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Ionic Dependence and Modulatory Factors of the Background Current Activated by Isoprenaline in Rabbit Ventricular Cells

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= ABSTRACT =

In order to elucidate the properties of the background current whole cell patch clamp studies were performed in rabbit ventricular cells. Ramp pulses of ±80 mV from holding potential of -40 mV(or -20 mV) at the speed of 0.8 V/sec were given every 30 sec(or 10 sec) and current-voltage diagrams(I-V curve) were obtained. For the activation of the background current isoprenaline, adenosine 3′,5′-cyclic monophosphate(cAMP), guanosine 3′,5′-cyclic monophosphate(cGMP), and N^6-2′-o-dibutyryladenosine 3′,5′-cyclic monophosphate(dBcAMP) were applied after all known current systems were blocked with 2mM Ba, 1 mM Cd , 5 mM Ni, 10 μM diltiazem, 10 μM ouabain, and 20 mM tetraethylammonium(TEA). The conductance of background current in control was 0.65± 0.69 nS at 0 mV, its I-V curves was almost linear and reversed near -50 mV. When there was no taurine in pipette solution, isoprenaline hardly activated the background current but when taurine existed in pipette solution, isoprenaline activated the larger background current. Cyclic AMP or cyclic GMP alone had little effect on the activation of the background current, while cGMP potentiated cAMP effect. When the background current was activated with cGMP and cAMP, isoprenaline could not further increased the background current. The background current activated by isoprenaline depended on extracellular Cl^- concentration and its reversal potential was shifted according to chloride equilibrium potential. The change of extracellular Na^+ concentration had little effect on reversal potential of the background current activated by isoprenaline.

Key Word: Rabbit ventricular myocytes, Whole cell patch clamp, Background current, Isoprenaline, Taurine, Cyclic nucleotide.

INTRODUCTION

In 1987 Egan et al. discovered a current activated by isoprenaline. This current could be elicited after suppression of all known currents with blockers and disappeared after substitution of extracellular Na with tetramethyl ammonium(TMA). They suggested this current be new background current dependent on extracellular Na and cAMP have a role as an intracellular second messenger(Egan et al, 1987a,b,c, 1988). But there were some discrepancies for ionic nature of the background current. Matsuoka et al. (1990) discovered this current was dependent on extracellular Cl^- using various kinds of substitutes for Na^+ and Cl^- and suggested that this Cl^- current activated by isoprenaline be dependent on extracelluar Na^+. Other investigators also reported similar results that isoprenaline-activated current was dependent on Cl^- ion(Bahinski et al, 1989; Harvey & Hume,1989). It has been reported that in guinea-pig, isoprenaline, β-agonist, exerted its action through the increase of intracellular cAMP(Harzell & Fischmeister, 1986; Harzell et

al, 1991) and its action was suppressed by cGMP through activation of cGMP-dependent-phosphodiesterase (Fischmeister & Harzell, 1987). On the contrary Ono et al. (1991) and our results indicated that cGMP potentiated cAMP action on Ca current. Therefore the action of cGMP was not so simple.

On the other hand, according to Earm et al. (1990) 5 mM of taurine (2-aminoethane sulfonic acid) could produce action potential in quiescent single cardiac cells isolated from the guinea-pig. And it has been known that intracellular concentration of taurine in cardiac muscle was 60 to 600 times as high as extracellular concentration(Baños et al, 1978) and intracellular taurine was closely related to regulation of $[Na]_i$ and Ca tolerance(Chapmann & Rodrigo, 1990).

We found effects of isoprenaline on the background current in rabbit ventricular cells was very different from those in guinea-pig. Thus we examined the effect of taurine and cyclic nucleotides on the background current and its ionic nature in rabbit ventricular cells. The preliminary results were presented in abstract form (Leem et al, 1991).

METHODS

Single ventricular cell isolation

Rabbits were anesthetized with pentobarbital sodium(50mg/Kg). Under artificial respiration, the chest was opened and the aorta was cannulated *in situ*. The heart was excised and perfused by using a Langendorff perfusion system with 80 cmH_2O pressure. Normal Tyrode solution was perfused until the blood was washed out completely. And then Ca^{2+}-free Tyrode solution was perfused for 5 min and Ca^{2+}-free Tyrode solution containing 0.01% collagenase(5mg/50cc, Yakult) was perfused for 15-25 min. After enzymatic treatment Krafts-Brühe(KB) solution(Isenberg & Klöckner, 1982) was perfused for washing out remaining enzyme in the heart and dispersed the ventricular cells by stirring in KB medium. Isolated ventricular cells were kept in KB medium at 4°C

Solutions

Normal Tyrode solution contained, in mM:NaCl 143; KCl 5.4; $CaCl_2$ 1.8; $MgCl_2$ 0.5; glucose 5.5; HEPES 5 and pH was adjusted to 7.4 with NaOH. Composition of internal solution was, in mM: KCl 100(110 without taurine); MgATP 5; di-Tris-creatine phosphate 2.5; di-Na-creatine phosphate 2.5; $MgCl_2$ 1; TEA-Cl 20; HEPES 5; taurine 20; EGTA 5 and pH adjusted to 7.4 with KOH. For isolation of the background current, K current was blocked by 2 mM $BaCl_2$ and 20 mM TEA-Cl, Na-K pump current by 10 μM ouabain, Ca current by 1 mM $CdCl_2$ and 10 μM diltiazem, and Na-Ca exchange current by 5 mM $NiCl_2$. For substitution of extracellular Na^+, N-methyl-D-glucamine(NMG) was used. Ascorbic acid was used to substitute Cl^-.

Electrophysiological recording

Glass electrodes of the resistance of 1-2 MΩ were used. The ventricular cells were transferred to the bath on the inverted microscope(Olympus IMT-2). The chamber was perfused at the velocity of 1 ml/min and the temperature of the solution was maintained at 37°C. After giga-seal was formed(seal resistance 1-10 GΩ), more negative pressure of 50-80 cmH_2O was applied to rupture the membrane and established the whole cell mode patch clamp(Hamill et al, 1981).

Using voltage clamping technique, membrane potential was held at -40 mV or -20 mV to inactivate Na current and ± 80 mV of ramp pulses(0.8 V/sec) were applied every 30 sec or 10 sec. The data were displayed on digital oscilloscope (Philips, PM3350), pen recorder (Gould,15-6327-57) and recorded on PCM (Medical systems Corp., PCM-4/8) and videocasette recorder(Thomson consumer electrinics, INC. VG7785) for later analysis. I-V relationships were plotted with plotter(Graphtec, MP 4100) or laser printer(Hewlette Packard, laserjet III).

RESULTS

The background current activated by isoprenaline

The typical background current activated by isoprenaline was shown in Fig. 1. In insets of pen records, the holding current shifted to inward direction, and the amplitudes of inward and outward current increased and then spontaneously decreased.

The amplitude of control current was about 80 pA and the reversal potential was near at -60 mV. Isoprenaline activated background current was obtained by substracting of the control current from the isoprenaline-activated current(b-a), the reversal potential was around at -5 mV and increased linearly.

Current responses by isoprenaline in guinea-pig and rabbit were shown in Fig. 2. In insets of pen records the holding current was shifted to inward direction, and amplitude of inward and outward current was increased by isoprenaline but the amplitude was much larger in guinea-pig than in rabbit. In I-V curve which was normalized with the membrane capacitance of 50 pF, the amplitude of the current in guinea-pig was 4 times as large as that in rabbit which was one of the largest response. In the ventricular cells of guinea-pig, 0.5 μM isoprenaline could activate the maximum response of the background current, but in the rabbit, 4 μM isoprenaline could not activate

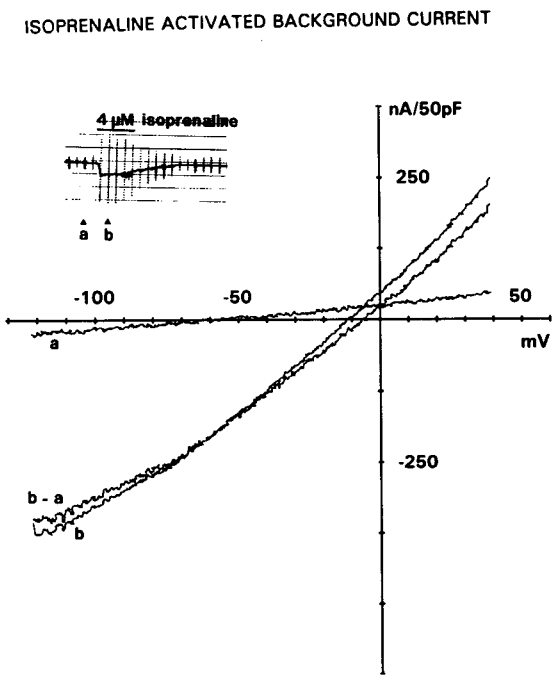

Fig. 1. The effect of 4 μM isoprenaline. a) control current. b) isoprenaline activated background current. b-a) The difference current which was obtained by subtracting the net current traces of before and after isoprenaline superfusion. Pipette solution contained 20 mM taurine.

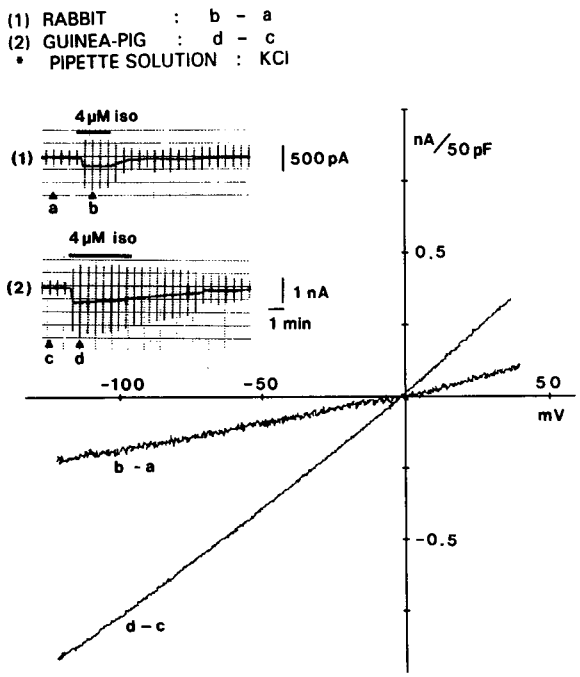

Fig. 2. Difference of isoprenaline effect between rabbit and guinea-pig. Pipette solution contained mainly KCl. (1) the isoprenaline response in rabbit. (2) the isoprenaline response in guinea-pig.

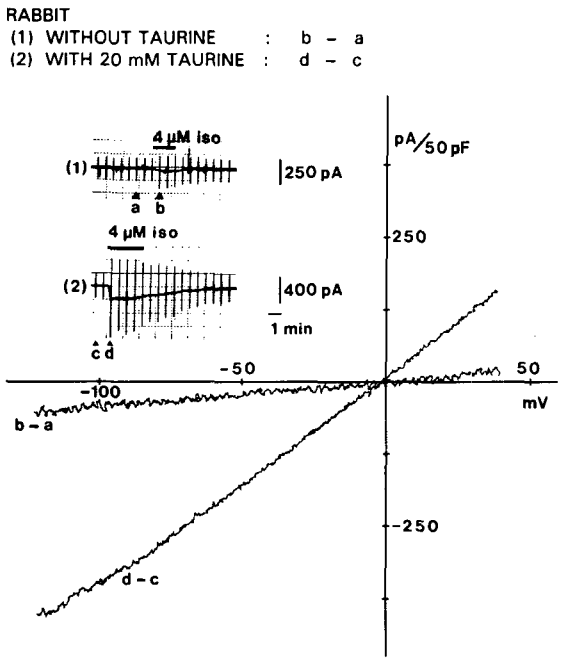

Fig. 3. The effect of taurine on isoprenaline activated background current: (1) the response in the absence of taurine, (2) the response in the presence of taurine

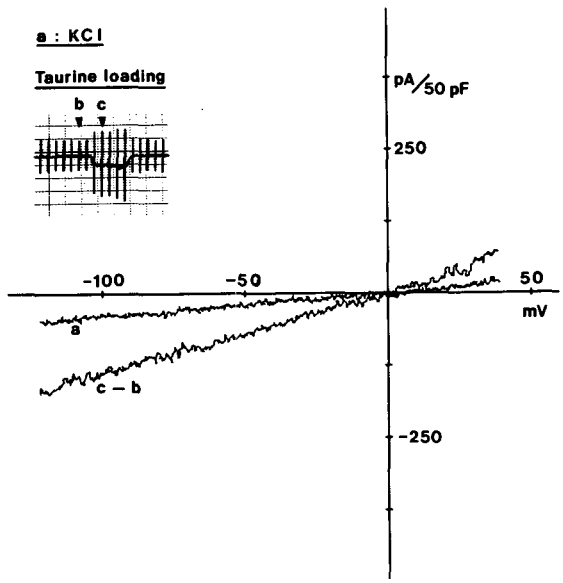

Fig. 4. The effect after 15 min loading of taurine.a) control current, c-b) difference current.

background current occasionally. The reason of such differences between them was not obvious. It is known there was very high physiological concentration of taurine in the cardiac cells(Jacobsen et al, 1968; Baños et al, 1978) and there was a results that action potential could be generated by taurine in guinea-pig ventricular cells(Earm et al, 1990). So the response to isoprenaline was studied under the condition of 20 mM taurine in internal solution.

The effects of taurine on the isoprenaline activated background current

In Figure 3 responses in the presence or absence of 20 mM taurine in the pipette solution were shown. In figure 3-1 a typical trace of the current activated by 4 μM isoprenaline in the absence of taurine was shown. In figure 3-2 the response to 4 μM isoprenaline was shown in the presence of 20 mM taurine and the typical appearance like in guinea-pig was shown, in which the holding current was shifted to inward direction and amplitudes of inward and outward current increased. The amplitude became smaller with time and it suggests there is desensitization. With taurine current amplitude was 650 ± 350 pA(n = 18) and without taurine it was 105 ± 110 pA(n = 28).

In figure 4 the reponse to isoprenaline after superfusing bath solution with 20 mM taurine for 15 minute was shown. In figure 4-a response without taurine in the bath solution was shown. After loading taurine the current activated by became larger as b-c.

Because taurine enhanced the isoprenaline activated background current, taurine was always added to the internal solution in all the following experiments.

The effect of cAMP and cGMP on the background current activated by isoprenaline

Since 5-20 μM of cAMP was known to elicit maximum effect on Ca current(Harzell, 1988),

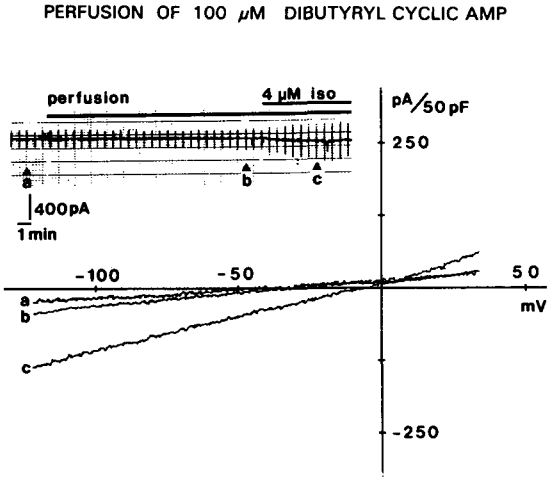

Fig. 5. Effect of perfusion of 100 μM dibytyryl cyclic AMP(dBcAMP): a) control I-V curve, b) perfusion of 100 μM dBcAMP, c) superfusion of 4 μM isoprenaline

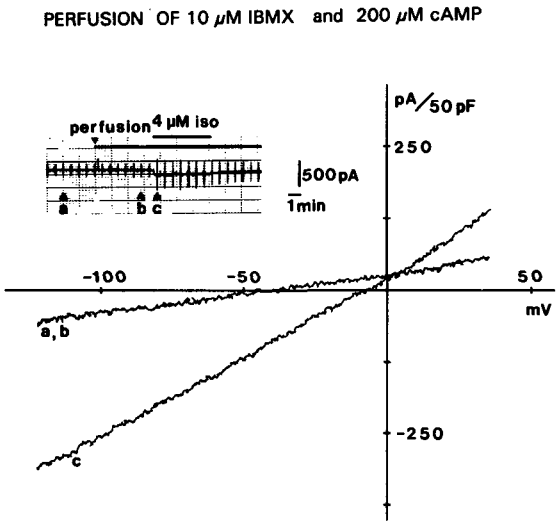

Fig. 6. The effect of perfusion of 10 μM IBMX and 200 μM cyclic AMP: a) control I-V curve, b) perfusion of 10 μM IBMX and 200 μM cyclic AMP.

30 μM cAMP was intracellularly perfused to see whether the background current was also activated by this concentration. But there was no effect(data was not shown). 100 μM dibutyryl cAMP(dBcAMP) which has more potent effect than cAMP(Miller et al, 1973) was also perfused intracellularly but there was no effect. In this condition, 4 μM isoprenaline could activate background current though it was small(Figure 5). 10 μM IBMX, which is known to block phosphodiesterase and 200 μM cAMP was also perfused intracellularly but there was no effect, but 4 μM isoprenaline could also activate background current(Figure 6).

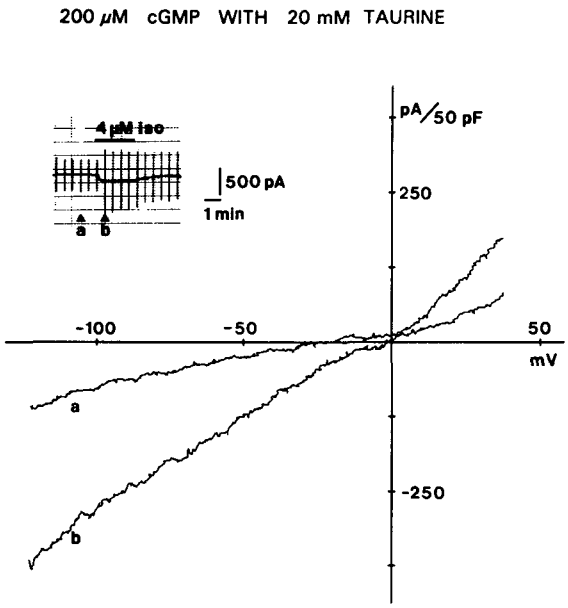

Fig. 7. The effect of 200 μM cGMP on isoprenaline activated background current.

200 μM cGMP was added to internal solution to see whether cGMP could inhibit the action of isoprenaline which was shown by Fischmeister & Harzell(1987)(Figure 7). cGMP alone has no effect on the background current, while application of 4 μM isoprenaline still activated the background current. From these results cGMP seems not to suppress the action of isoprenaline. When cGMP and cAMP were included in internal solution(Figure 8), the background current became larger as the internal pipette solution diffused into the cell after

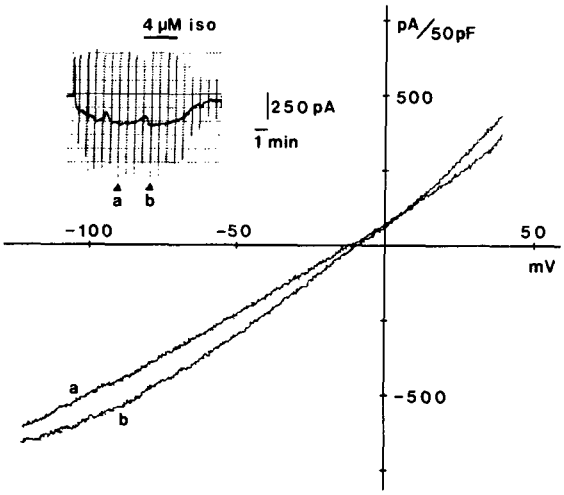

Fig. 8. The effect of direct administration with pipette solution of 200 μM cAMP, 200 μM cGMP: a) after rupture of membrane with pipette solution of 200 μM cAMP, 200 μM cGMP, b) superfusion of 4 μM isoprenaline

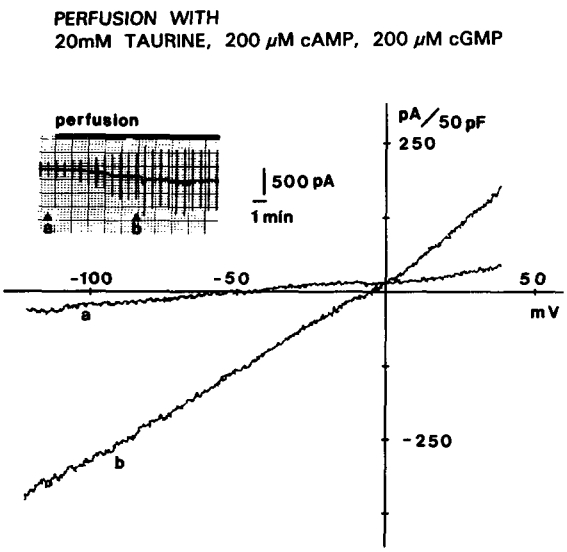

Fig. 9. The effect of perfusion of cAMP and cGMP: a) control current. Pipette solution contained cGMP 200 μM. b) After perfusion of cAMP and cGMP.

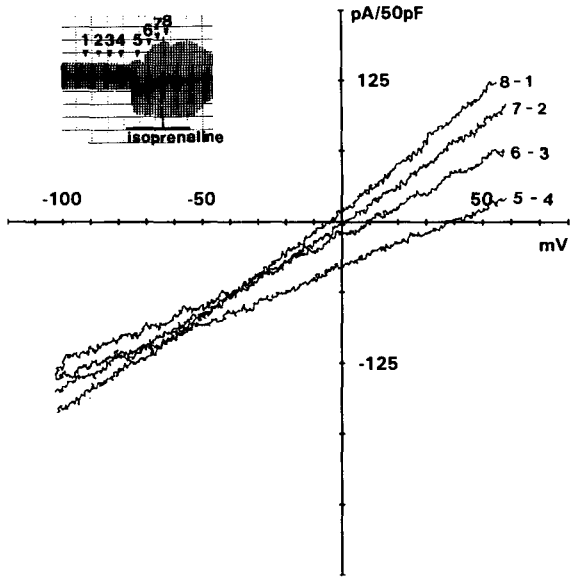

Fig. 10. The effect of the change of Cl^- ion concentration. 1,2,3,4: Control current. Cl ion concentration of bath solution was 16mM, 76mM, 136 mM, 166mM respectively. 5,6,7,8: Isoprenaline effect. Cl concentration of bath solution was 166mM, 136mM, 76 mM, 16mM respectively. Pipette solution contained 122 mM Cl ion. All isoprenaline activated background currents were subtracted with control current.

the rupture of cell membrane. Superfusion of isoprenaline slightly increased but there was no significant change. In order to confirm the effect of cGMP, 200 μM cAMP was perfused in the presence of cGMP in the pipette solution(Figure 9). As the pipette solution was diffused into the cell, the holding current shifted to inward direction and the amplitude of current increased.

Ionic nature of background current

In order to know the ionic nature of the background current, extracellular concentra-

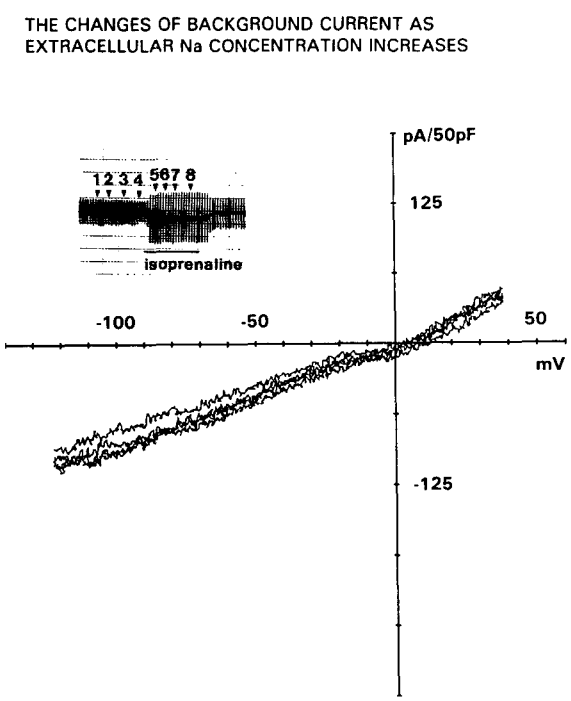

Fig. 11. The effect of the change of Na ion concentration. 1,2,3,4: Control current. Na ion concentration of bath solution was 0mM, 60 mM, 120 mM, 150 mM respectively. 5,6,7,8,: Isoprenaline effect. Na ion concentration of bath solution was 150 mM, 120 mM, 60 mM, 0mM repectively. Pipette solution contained 5 mM Na. All isoprenaline activated background currents were subtracted with control current.

tions of Na^+ ion and Cl^- ion were changed. Figure 10 showed pen records(inset) and I-V curves of the background current when the concentrations of extracellular Cl^- ion were changed to 16,76,136,166 mM while internal Cl^- ion concentration was 122 mM. As shown in insets of pen records the amplitude of control current was changed little by increasing extracellular Cl^- concentration. And while isoprenaline was superfused, the extracellular concentration of Cl^- ion was increased. Then outward current was increased and reversal potential was shifted to negative potential. These changes were well fitted with the predicted chloride equilibrium potential.

Figure 11 showed the pen records(inset) and I-V curves of the background current as the concentration of extracellular Na ion were changed to 150,120,60,0 mM while internal Na ion concentration was 5 mM. The current amplitude and reversal potential were not changed by Na^+ concentration in control and 4 μM isoprenaline.

DISCUSSION

The major results from this experiment were summarized as follows 1) isoprenaline activated the background current to inward and outward direction 2) in rabbit ventriucluar cells the magnitude of isoprenaline—activated background current was smaller than that in guinea-pig 3) intracellular taurine had modulatory effect on isoprenaline-activated background current in rabbit 4) cGMP potentiated cAMP effect 5) the background current activated by isoprenaline was dependent on Cl^- ion and not dependent on Na^+ ion.

The meaning of the background current activated by isoprenaline

Inward background current was postulated to have an important role to the generation of the rhythm in the cardiac muscle(Noble et al, 1989). And whether this background current is affected by neuronal or hormonal control is also important to understand the generation of normal or abnormal rhythm(Noble et al, 1989). Until now there has not been any report that the change of the background current directly affected to the rhythm of the heart. But there was an evidence that the automaticity was generated in the quiescent atrial muscle after small inward current was applied(Brown & Noble,1969) and the pacemaker activity was generated by inward current injection in ventricular muscle(Katzung & Morgenstern, 1977). Normal atrial or ventricluar muscle do not generated action potential but if the membrane

potential was depolarized over -60 mV by certain inward current, then they could produce spontaneous action potential(Noble et al, 1989).

So if isoprenaline produce depolarization and elicit spontaneous activity, it must be very important factor for production ventricular arrythmia. During infarction of the heart the concentration of adrenaline and noradrenaline was increased and the high concentration of catecholamine can produce arrhythmia(Jewitt et al, 1969; Richardson, 1968; Videbaek et al, 1972). Also 0.1 μM isoprenaline could produce oscillatory activity in quiescent guinea-pig ventricular cells(Egan et al, 1987). And they found that this current was dependent on extracellular Na^+ ion. After then there were many results that isoprenaline-activated current is dependent on chloride ion(Bahinski et al, 1989; Harvey & Hume, 1989; Harvey et al, 1990; Matsuoka et al, 1990). Matsuoka et al.(1990) suggested that the background current can be modulated by extracellular cation such as Na^+ ion because the reversal potential was not affected by the change of the concentration of Na ion but the amplitude of the background current was reduced when extracellular Na concentration was decreased. If the background current activated by isoprenaline is dependent on Cl^- ion, its reversal potential should be changed with predicted chloride equilibrium potential. If we assume E_{Cl} is positive than -60mV then this current in physiological condition will be a activated as an inward current and it will depolarize the membrane potential and elicit the spontaneous action potential. If its reversal potential is negative than -60 mV, this background current could depress the arrhythmogenic effect by strong sympathetic activity. Because the direction of Cl current is outward in physiological condition and this current was activated instantaneously, it can repolarize action potential and is contrary to the depolarizing effect of Ca current increased by catecholamine(Bahinski et al, 1989).

It has been known that the distribution of free chloride ion in physiological condition was passive and its reversal potential was very near potassium equilibrium potential. So Bahinski et al. (1989) suggested that this chloride current activated by isoprenaline have a role of speeding repolarization and so might protect the heart from arrhythmogenic effect of strong sympathetic stimulation. But there were many results that chloride ion between cell membrane was not distributed passively according to Nernst equation but intracellular concentration of free chloride ion was higher than that predicted by Nernst equation and ranged from 15 mM to 25 mM(Spitzer & Walker, 1980; Baumgarten & Fozzard, 1981; Fong & Hinke, 1981). Ehara et al. (1990) recorded the single chloride channel current using cell attached patch clamp technique in guinea-pig heart muscle. Their results showed that when intrapipette concentration of chloride was 150 mM the reversal potential was -40 mV and by Nernst equation it could be concluded that normal intracellular concentration of chloride was about 30 mM. If $[Cl]_i$ is from 15 mM to 25 mM and $[Cl]_o$ is 140 mM, the chloride euilibrium potential would be or positive less negative than -60 mV. If this is the case then chloride current activated by isoprenaline could depolarize the membrane and produce action potential. All of these results could explain the arrhythmia produced by catecholamine.

The role of taurine in ventricular cells

It was found that taurine was highly accumulated in excitable cells such as cardiac muscle and neuron. It has been thought that it had certain role for cell function and control of the excitability of cell membrane. The effects of taurine on heart mostly related to the protection effect of abnormal heart function. In myocardial infarction taurine was released from heart cell to blood(Cooper & Lombardini, 1982), and the concentration of taurine of heart cells was rather decreased(Kramer *et al.*, 1981). But in congestive heart failure the concentration of taurine was rather increased(Huxtable & Bressler, 1974). There were results that taurine have a protective effect on the arrhythmia produced in ischemic heart disease and it was explained by the mechanism that taurine protected the decrease of the activity of

ATPase accompanied with ischemia(Kramer et al, 1981; Franconi et al, 1985). Taurine also had the effect on oxygen paradox and arrhythmia occured when O_2 was infused after hypoxia(Read & Welty, 1963; Chazow et al, 1974; Franconi et al, 1985). It was explained mainly by the mechanism that taurine inhibited the Ca overload in the heart cells(Azari et al, 1980; Kramer et al, 1981). Such results were supported by the result of Chapmann & Rodrigo (1990) that taurine inhibited the accumulation of Na in heart cell, therefore inhibited the increase of Ca concentration by Na-Ca exchange. Since taurine in cardiac cells was originated physiologically from extracelluar fluid(Wright et al, 1986) and the concentration gradient reached to 630 fold, it could be suggested taurine be actively uptaked into cardiac cells from the outside(Baños et al, 1978).

In our results taurine facilitated the isoprenaline effect to elicit the background current in rabbit ventricular cells. From the results of Earm et al.(1990) taurine activated action potential by increasing inward current or activated outward current. The increase of inward current was not agreed to above results that taurine protected the arrhythmia but it was possible the increase of outward current suppressed the abnormal rhythm and stabilized the heart depolarized by ischemia. Moreover, there was result that isoprenaline could facilitated the transport the taurine into the heart cells(Huxtable & Chubb, 1977). There must be more studies to elucidate the mechanism of taurine action, and taurine transport mechanism across the membrane.

The intracellular signal transduction of isoprenaline

It was generally accepted that isoprenaline could exhibit its action through the increase of intracellular cAMP(Morad et al, 1981; Bean et al, 1984; Fischmeister & Harzell, 1987). And the increase of cAMP was suppressed by cGMP through the activation of type II cAMP phosphodiesterase(Fischmeister & Harzell, 1987). But Ono et al. (1991) reported cGMP could potentiate the increase of the Ca current amplitude by β-adrenergic stimulation. And the background current activated by isoprenaline was also potentiated by cGMP(Ono et al, 1991).

Horie et al. (1991) reported that desensitization phenomena of isoprenaline was prevented by addition of GTP to pipette solution. But in our experiment ,though we added 1 mM GTP into the pipette solution, the response of isoprenaline became much smaller with time and repeated exposure. Egan et al. (1988) reported that the desensitization was related to cAMP and also observed in forskolin which increased cAMP by facilitating adenylate cyclase and by IBMX which increased cAMP through inhibiting phosphodiesterase.

In our laboratory, we found that cGMP increases Ca current and potentiate cAMP effect in rabbit ventricular cell. cGMP does not inhibit the action of isoprenaline on the background and neither cAMP nor cGMP alone could increase the background current. Both cAMP and cGMP together could increase the background current and additional superfusion of isoprenaline could not increase significantly the background current. From the above results, isoprenaline may activate the background current through increasing cAMP and cGMP.

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