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A dual action of taurine on the delayed rectifier K^+ current in embryonic chick cardiomyocytes

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Summary. Effects of taurine on the delayed rectifier K^+ channel in isolated 10-day-old embryonic chick ventricular cardiomyocytes were examined at different intracellular Ca^{2+} concentrations ($[Ca]_i$), using whole-cell voltage and current clamp techniques. Experiments were performed at room temperature (22°C). Test pulses were applied between −20 to +90 mV from a holding potential of −40 mV. When $[Ca]_i$ was pCa 7, addition of 10 and 20 mM taurine to the bath solution reduced the delayed rectifier K^+ current (I_K) at +90 mV by 17.4 ± 2.8% ($n = 5$, $P < 0.01$) and 25.5 ± 2.6% ($n = 5$, $P < 0.001$), respectively. In contrast, when $[Ca]_i$ was pCa 10, I_K at +90 mV was enhanced by 19.1 ± 3.1% ($n = 7$, $P < 0.01$) at 10 mM taurine, and by 29.3 ± 2.4% ($n = 7$, $P < 0.001$) at 20 mM taurine. The voltage of half-maximum activation ($V_{1/2}$) was shifted in a hyperpolarizing direction; at pCa 7, the value was +0.2 ± 2.2 mV ($n = 5$) in control and −10.6 ± 1.8 mV ($n = 5$) in 20 mM taurine. At pCa 10, the $V_{1/2}$ value was +18.5 ± 4.6 mV ($n = 5$) in control and +6.6 ± 5.2 mV ($n = 5$) in taurine (20 mM). Taurine decreased the action potential duration (APD) at pCa 10, but at pCa 7 did not affect it. In addition, taurine enhanced the transient outward current in a concentration-dependent manner. These results indicate that taurine modulates the delayed rectifier K^+ channel, an effect dependent on $[Ca]_i$ and capable of regulating APD.

Keywords: Amino acids – Taurine – Delayed rectifier K^+ channel – Action potential duration – Transient outward current – Embryonic chick heart cells – Whole-cell voltage and current clamps

Introduction

Taurine (2-aminoethanesulfonic acid), an abundant sulfur-containing amino acid in cardiac myocardium (20 to 38 μmol/g wt tissue in rat), modulates ion fluxes across the cell membrane (Schaffer et al., 1980; Huxtable and Sebring, 1983). Many mechanical and electrophysiological actions of taurine have already been described (Franconi et al., 1982; Schaffer et al., 1987; Sawamura

et al., 1990). Satoh and Sperelakis (1992, 1993) have recently reported that in embryonic chick cardiomyocytes taurine inhibits the TTX-sensitive Na^+ current (I_{Na}) and at pCa 7 the L-type Ca^{2+} current ($I_{Ca(L)}$). It also enhances $I_{Ca(L)}$ at pCa 10. This is consistent with the view that taurine modulates the actions of some pharmacological agents, the effect dependent on extracellular Ca^{2+} concentration ($[Ca]_o$) (Schaffer et al., 1980; Franconi et al., 1982; Sawamura et al., 1990). The results also indicate that taurine possesses cardioprotective activity related to its ability to alter Ca^{2+} movement (Huxtable and Sebring, 1983; Satoh and Sperelakis, 1993). Thus, taurine exerts "Ca^{2+} normalizing action" (or Ca^{2+} homeostasis).

Outward K^+ currents play an important role in the regulation of repolarization of the action potential in excitable membranes. Blockade of K^+ channels prolongs the action potential duration. The delayed rectifier K^+ current (I_K) is regulated by $[Ca]_o$ or $[Ca]_i$ (Meech, 1974; Isenberg, 1975). In the present experiments, therefore, the modulation by taurine of I_K and the action potential duration in cardiac cells at two different $[Ca]_i$ levels (pCa 7 and 10) was examined. A preliminary report of this work was presented at a recent symposium (Sperelakis et al., 1992).

Materials and methods

Cell culture preparation

Cell cultures were prepared from tissue taken from the ventricle muscle of 10-day-old embryonic chick hearts, using methods similar to those described previously (Satoh and Sperelakis, 1991, 1992). Twelve dozen fertilized White Leghorn chick embryos were incubated for 3 days at 37.5°C and staged to confirm their degree of development. Hearts were sterilely removed and collected in a balanced salt solution (4°C). Tissue dissociation was accomplished by gentle rotation of the tissues in a Mg^{2+}-free and Ca^{2+}-free Ringer solution containing 0.1–0.4% trypsin (Sigma Chemical, St. Louis, MO, USA). The cell suspensions were harvested at 5 min intervals, pooled, and pelleted by centrifugation (85 ×g). The cells were washed three times in tissue culture medium (M199, GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum. The cells were placed onto glass coverslips placed in 35 mm plastic petri dishes (Falcon, Becton Dickinson, NJ, USA) at a concentration of 10^5–10^6 cells/ml. The myocyte cultures were incubated at 37°C in a humidified atmosphere (5% CO_2 and 95% air) until used for experimentation.

Whole-cell voltage-clamp and current-clamp experiments

Whole-cell voltage-clamp and current-clamp recordings were made using a Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, USA) and standard techniques. Patch pipettes were fabricated using a two-stage puller, and had a resistance of 3–5M . The series resistance error was less than 3–7 mV, and no compensation was used. Experiments were carried out at room temperature (22°C). 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. All values are given as mean ± S.E.M. The differences of the mean values were analyzed by the Student's *t*-test for paired data, and a P value less than 0.05 was considered significant.

Experimental solutions

Glass coverslips containing the cells were placed in a bath-chamber and were superfused with a modified Tyrode solution. The composition of the modified 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. To avoid the interference of other currents, 10 μM tetrodotoxin (TTX), 1 μM nifedipine, and 15 mM 4-aminopyridine (4-AP) were added to the external Tyrode solution to block the fast Na^+ current (TTX), the Ca^{2+} current (nifedipine), and the transient outward current (I_{TO}) (4-AP). When measuring I_{TO}, 4-AP was not added. Taurine (Sigma Chemical Co., St. Louis, MO, USA) was dissolved to the desired concentrations directly in the bath solution, and the solution was superfused. The pipette solution (intracellular) contained (in mM): K-aspartate 110, KCl 20, $MgCl_2$ 2, EGTA 10, Mg-ATP 5, creatine phosphate 5, and HEPES 5 (pH 7.2). The concentration of calcium in the pipette solution was determined according to the calculation of Fabiato and Fabiato (1979) and the correction of Tsien and Rink (1980).

Results

Taurine action on I_K

The I_K current was measured by applying test pulses (2 sec duration) between −20 to +90 mV from a holding potential of −40 mV. The current density of the I_K current at +90 mV in 10-day-old embryonic chick ventricular cells was increased from 15.0 ± 0.8 pA/pF to 24.7 ± 0.9 pA/pF by increasing $[Ca]_i$ from pCa 10 to pCa 7 in the pipette (Figs. 1A and 2A). The average capacitance was 10.9 ± 2.1 pF (n = 14). At pCa 7, cumulative administrations of taurine (10

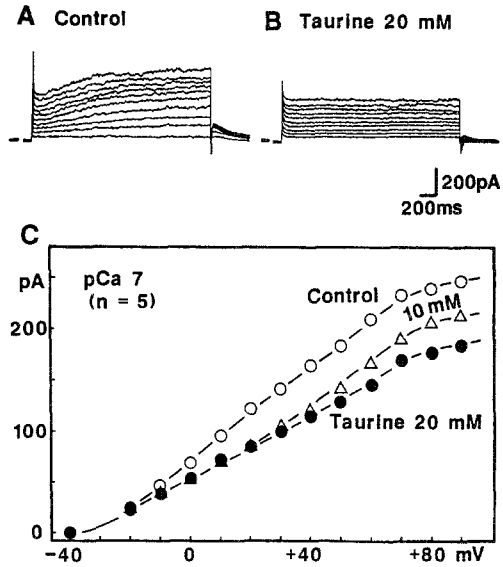

Fig. 1. Inhibition of I_K induced by taurine at pCa 7. **A–B** Current traces in a 10-day-old cell in the absence and presence of 20 mM taurine. A test pulse was applied between −20 mV to +90 mV from a holding potential of −40 mV. The short line at the left of the current records represents the zero current level. **C** Current-voltage relationship for I_K. Symbols used are: control (open circles), 10 mM taurine (triangles) and 20 mM taurine (filled circles). The values represent means ± S.E.M. (n = 5)

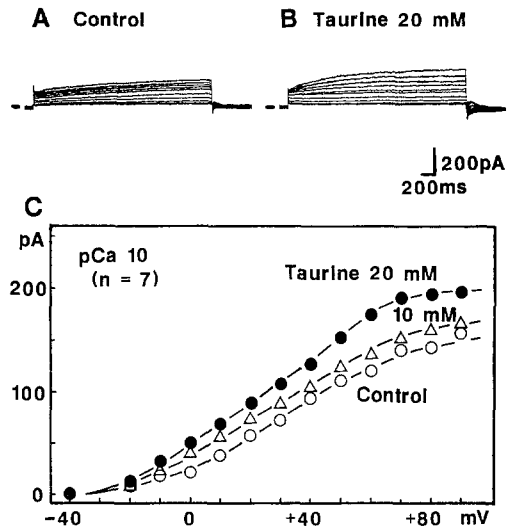

Fig. 2. Enhancement of I_K induced by taurine at pCa 10. **A–B** Current traces in a 10-day-old cell in the absence and presence of 20 mM taurine. A test pulse was applied between −20 mV to +90 mV from a holding potential of −40 mV. The short line at the left of the current records represents the zero current level. **C** Current-voltage relationship for $I_{Ca(L)}$. Symbols used are: control (open circles), 10 mM taurine (triangles) and 20 mM taurine (filled circles). The values represent means ± S.E.M. (n = 7)

and 20 mM) to the bath solution reduced I_K by 35.2% at 10 mM taurine, and by 67.6% at 20 mM (Fig. 1A and B). Current-voltage (I-V) relations in the absence and presence of taurine (10 and 20 mM) are given in Fig. 1C. The average decrease at +90 mV was by 17.4 ± 2.8% ($n = 5$, $P < 0.01$) at 10 mM, and by 25.5 ± 2.6% ($n = 5$, $P < 0.001$) at 20 mM.

On the other hand, the I_K at pCa 10 was enhanced by application of taurine in a concentration-dependent manner (Fig. 2A and B). The I_K amplitude and its tail current were much smaller compared with those at pCa 7. Figure 2C shows the I-V relationship for I_K from 7 cells. The I_K value at +90 mV on the I-V curve was enhanced by 19.1 ± 3.1% ($P < 0.01$) at 10 mM taurine, and by 29.3 ± 2.4% ($P < 0.001$) at 20 mM. The responses to taurine were not reversed by a 30-min washout. Low concentrations (1 to 5 mM) of taurine had little or no effect on I_K.

Effects on activation curve for I_K

To examine the degree of activation of the I_K current, the amplitude of the outward tail current of I_K was plotted along the voltage axis. At pCa 10, 10 and 20 mM taurine enhanced the tail current at +80 mV by 8.6 ± 2.4% ($n = 5$, $P < 0.01$) and 33.3 ± 1.8% ($n = 5$, $P < 0.01$), respectively (Fig. 3A). The curves in Fig. 3B were fitted by an empirical equation; $p = 1/\{1 + \exp[(V_m - V_{1/2})/S]\}$. $V_{1/2}$ is the potential of half-activation, and S is the slope factor. The slope factor at pCa 10 was 9.8 ± 2.1 ($n = 5$) in the control and 9.9 ± 1.3 ($n = 5$) in the taurine (10 and 20 mM) treated group. The $V_{1/2}$ value in 5 cells was

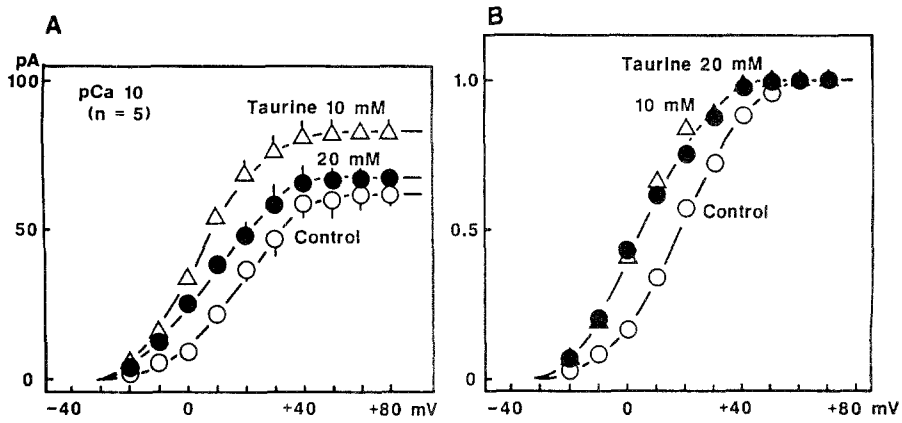

Fig. 3. Effects of taurine on the activation process for I_K at pCa 10 in a 10-day-old embryonic chick heart cell. A test pulse was applied between −30 mV to +80 mV from a holding potential of −40 mV. **A** Outward tail current in control and in 10 to 20 mM taurine. The values represent means ± S.E.M. (n = 5). **B** Normalized activation curves for I_K. The values represent only the means

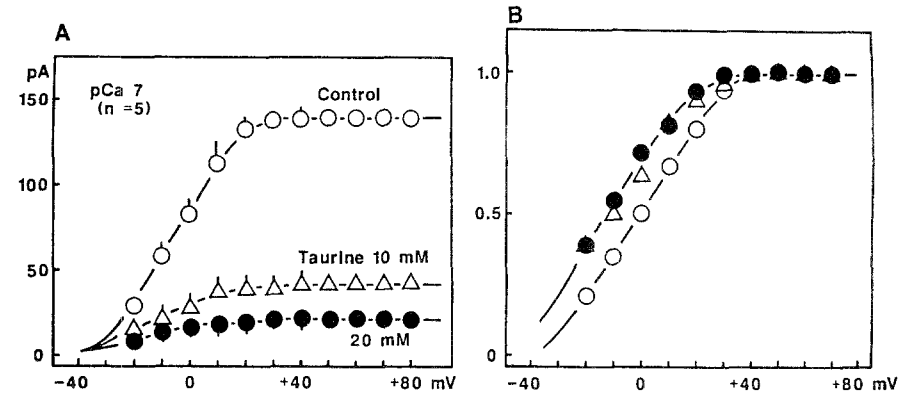

Fig. 4. Effects of taurine on the activation curve for I_K at pCa 7 in 10-day-old embryonic chick heart cells. **A** Outward tail currents in control and 10 to 20 mM taurine. The values represent means ± S.E.M. (n =5). **B** Normalized curves of the activation of I_K. The values represent only the means

+18.5 ± 4.6 mV (n = 5) in control and +6.6 ± 5.2 mV (n = 5) at 20 mM taurine; the difference between the values (by 11.9 mV) was significant ($P < 0.05$).

At pCa 7, the tail current at +80 mV was inhibited by 67.4 ± 3.9% (n = 5, $P < 0.001$) at 10 mM taurine and by 81.1 ± 6.9% (n = 5, $P < 0.001$) at 20 mM taurine (Fig. 4A). Increasing $[Ca]_i$ (from pCa 10 to 7) shifted the normalized curve to a more negative potential in normal Tyrode solution; by 16.5 mV at $V_{1/2}$ from the curves in Figs. 3B and 4B. The slope factors were 10.2 ± 0.3 (n = 5) in the control, and 10.8 ± 0.4 in the presence of taurine (10 and 20 mM). The $V_{1/2}$ value was +0.2 ± 2.2 mV (n = 5) in the control, and −10.6 ± 1.8 mV (n = 5) in the presence of 10 and 20 mM taurine; the difference was significant ($P < 0.05$).

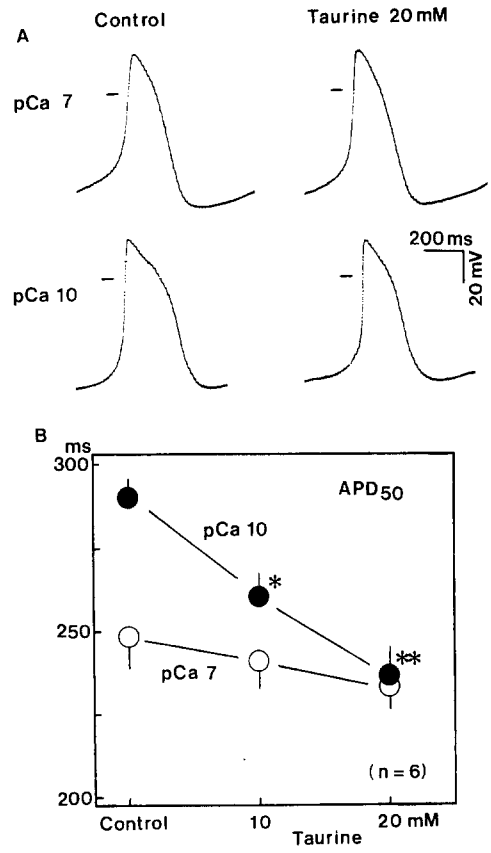

Fig. 5. Effects of taurine on the duration of spontaneous action potential from a 10-day-old embryonic chick ventricular cell. **A** Action potentials at pCa 7 and 10, and before and after 20 mM taurine application. **B** Summarized taurine effects on the action potential duration at 50% repolarization (APD_{50}). Taurine (10 and 20 mM) was added to the bath solution. Values represent means ± S.E.M. *: $P < 0.05$, **: $P < 0.01$, with respect to control value

Effects on the action potential duration

To examine the effect of taurine on APD, current-clamp experiments were performed in 10-day-old embryonic chick ventricular myocytes. The single cells were spontaneously beating. As shown in Fig. 5A, taurine (20 mM) markedly shortened the action potential duration (APD) at pCa 10, but did not affect it at pCa 7. The 50% repolarization of APD (APD_{50}) at pCa 7 tended to shorten in the presence of 10 and 20 mM taurine (by 3.3 ± 2.8% and 6.5 ± 2.2%, $n = 6$, $P > 0.05$) (Fig. 5B). At pCa 10, taurine shortened APD_{50} by 8.2 ± 2.5% ($P < 0.05$) at 10 mM and by 16.0 ± 2.0% ($P < 0.01$) at 20 mM. The resting potential tended to depolarize, although the effect was not significant.

Effects on the transient outward current

Effects of taurine on the transient outward current (I_{TO}) in 10-day-old cells were also examined. Test pulses were applied between −20 and +70 mV from

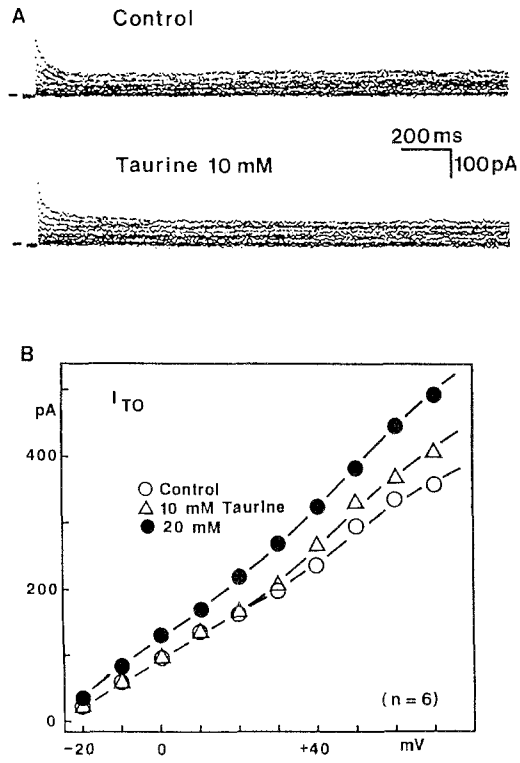

Fig. 6. Enhancement of I_{TO} by application of taurine in a 10-day-old cell. **A** Current traces in control and taurine 10 mM. A test pulse was applied to −40 to +70 mV from a holding potential of −70 mV. The short line at the left of the current records represents the zero current level. **B** Current-voltage curves. Symbols used are; control (open circles), 10 mM taurine (triangles), and 20 mM taurine (filled circles). Values represent means ± S.E.M.; the S.E.M. bars are less than the thickness of the symbols

a holding potential of −70 mV. The I_{TO} current was not always observed in all the prepared cells. The experiments were performed only at pCa 10. The average amplitude of I_{TO} current at +70 mV was 327 ± 5.1 pA (n = 6). The I_{TO} was inhibited by 35.2 ± 2.3% (n = 3, $P < 0.01$) by 2 mM 4-AP, and was abolished almost completely by 5 mM 4-AP. At +70 mV, I_{TO} was enhanced by 15.1 ± 1.3% (n = 6, $P < 0.05$) and 37.2 ± 2.1% (n = 5, $P < 0.001$) by 10 and 20 mM taurine, respectively (Fig. 6A and B).

Discussion

Taurine, an amino acid largely obtained from the diet, exerts many important physiological functions. Its electrical and mechanical actions are independent of many known regulators, such as cAMP and cGMP levels, (Na,K)-ATPase activity and calmodulin-dependent protein kinase action, although taurine may inhibit protein kinase C activity (Segawa et al., 1985; Lombardini, 1992). Many reports on the cardiovascular actions of taurine have already been described (Schaffer et al., 1980, 1987; Huxtable and Sebring, 1983; Franconi et al., 1982; Sawamura et al., 1990; Satoh and Sperelakis, 1992, 1993; Sperelakis et al., 1992). The experiments were designed to examine the effects of taurine

on the delayed rectifier K^+ current at low and high $[Ca]_i$ in single isolated 10-day-old embryonic chick heart cells. The present experiments showed the following: (a) Taurine stimulated I_K at low $[Ca]_i$, but depressed I_K at high $[Ca]_i$. These results are similar to the effects of taurine on $I_{Ca(L)}$, which are also dependent on $[Ca]_i$ and $[Ca]_o$ (Sawamura et al., 1990; Satoh and Sperelakis, 1993). (b) The voltage of half-maximum activation of I_K was shifted in the hyperpolarizing direction by taurine (by ca. 10 mV) at both pCa 10 and pCa 7. (c) The APD_{50} was decreased by taurine at pCa 10, but not at pCa 7. (d) Taurine enhanced the I_{TO} current in a concentration-dependent manner. The taurine-induced effects were irreversible even after a 30-min washout. The basis for this is unclear, although some possibilities exist: (a) recovery from taurine may occur slowly and may not be observed by 30 min, (b) the recovery may be masked by time-dependent damage to the myocytes.

During ischemia and hypoxia, taurine levels in the heart decrease, with the depletion correlated with the degree of mechanical dysfunction (Lombardini, 1980, 1981; Kramer et al., 1981). This suggests that taurine may be essential to maintain cardiac function and thus plays an important role in cardioprotection.

I_K at different pCa

In the present experiments, two $[Ca]_i$ were used. The amplitude of I_K current was greater at pCa 7 than at pCa 10, and its activation curve was shifted at $V_{1/2}$ by 16.5 mV to the negative potential (see Figs. 3B and 4B). The potentiation of I_K amplitude is consistent with previous reports (Meech, 1974; Isenberg, 1975). It has also been reported that a negative shift of $V_{1/2}$ is caused by Ca^{2+} ionophores in sino-atrial node cells (Satoh et al., 1989). Thus, $[Ca]_i$ elevation itself produces an enhancement in I_K amplitude and a shift to a more negative potential in embryonic chick heart cells.

Taurine inhibited I_K at pCa 7, but enhanced I_K at pCa 10. Nevertheless, the negative shift of the activation curve for I_K occurred to almost the same extent at both pCa 7 and 10. Since in the present experiments, $[Ca]_i$ was fixed at pCa 7 or 10 (EGTA 10 mM in the pipette), the shift and the dual action on I_K induced by taurine are independent of $[Ca]_i$. Although the mechanism is still unknown, taurine might alter the density of charge on the inner surface of the membrane, thereby causing a negative shift of I_K in a voltage-dependent manner. Similarly, taurine shifted the reversal potential for the fast Na^+ current (I_{Na}) in 17-day-old embryonic chick cardiomyocytes (Satoh and Sperelakis, 1992). Amino acids (such as taurine) and Na^+ can cotransport across the membrane (Suleiman et al., 1992; Benyajati and Bay, 1992). Also, Ca^{2+} may alter the permeation properties by interacting with the external mouth of the channel.

Action potential duration

At the plateau, the inward and outward currents are practically in balance (McAllister et al., 1975), and a small change in one of the currents will greatly

affect the course of the potential. From a theoretical point of view, APD is strongly regulated by the I_K current. Also, the time-dependent activated K^+ current should contribute to the repolarization of the action potential. Taurine stimulated I_K at pCa 10, whereas it depressed I_K at pCa 7. Since the I_K current in cardiac cells is dependent on $[Ca]_i$ and $[Ca]_o$ (Meech, 1974; Isenberg, 1975), the Ca^{2+}-activated I_K might be stimulated through the enhancement of I_{Ca} even when $[Ca]_i$ is low. It has been reported that taurine regulates $[Ca]_i$ level (Failli et al., 1992). In the present experiments, however, the $[Ca]_i$ level was fixed, according to the equations (Fabiato and Fabiato, 1979; Tsien and Rink, 1980). Therefore, it is unlikely that the dual action may be due to the modulation of Ca^{2+}-activated K^+ current by taurine.

Taurine decreased the APD at pCa 10, but did not affect it at pCa 7. These results agree with the taurine effects on I_K; namely, I_K was inhibited at high pCa and enhanced at low pCa. One would have predicted that inhibition should prolong APD, whereas enhancement should shorten APD. At pCa 7, however, taurine tended to shorten APD (but not significantly). In guinea pig ventricular muscle, taurine at high $[Ca]_o$ decreased APD and at low $[Ca]_o$ increased it (Sawamura et al., 1990; Satoh, 1994). This apparent discrepancy is still unclear, but some possibilities exist; (a) it may relate to differences between embryonic and adult heart muscle cells, and/or (b) differences between single cellular and multicellular preparations (Satoh, 1993a). Since the change in $[Ca]_o$ would simultaneously alter $[Ca]_i$ in multicellular preparations, the other factors regulating APD might be present. In this study, $[Ca]_i$ level was either pCa 10 or 7 (EGTA 10 mM in the pipette). Thus, the effect of taurine on APD is considered to be a direct action. The failure to observe an effect of taurine on APD at pCa 7 indicates that APD is not modulated solely by a change in I_K; the effect might be induced by other ionic currents (including other K^+ channels).

The prolongation or shortening of APD may be regulated by the magnitude and inactivation of the fast Na^+ current (I_{Na}) (Carmeliet and Saikawa, 1982) and of $I_{Ca(L)}$. The inactivation of $I_{Ca(L)}$ is composed of two (fast and slow) exponentials (Kass and Sanguinetti, 1984; Lee et al., 1985; Satin and Cook, 1989). The fast component is $[Ca]_i$-dependent, and the slow component is voltage-dependent (Eckert and Chad, 1984; Carbone and Swandulla, 1989). In embryonic chick ventricular myocytes, taurine increases the slow time constant for I_{Ca} inactivation at both pCa 7 and 10, but the fast time constant is unaffected, which is indicative of no change in $[Ca]_i$ level (Satoh and Sperelakis, 1993). Thus, the increase in the time constant for inactivation results in APD prolongation independent of $[Ca]_i$ level. On the other hand, the time constant for I_{Na} inactivation (which is a single component in embryonic chick heart) is not modified by taurine application (Satoh and Sperelakis, 1992). These results suggest that taurine-induced APD modulation is mainly due to its actions on I_K, and in part by the slow inactivation of I_{Ca}, although APD may also in part be regulated by I_{TO}. Further experiments are required to elucidate this mechanism.

Cardioprotective action

Our recent experiments showed that taurine possesses potent cardioprotective action in embryonic chick cardiomyocytes and guinea pig ventricular myocytes (Sawamura et al., 1986; 1990; Satoh and Sperelakis, 1992). Taurine enhanced I_{Ca} at low pCa, but inhibited I_{Ca} at high pCa. Sperelakis and colleagues (Sperelakis and Satoh, 1993; Sperelakis et al., 1988) concluded that one of taurine's actions is to normalize Ca^{2+} movement through the sarcolemma, dampening the effects of variation in perfusate Ca^{2+} concentration. These results indicate that taurine acts on $I_{Ca(L)}$ in a manner to keep $[Ca]_i$ levels relatively constant, and consequently plays an important role in maintaining cell viability and cardiac function.

The findings that taurine inhibits the fast Na^+ (TTX-sensitive) current (Satoh and Sperelakis, 1992) may be one way to protect heart muscles against arrhythmias and decrease $[Ca]_i$ via Na-Ca exchange. The decline in $[Ca]_i$ induced by taurine should lead to a reduction in cellular calcium overload during ischemia. Taurine also promotes the sudden emergence of a dormant $I_{Ca(T)}$ (Satoh and Sperelakis, 1993). Since $I_{Ca(T)}$ contributes to the generation of pacemaker activity during diastole (Hagiwara et al., 1988), and automaticity is depressed under calcium overload (Satoh and Hashimoto, 1988; Satoh et al., 1989), the stimulation of $I_{Ca(T)}$ may also be one of the protective actions of taurine. In the present experiments, high pCa itself shortened APD, whereas low pCa itself prolonged APD. Taurine decreased prolonged APD at pCa 10. Extreme prolongation of APD may elicit arrhythmias (Satoh, 1993b). These results indicate that the effects of high and low $[Ca]_i$ on APD are antagonized by taurine application, indicative of a cardioprotective action of taurine. Although many of taurine's actions on heart still remain unclear, further studies should reveal possible therapeutic uses of taurine.

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