this figure, the length of I is measured at 5 points divided by 100 msec on the abscissa. The approximation to the changes of the membrane ionic conductance was calculated using a resiprocal of I^2.

Figure 11 shows the successive changes of the membrane conductance membrane conductance, shematically. The length of I, the vertical distance of the two curves in this figure is shown at 5 points separated by 100 msec on the abscissa. Since the membrane resistance is relatively proportional to the square of I (15), Table

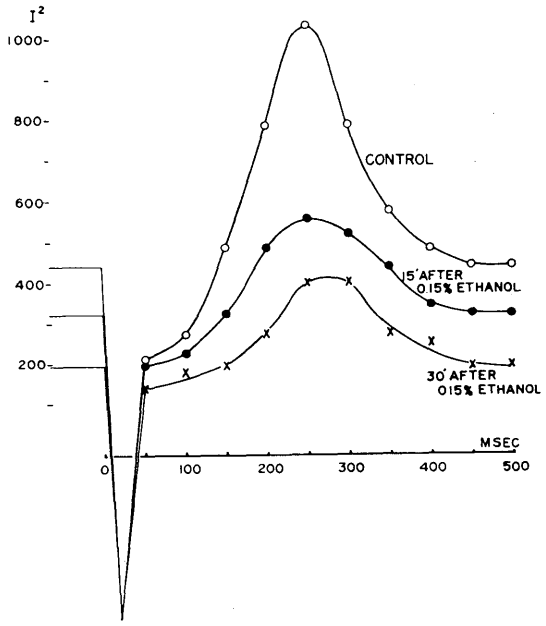

Fig. 12. Effect of ethanol on Impedance during the action potential. Ordinate: the value of I^2. Abscissa: time after onset of the action potential. Open circle: control. Filled circle: at 15 minutes after 0.15% ethanol application. crosses: at 30 minutes after 0.15 % ethanol application. Impedance could not be measured furing the rapid phase of the action potential.

* / **	REST	50	100	150	200	250	300	350	400	450	500
CONTROL	441	210	272	484	784	992	784	576	484	441	441
ETHANOL 15′	324	196	225	324	484	552	529	441	342	324	324
30′	196	144	182	196	272	400	400	272	256	196	196

Table 2. Shows the numerical value of 1(**) was measured at each points divided by 50 msec on the abscissa in Fig. 11(*). The effect of ethanol on the membrane ionic conductance during the plateau phase was examined.

2, which denotes the numerical values of I measured at each point, is written in order to analyse the conductance-value; and Fig. 12 is the graphical description of them. The increase in the membrane conductance accompanied with the action potential in the control is in accordance with what has been experimentally observed by Weidmann (14). The membrane resistance of the preparation treated with 0.15 % ethanol also rises during the plateau, falls at the end of the

repolarization and then rises slightly during the following slow depolarization. Comparing to the control, it is clear that the membrane conductance of the purkinje fiber is increased by the ethanol-administration both in plateau-phase and in resting period, and that the former is more remarkable than the latter. It must be noted that the increase of the membrane resistance in plateauphase is most remarkable in the period from 200 to 300 msec.

DISCUSSION

It was shown that the turtle ventricle, in vitro, often fibrillated when the perfusate containing the higher concentrations of ethanol was used (3). Gimeno et al (16) also reported that such a higher concentration (96mM or more) of ethanol induced the decrease in both duration and area of the action potential which predispose the heart to such dysrhythmias as fibrillation. Contrary to these observations, the author has shown that the fibrillation induced by aconitine in the Purkinje fiber was disappeared by ethanol (4). It was also inferred that the disappearance of this fibrillation was caused mainly by both membrane hyperpolarization and decrease of spike frequency in the action potential after the application of ethanol (4).

In the present study, ethanol also induced the decrease of spike frequency in the action potential. Indeed, ethanol increased the level of threshold potential which was required for firing of an action potential, however no significant changes in both the slow depolarization and rising time in the action potential were observed. Since the initial membrane excitation would depend upon the conditions in slow depolarization, threshold and rising time (8), thus the present results suggest that spike frequency in the action potential of the cardiac Purkinje fiber may be decreased by the increase of threshold potential after the application of ethanol. Ethanol also induced the shortning in the duration of the action potential. In the present experiments, as shown in Fig. 12, ethanol increased the membrane conductance for potassium ion (gk) during the action potential plateau, though sodium conductance (gNa) was not examined. It is well known that the long lasting plateau phase of the action potential in the Purkinje fiber is associated with the reduction in gk during the plateau (14, 17). Although it is difficult to explaine how the plateau was shortened in the present experiments, the significant increase in gk during the plateau would, at least, be an important factor of the shortning in the duration of the action potential. It is generally accepted that the rising phase in cardiac muscle depends on the increase in sodium permeability (18). However, as shown in Fig. 2, 3, 5, 6, no significant changes of the rising time in the action potential were observed. In the Purkinje fiber, therefore, it appears likely that ethanol has no significant effect on the membrane permeability for sodium ion.

David (19) has reported that, in the squid axon at room temperature, alcohols reduced only the maximum gNa and no large changes in gK occurred. However, ethyl alcohol reduced both gNa and gK at low temperature (20). In regard to the effect of ethanol on the membrane conductance, therefore, there were several discrepancy between the present results and the earlier observations, as described above.

In both squid and lobster axon, the higher concentration of ethanol (tenfold in the Purkinje fiber) was used at lower temperatures. Such a higher concentration of ethanol may also induce the changes in the membrane structure by means of mobilization of tightly bound calcium from the membrane sites, as was reported in smooth muscle (21). However, the question as to why the gK in the Purkinje fiber increases by ethanol remains unanswered. There is no direct explanation from the present experiments. However, it has been reported that ethanol may reduce the calcium ion being required to restrict the outward movement of potassium ion and, therefore, enhances the rate of potassium efflux in the unexcited smooth muscle (22), Consequently, in the present experiment, the increase in gK by ethanol may be caused by such an analogous effect as the case in the smooth muscle.

As described before, the spike frequency in the action potential decreased in the presence of ethanol, though the duration in the action potential was shortened by ethanol. Presumably, the decrease of the spike frequency would be caused by the increase of the threshold potential. It was difficult to explaine how the threshold was increased in the presence of ethanol. However, it appears likely that the increase of the threshold is related to the increase of gK resulting from the membrane depolarization (23), without the significant changes in gNa.

Thus, the disappearance of the cardiac fibrillation in the presence of ethanol would be explained by the increase of threshold potential followed by the decrease of the spike frequency in the action potential.

SUMMARY

The effects of ethanol on the slope of slow depolarization, rising time, threshold potential, membrane conductance and plateau duration in the action potential of dog Purkinje fiber were studied by means of intracellular electrode techniques.

The concentration of ethanol used in the present experiments was ranged between 0.1 % and 0.2 %.

It was observed that 0.1 % ethanol lowered the beating frequency with the change of excitability at the level of threshold potential and shortened the plateau because of increased membrane conductance for potassium ion. No significant changes in both the slow depolarization phase and rising time in the action po-

tential were observed.

It was discussed that ethanol was effective to inhibit the fibrillation by the mechanisms of elevating the threshold in slow depolarization and of increasing the membrane conductance for potassium in plateau. This would be the reason why ethanol is clinically useful for preventing the fibrillation which appears at warming-up stage after the operation of heart with hypothermia.

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