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Inhibition by taurine of the inwardly rectifying K^+ current in guinea pig ventricular cardiomyocytes

Hiroyasu Satoh *

Department of Pharmacology, Division of Molecular and Cellular Biology, Nara Medical University, Kashihara, Nara 634-8521, Japan

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Abstract

Effects of taurine on the inwardly rectifying K^+ current (I_{K1}) in isolated guinea pig ventricular cardiomyocytes were examined using patch voltage-clamp methods. All experiments were performed at 36°C. Taurine (10–20 mM) increased the action potential duration, but failed to affect the resting potential. Holding potential was maintained at −30 mV. The current was activated with an inwardly going rectification, and was completely blocked by Ba^{2+} (2 mM). Taurine inhibited I_{K1} at −120 mV by 28.3 ± 1.1% ($n = 6$, $P < 0.05$) at 10 mM and by 36.0 ± 2.1% ($n = 6$, $P < 0.01$) at 20 mM. The reversal potential was shifted in the hyperpolarizing direction by 3.7 ± 0.6 mV ($n = 6$) at 20 mM. In inside-out patch-clamp experiments, the amplitude of unitary channels was −2.7 ± 0.3 pA ($n = 21$) at −90 mV. Symmetrical high-K^+ (150 mM) solutions in both bath and pipette were used. The channel conductance was 32 ± 2 pS ($n = 9$). Taurine did not affect channel conductance, but markedly decreased the open probability at −120 mV of channel by 21.5 ± 2.4% ($n = 8$, $P < 0.01$) at 10 mM, and by 56.7 ± 3.8% ($n = 8$, $P < 0.001$) at 20 mM. These responses were almost reversible. These results suggest that taurine directly modulates the open probability of the inwardly rectifying K^+ current, resulting in regulation of the functions of heart cells. © 1998 Elsevier Science B.V.

Keywords: Taurine; K^+ current, inwardly rectifying; Channel current, unitary; Voltage-clamp, cell-attached patch; Voltage-clamp, whole-cell patch; Cardiomyocyte, guinea pig, ventricular

1. Introduction

Under disease conditions such as ischemia, hypoxia and cardiac failure, there occur cardiac dysfunctions. Simultaneously, the myocardial level of taurine, 2-aminoethanesulfonic acid falls, with the depletion correlated with the degree of mechanical dysfunction (Lombardini, 1980; Kramer et al., 1981). As one of the sulfur-containing amino acids, taurine is largely obtained from the diet, and is abundant in cardiac myocardium (9.3–12.3 μmol/g wet weight in guinea pig). This suggests that taurine may be essential to maintain cardiac functions and may play an important role in cardioprotective functions.

Taurine produces a positive inotropic effect, and modulates the ionic currents across cell membrane (Schaffer et al., 1980; Huxtable and Sebring, 1983; Satoh, 1994b, 1996b). In our laboratory, the following actions of taurine on the ionic currents have been shown: (1) taurine stimulates a tetrodotoxin-insensitive Na^+ current and depresses the fast (tetrodotoxin-sensitive) Na^+ current (I_{Na})(Sperelakis et al., 1989, 1992; Satoh and Sperelakis, 1992; Satoh, 1997), (2) taurine inhibits the L-type Ca^{2+} current (I_{Ca}) and the delayed rectifier K^+ current (I_{Krec}) at pCa 7, whereas taurine enhances both currents at pCa 10 (Satoh and Sperelakis, 1993; Satoh, 1995a,b), and (3) taurine inhibits the ATP-sensitive K^+ current (I_{KATP})(Satoh, 1993), and enhances the transient outward current (I_{to})(Satoh, 1995b, 1996a). Thus, the effects of taurine on cardiac cells are complex, and are dependent on intracellular and extracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$ and $[Ca^{2+}]_o$). These results suggest that taurine possesses cardioprotective actions which are presumably related to the regulation of Ca^{2+} movement in cytoplasm (Ca^{2+} homeostasis).

The configuration of action potential of cardiac cells is regulated by ionic currents, largely such as I_{Na}, I_{Ca} and I_{Krec} currents. The inwardly rectifying K^+ current (I_{K1}) is also one of the modulators, and is considered to be mostly responsible for regulation of the resting potential of car-

* Corresponding author. Tel.: +81-744-29-8831; fax: +81-744-25-7657; e-mail: hysat@naramed-u.ac.jp

diac myocytes. Taurine failed to alter the resting potential in guinea pig papillary muscles (multiple cell preparations)(Satoh, 1994a). However, little is yet known concerning the modulations by taurine of the I_{K1} itself. Therefore, the aim of the present experiments was to examine whether taurine affects the I_{K1} and to find how to modulate the I_{K1} channel of single cardiac cells, using patch-clamp methods.

2. Materials and methods

2.1. Cell preparation

Cells were prepared from tissue taken from the ventricle muscle of guinea pig hearts, using methods similar to those described previously (Satoh and Sperelakis, 1992, 1993; Satoh and Horie, 1997). Under anesthesia with sodium pentobarbital (30 mg/kg, i.p.), the chest was opened and the aorta was cannulated in situ. The heart was dissected out and perfused with normal Tyrode solution on the Langendorff apparatus. After washout of blood, the heart was perfused with Ca^{2+}-free Tyrode solution, and beating ceased spontaneously. Then, the perfusate was switched to low-Ca^{2+} (30 to 60 μM) Tyrode solution containing 0.4 mg/ml collagenase (Type I, Sigma Chemical, St. Louis, MO, USA) for about 20 min. The heart was washed out with high-K^+ and low-Cl^- solution (KB solution), and was dissected out with scissors. The temperature of all solutions was maintained at 36°C.

2.2. Patch-clamp experiments

Whole-cell, current- and cell-attached patch-clamp experiments were performed according to the methods of Hamil et al. (1981). The experiments involved use of an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, USA) and standard techniques. Patch pipettes were fabricated using a two-stage puller. The resistance of the patch electrode was 3–5 MΩ for whole-cell and 2–3 MΩ for inside-out patch-clamp experiments. The tip of the electrode was sometimes coated with Sylport (KE106, Shin-etsu Chemical). The data were stored and analyzed on an IBM-AT microcomputer, using the PCLAMP analysis program (Axon Instruments). Current traces were filtered using a cut-off frequency of 1 kHz for plotting (FV-625, NF, Tokyo). All values are given as means $\pm$ S.E.M. The significance of differences between mean values was analysed by analysis of variance (ANOVA) and the Bonferroni method, and a P value less than 0.05 was considered significant.

2.3. Experimental solutions

The composition of normal Tyrode solution was (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.3, glucose 5.0, and HEPES 5.0. The pH was adjusted to 7.4 with NaOH. In whole-cell clamp experiments, tetrodotoxin (10 μM) and $CoCl_2$ (1 mM) were added to the bath solution. The pipette solution (intracellular medium) contained (mM): K-aspartate 140, $MgCl_2$ 2, Mg-ATP 5, Na_2-creatine phosphate 5, EGTA 10, and HEPES 5 (pH 7.2). The Ca^{2+} concentration was adjusted to pCa 7. Taurine (Sigma Chemical, St. Louis, MO, USA) was dissolved to the desired concentrations directly in the bath solution, and the solution was superfused. In inside-out patch-clamp experiments, the bath solution (intracellular medium) contained (mM): KCl 140, K-gluconate 40, EGTA 5 and HEPES 5 (pH 7.4). The pipette solution (extracellular medium) contained (mM): KCl 140, $CaCl_2$

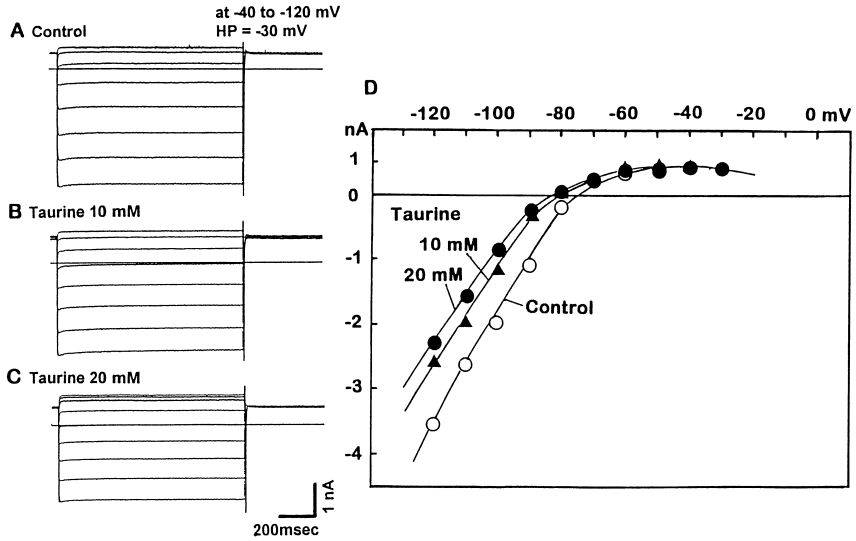

Fig. 1. Inhibition of the inwardly rectifying K^+ current in a ventricular muscle cell of guinea pig. Holding potential was maintained at -30 mV. (A) Current traces in the absence and presence of taurine (10 and 20 mM). Line on the current records represents the zero current level. (B) Current–voltage curves in control and in taurine-containing solutions. Symbols used are: control (open circles), 10 mM (triangles) and 20 mM (filled circles) of taurine.

1.8, $MgCl_2$ 0.5, and HEPES 5 (pH 7.4). Taurine was added in the desired concentrations directly in the pipette solution. All experiments were performed at 36°C.

3. Results

The time-dependent change in steady current of I_{K1}, using a whole-cell patch-voltage clamp mode, was measured during exposure to taurine. Taurine (10 and 20 mM) inhibited the I_{K1} current in a concentration-dependent fashion. Fig. 1 shows the current–voltage relationship. Test pulses were applied from −40 to −120 mV in increments of 10 mV from a holding potential of −30 mV (Fig. 1A). The duration was 1 s. The current–voltage curve showed a markedly inward going rectification. The amplitude at −120 mV was -3.7 ± 2 nA ($n = 6$) in normal Tyrode solution. Addition of Ba^{2+} (2 mM) completely blocked the current. Taurine (10 and 20 mM) decreased the I_{K1} in a concentration-dependent manner. Taurine was added cumulatively to the bath solution. However, taurine had little or no effect on the outward current over a range of potentials between approximately −70 and −30 mV. The current–voltage curves in the absence and presence of taurine are shown in Fig. 1B. The averaged inhibition at −120 mV was $28.3 \pm 1.1\%$ ($n = 6$, $P < 0.05$) at 10 mM, and $36.0 \pm 2.1\%$ ($n = 6$, $P < 0.01$) at 20 mM taurine. Simultaneously, the reversal potential was shifted from -77.6 ± 2.1 mV ($n = 6$) to -79.8 ± 2.1 mV ($n = 6$, $P > 0.05$) at 10 mM and to -81.3 ± 2.3 mV ($n = 6$, $P < 0.05$) at 20 mM of taurine. After 10-min washout, the I_{K1} recovered to 60–70% of the control level.

Taurine prolonged the action potential duration, when a current-clamp method was used, in a concentration-depen-

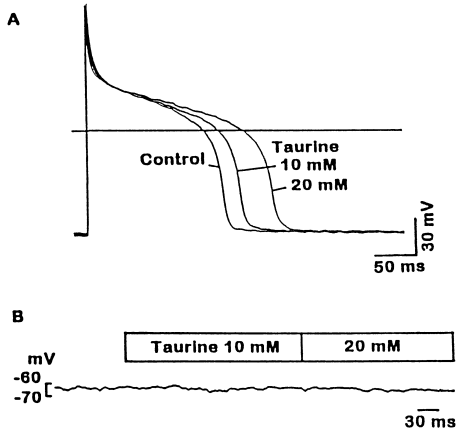

Fig. 2. The effects of taurine on the action potential configuration in a guinea pig ventricular myocyte. A current-clamp mode was used for recordings. (A) Superimposed action potentials in the absence and presence of taurine. Taurine (10–20 mM) was added cumulatively at pCa 7. Horizontal line indicates zero current level. (B) Effect of taurine on the resting membrane potential in a non-stimulated ventricular myocyte. The resting potential was −73 mV in normal Tyrode solution.

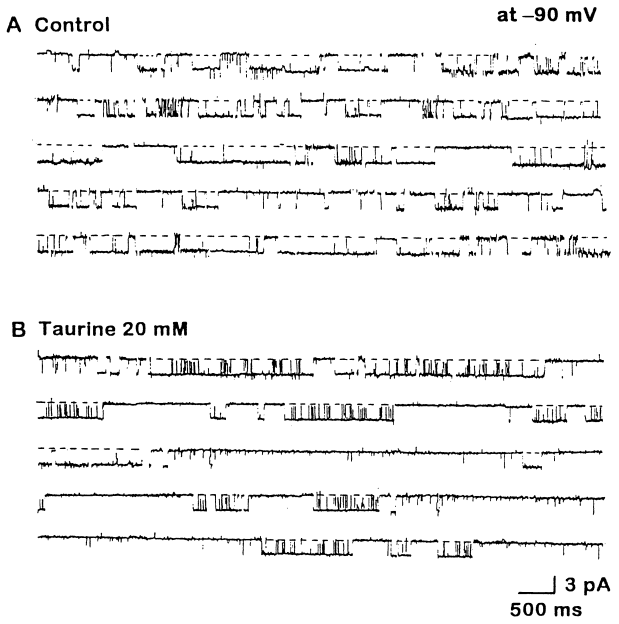

Fig. 3. The action of taurine on the inwardly rectifying K^+ channel opening. Inside-out patch clamp experiment was carried out. Holding potential was maintained at −90 mV. (A) Unitary current in control. (B) Current in 20 mM taurine.

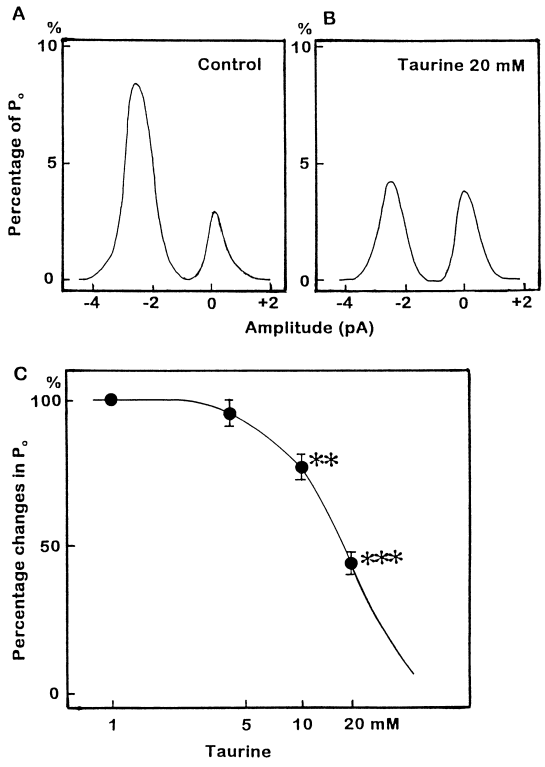

Fig. 4. Modulation by taurine of open probability for the inwardly rectifying K^+ current in guinea pig ventricular myocytes. (A,B) Percentage changes in the open probabilities in control and in 20 mM taurine. (C) Percentage inhibition by taurine of the open probability for the inwardly rectifying K^+ channel. Averaged values at −120 mV ($n = 8$) were obtained from changes in the open probability of channel for 30 s in the presence of taurine (1 to 20 mM). Values represent means ± S.E.M. $**$: $P < 0.01$, $***$: $P < 0.001$, with respect to control value.

dent manner (Fig. 2A). The cellular Ca^{2+} concentration was adjusted at pCa 7. However, the resting potential was unaffected. The resting potential of non-stimulated cells was also not modified in the presence of 10 and 20 mM taurine (Fig. 2B). The resting potential was -73 ± 3 mV ($n = 7$) in normal Tyrode solution, and -74 ± 3 mV ($n = 7$) at 20 mM taurine.

In inside-out patch-clamp experiments, the channel was opened inwardly at -90 mV (Fig. 3A). The amplitude of unitary channels was -2.7 ± 0.3 pA ($n = 21$) at -90 mV. A high-K^+ (150 mM) solution was used in both bath and pipette solutions. Taurine (20 mM) inhibited the opening of unitary channels as shown in Fig. 3B. Conductance was unaffected; 32 ± 2 pS ($n = 9$) in control, and 33 ± 4 pS ($n = 6$) in 20 mM taurine. The open probability (P_o) was $8.7 \pm 1.4\%$ ($n = 8$) at -120 mV in normal Tyrode solution. Taurine at low concentrations (1 and 5 mM) did not affect significantly, but at 10 and 20 mM, it decreased the P_o in a concentration-dependent manner: by $21.5 \pm 2.4\%$ ($n = 8$, $P < 0.01$) at 10 mM and $56.7 \pm 3.8\%$ ($n = 8$, $P < 0.001$) at 20 mM taurine (Fig. 4A–C). The responses were reversible.

4. Discussion

The present experiments were designed to examine the effects of taurine on the inwardly rectifying K^+ current (I_{K1}) in isolated single guinea pig ventricular cardiomyocytes. The results were the following: (a) taurine inhibited the I_{K1} current in a concentration-dependent manner, (b) taurine did not affect the resting potential, (c) taurine decreased the open probability of unitary I_{K1} channels in a concentration-dependent manner, (d) taurine did not affect channel conductance (approximately 33 pS), and (e) the responses to taurine were almost reversible.

We have already reported on the modulations by taurine of several ion currents and channels of cardiomyocytes (Satoh, 1996b). In embryonic and adult cardiomyocytes, taurine stimulates a tetrodotoxin-insensitive Na^+ current, and depresses the I_{Na} (Sperelakis et al., 1989; Satoh and Sperelakis, 1992; Satoh, 1997; Sada et al., 1996). Furthermore, taurine reverses the cellular Ca^{2+}-dependent actions on the ionic currents in embryonic chick cardiomyocytes, guinea pig ventricular muscle and rabbit sino-atrial nodal cells. Taurine inhibits the I_{Ca} and I_{Krec} at pCa 7, whereas it enhances both currents at pCa 10 (Satoh and Sperelakis, 1993; Satoh, 1995a,b).

The I_{K1} current can be measured as an inward current by hyperpolarizing pulses to a potential more negative than -70 to -80 mV in ventricular myocytes, indicating a contribution to maintenance of the resting potential of cardiac cells. The current was highly sensitive to Ba^{2+}. The resting potential is regulated by the ratio of membrane K^+ permeability (P_K) and membrane Na^+ permeability (P_{Na}), according to the Goldmann–Hodgkin–Katz equation (Goldman, 1943; Hodgkin and Katz, 1949). Of the inwardly rectifying K^+ channel family, the I_{K1} plays an important role in the balance of P_K/P_{Na}. Thus, the I_{K1} brings the resting potential progressively close to the K^+ equilibrium potential (E_K), provided that the ion distribution across the cell membrane is at steady state. On the other hand, the P_{Na} at the level of the resting potential may be maintained by a background cation current system in cardiomyocytes (Kiyosue et al., 1993).

Taurine application caused a markedly concentration-dependent decrease in the I_{K1} current. In addition, taurine markedly inhibited the open probability of the channel, although it failed to affect channel conductance. Taurine had similar pharmacological effects on the I_{Ca} channel (Satoh and Horie, 1997). This I_{K1} inhibition might be expected to lead to partial depolarization of the membrane. However, taurine had little or no effect on the resting potential. This is consistent with the results obtained for embryonic (17-day-old) chick cardiomyocytes (Satoh, 1995a,b), and multicellular preparations of guinea pig ventricular muscles (Satoh, 1994a). Thus, it is considered that the resting potential may not necessarily be affected, even if the I_{K1} current decreases, because the lack of change in the resting potential is the result of the resting potential being close to the E_K of cardiac cells, and also, the resting potential may not be dependent on only I_{K1}. Actually, the change in I_{K1} current was not marked at voltages around -80 mV, considered as a resting potential (see Fig. 1B).

Another member of the K^+ channel family, I_{Krec}, also contributes to the AP configuration. This channel largely regulates the action potential duration. Taurine inhibits I_{Krec} (Satoh, 1995a,b), and the action potential duration would thereby be prolonged in the present experiments. The I_{Krec} inhibition would reduce the K^+ efflux from whole heart during ischemia. Since, in this study, the $[Ca^{2+}]_i$ level was buffered at pCa 7, no effect on the Ca^{2+}-activated K^+ current and Na^+/Ca^{2+} exchange current would occur indirectly. The effect of taurine on Na^+/Ca^{2+} exchange current is not still uniform. Taurine has no direct effect on the Na^+/Ca^{2+} exchange current (Katsube and Sperelakis, 1996), but inhibits the Na^+/Ca^{2+} exchange current (Earm et al., 1993). Under diseased conditions, the membrane potential would be depolarized. The altered resting potential may be affected by taurine. The decrease of resting potential would depress the activation of I_{Na} and the T-type Ca^{2+} current (I_{CaT}), resulting in membrane stabilization and the production of cardioprotective actions. Because taurine can decrease the $[Ca^{2+}]_i$ level through a Na^+/Ca^{2+} exchange, it might exert an antiarrhythmic or an antihypertensive action. Therefore, under diseased conditions, the inhibitions of I_{K1} and I_{Krec} may also play a key role as one of the cardioprotective actions of taurine.

Thus, the electrical effects of taurine on the ionic currents of cardiac cells are complex. The effects of high and low $[Ca^{2+}]_i$ on electrophysiological functions are re-

versed by taurine application, indicative of cardioprotective actions of taurine. Therefore, these results suggest that taurine may possess cardioprotective actions associated with its ability to regulate Ca^{2+} movement in cytoplasm, including the inhibition of I_{K1} channels. The mechanisms of many of taurine's actions on heart muscles still remain unclear. Further studies may reveal possible therapeutic uses of taurine.

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