Action Potential Duration-Stabilizing Action of Taurine in Guinea Pig Ventricular Myocytes

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ABSTRACT—To examine taurine actions on the rate of repolarization of action potentials (AP), L-type Ca²⁺ (I₈Ca) and the inward rectifier currents as affected by the external Ca²⁺ concentrations ([Ca²⁺]₀), whole-cell voltage-clamp and current-clamp experiments were conducted in guinea pig ventricular myocytes. At a high (3.6 mM) [Ca²⁺]₀, 10 mM taurine suppressed both I₈Ca and I₈K, shortened AP duration and decelerated the rate (-dV/dt) of terminal repolarization of AP. In contrast, at a low (0.9 mM) [Ca²⁺]₀, taurine intensified both I₈Ca and I₈K, lengthened AP duration and accelerated -dV/dt. However, at either [Ca²⁺]₀, the resting membrane potential was slightly hyperpolarized, and the inward rectifier current examined by the ramp-pulse protocol remained unaffected by taurine. Taurine is suggested to maintain a stable AP duration by altering the inward Ca²⁺ and IK in the opposite directions, depending on [Ca²⁺]₀. The relevance of the stabilizing action of taurine on the AP duration to its reported antiarrhythmic efficacies is discussed.

Keywords: Taurine, Action potential, Ca²⁺ channel, K⁺ channel, Antiarrhythmic action

Taurine, 2-aminoethanesulfonic acid (H₂NCH₂CH₂SO₃H), is the most abundant amino acid of the amino acid pool of the heart (50%). Although its plasma level is as low as 0.05 to 0.22 mM, the myocardial level is hundreds of times higher than the plasma level: 5.6 (humans)–28 (rats) mM/kg wet weight (1).

During the last decade, much evidence for the cardiac effects of taurine was found (2). Sawamura et al. (3) observed its Ca²⁺ channel current (I₈Ca)-modulating action that was influenced by the external Ca²⁺ concentration ([Ca²⁺]₀) in guinea pig ventricular myocytes: enhanced I₈Ca at a low [Ca²⁺]₀ and depressed I₈Ca at a high [Ca²⁺]₀. Likewise, Sato and Sperelakis (4) reported that taurine actions on the L-type I₈Ca was also modified by the intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ): I₈Ca-stimulatory action at a low [Ca²⁺]ᵢ (pCa = 10) and inhibitory action at a high [Ca²⁺]ᵢ (pCa = 7). Although it long has been known that taurine possessed either negative or positive inotropic actions depending on [Ca²⁺]ᵢ (5), the above two voltage-clamp data, in turn, apparently lead to the conclusion that taurine functions to normalize the intracellular free Ca²⁺ content.

In 1963, Read and Welty (6) first described that taurine, intracellularly given, could prevent the development of epinephrine-induced premature contractions and ceased the arrhythmias of acute or chronic digitalis toxicity. Later, Chazov et al. (7) (1974) in guinea pig and dog hearts treated with toxic doses of strophanthidin-K, Franchini et al. (8) (1985) in Langendorff-perfused guinea pig hearts exposed to the hypoxia-reoxygenation sequence, and Takahashi et al. (9) (1988) in cultured embryonic mouse heart cells exposed to high and low [Ca²⁺]₀ also confirmed the antiarrhythmic activities of taurine in concentrations of 1–20 mM.

The action potential (AP) duration is a major determinant for the electrical refractory period of the cardiac muscle, and the AP duration is ionically controlled by mainly the inward Ca²⁺ and the delayed rectifier currents. Also, the inward rectifier current plays a key role in maintaining a stable resting potential (10). The present study was designed to test how taurine altered AP parameters, whether the taurine-induced alterations in AP parameters could provide an antiarrhythmic effect and how the AP alterations are related to the changes in the underlying ionic currents. For this purpose, we examined taurine-induced changes in ionic currents in the same ionic environment as in AP recordings for a low and high [Ca²⁺]₀ without using any channel-specific blockers.
We report that taurine physiologically functions not only to normalize the Ca\(^{2+}\) supply to contractile apparatuses, but also to maintain a stable AP duration and the resting membrane potential, thereby preventing the occurrence of arrhythmias.

**MATERIALS AND METHODS**

**Preparation and electrode**

The heart was dissected out from guinea pigs (Sciwa, Fukuoka) that were under deep anesthesia from urethane (600 mg/kg). The heart was quickly mounted in a Langendorff apparatus (1-m-high) for the coronary perfusion. The method of obtaining single ventricular myocytes using both collagenase and pronase was essentially the same as described by others (11). The animal experiments were performed under the control of the Guideline for Animal Experiment in Yamaguchi University and The Law (No. 105) and Notification (No. 6) of the Government.

Glass pipette electrodes were fabricated by a two-step pulling of Pyrex glass capillary tubes and coated with Sylgard. To minimize the time-related run-down of any currents and the spontaneous hyperpolarizing shift in the voltage-dependent channel kinetics and to maintain the access resistance at a stable value throughout the experiments, the nystatin method (12) was used (100 \(\mu\)g/ml). Voltage values reported herein were all corrected for the liquid junction potential of 15 mV.

**Solution**

To record and compare AP, L-type I\(_{\text{Ca}}\) and the late outward K\(^+\) current (I\(_{\text{K}}\)) simultaneously in the same ionic environment, no specific channel blocker was included in the bath or internal (pipette) solutions. The bath solution had the following composition: 140 mM NaCl, 5.4 mM KCl, 1.0 mM MgCl\(_2\), 10 mM glucose, 10 mM sucrose and 10 mM HEPES; pH 7.3. The Ca\(^{2+}\) concentration was either 0.9 or 3.6 mM. The bath was continuously superfused at a rate of 3 ml/min, and temperature was controlled at 25°C by a Peltier-effect unit. The change in osmolality of the bath solution after taurine addition (10 mM) was corrected by removal of equimolar sucrose, which is electrophysiologically inactive (13). The composition of the pipette solution for voltage-clamp (v-c) and current-clamp (c-c) was: 130 mM K-aspartate, 11 mM EGTA, 1.0 mM CaCl\(_2\), 10.0 mM HEPES, 2.0 mM MgCl\(_2\), 5 mM ATP-Na\(_2\) and 5 mM glucose; pH 7.0.

**Passive membrane properties**

The membrane capacitance (\(C_m\)) was determined either from time integration (\(\int \Delta t\)) of the capacitive spike for 10-mV step pulses or from the current in response to ramp pulses (dV/dt=0.5 V/sec). The \(C_m\) values of myocytes were 50–200 pF, and data from myocytes with \(C_m\) of ca. 100 pF were presented in this paper. The falling phase of the capacitive transients upon 10-mV step pulses was approximated by a single exponential function. Using the time constants (\(\tau\)) of the exponential fits, the series resistance (\(R_s\)) values were determined simply by \(\tau = R_s \times C_m\). The \(R_s\) thus determined before its compensation was 60–70%. The membrane capacitance was compensated with analog circuits in an amplifier (List EPC-7; Darmstadt, Germany). The uncompensated fraction of the capacitive transient was digitally corrected by means of the conventional \(-P/4\) method.

**Experimental procedures and data analyses**

Experiments were started 10–15 min after the tight-seal was established. In both v-c and c-c experiments, the clamp pulse was delivered at 1/4 sec. Current and voltage signals were sampled at either 10 (v-c) or 100 (c-c) kHz. The holding potential (\(V_h\)) in v-c experiments were -40 mV. The magnitude of I\(_{\text{Ca}}\) herein was defined as the difference in current from the current level at the end of the 300–500-msec pulse, whereas that of I\(_{\text{K}}\) was the absolute level of current measured at the end of the 300–500-msec pulse. To examine the linear (chord) conductance of I\(_{\text{K}}\), the amplitudes of the currents between -10–0 mV and +30–+50 mV were fitted to a linear function (using the non-linear least-squares method (simplex or Marquardt)). To obtain the slope conductance of I\(_{\text{K}}\), the magnitudes of the currents as a function of voltage were approximated by an exponential function: \(I(\text{mA}) = \text{EXP}(A \times (V_m + B))\). The slope conductance at each voltage (\(V_m\)) was thus given by the differentiation of the above equation: \(A \times \text{EXP}(A \times (V_m + B))\). The inward rectifier current was examined by the ramp-pulse protocol, the slope of which was 0.012 V/sec.

Statistical tests were made by the paired t-test, unless indicated otherwise.

**RESULTS**

**L-type Ca\(^{2+}\) current (I\(_{\text{Ca}}\))**

The L-type I\(_{\text{Ca}}\) was recorded before and after 10 mM taurine addition at a holding potential of -40 mV. In the control period, I\(_{\text{Ca}}\) began to flow at \(-22.8 \pm 1.7\) mV (at 3.6 mM \([\text{Ca}^{2+}]_o\), \(N=10\)) or \(-25.1 \pm 0.9\) mV (at 0.9 mM \([\text{Ca}^{2+}]_o\), \(N=10\)). At 3.6 mM \([\text{Ca}^{2+}]_o\), the I\(_{\text{Ca}}\) reached its maximum of \(-14.1 \pm 0.9\) pA/pF at \(+7.2 \pm 2.3\) mV. At 0.9 mM \([\text{Ca}^{2+}]_o\), the I\(_{\text{Ca}}\) reached its maximum of \(-3.5 \pm 1.7\) pA/pF at \(-1.2 \pm 1.1\) mV. Hence, at the high \([\text{Ca}^{2+}]_o\), the maximum I\(_{\text{Ca}}\) was significantly (\(P<0.01\), un-
paired) larger and the voltage for the maximal $I_{ca}$ was significantly ($P<0.01$, unpaired) less negative, as compared with those at the low $[Ca^{2+}]_o$.

Taurine modified $I_{ca}$, depending on $[Ca^{2+}]_o$. When $[Ca^{2+}]_o$ was high, exposure of myocytes to 10 mM taurine was followed by 5–40% reductions of the peak amplitude of $I_{ca}$. Figure 1 shows a representative experiment. Such taurine-induced depression of $I_{ca}$ at a high $[Ca^{2+}]_o$ was in line with a report of Sawamura et al. (3). The depressant action of taurine was essentially reversible.

In contrast, as $[Ca^{2+}]_o$ was lowered, taurine at the same concentration tended to intensify $I_{ca}$ slightly. Figure 2 shows a typical instance for very minor stimulatory actions of taurine at $[Ca^{2+}]_o$ of 0.9 mM. The weak $I_{ca}$-stimulatory action at low $[Ca^{2+}]_o$ was also consistent with an earlier report (3). Concomitantly with changes in $I_{ca}$ magnitude after taurine-exposure, the voltage at which the maximal $I_{ca}$ was observed was displaced by a few mV to the negative potential direction, as in a study of the taurine action on Na$^+$ currents reported by others (14). At $[Ca^{2+}]_o$ of 1.8 mM, taurine actions on $I_{ca}$ were less obvious (data not shown).

In summary, the values of the maximal $I_{ca}$ measured at 5–15 min after exposure to taurine relative to the control (in %) were 85.3±2.9 ($P<0.01$) at the high and 104.8±2.1 ($P<0.05$) at the low $[Ca^{2+}]_o$ (means ±S.E.M.).

**Action potential (AP) parameters and rate of repolarization**

Taurine-induced $I_{ca}$ reductions at the high $[Ca^{2+}]_o$ and $I_{ca}$-augmentations at the low $[Ca^{2+}]_o$ were reflected in the changes in AP parameters. Figure 3 illustrates representa-
At the high \([Ca^{2+}]_o\), addition of 10 mM taurine was followed by reversible decreases in the overshoot and duration of APs. Concomitantly, the resting membrane potential (RMP) was also often slightly hyperpolarized by a few mV. In contrast, at the low \([Ca^{2+}]_o\), 10 mM taurine lengthened the AP duration. However, RMP was likewise slightly hyperpolarized, as it was at the high \([Ca^{2+}]_o\). On average, the change in overshoot following taurine-exposure was \(-3.7\pm0.6\) mV (\(P<0.01\)) at the high \([Ca^{2+}]_o\) (\(n=7\)) and \(+0.5\pm1.2\) mV (not significant) at the low \([Ca^{2+}]_o\) (\(n=13\)). The AP duration at 90% repolarization level was shortened at the high \([Ca^{2+}]_o\) by 63.2\pm11.7 msec and conversely lengthened at the low \([Ca^{2+}]_o\) by 87.6\pm21.7 msec by taurine. The alterations in the AP parameters can be elucidated, at least in part, by \(I_{Ca}\) changes (shown as above).

We examined the rate of terminal repolarization \((-dV/dt)\) of AP under the same experimental conditions. To reduce small but random fluctuations in AP signals which were large enough to hinder the distinction of a true repolarization signal from the background noise in \(dV/dt\) traces, AP data were treated with the adaptive smoothing procedure (15). Figure 3 shows the \(dV/dt\) signals through the smoothing treatments, revealing the rate of repolarizations at the high \([Ca^{2+}]_o\) (Fig. 3A(a), downward deflection in the lower trace) and at the low \([Ca^{2+}]_o\) (Fig. 3B(a)). The result is summarized in Table 1. It is obvious that taurine suppressed the repolarization velocity at the high \([Ca^{2+}]_o\) and, conversely, enhanced it at the low \([Ca^{2+}]_o\). At \([Ca^{2+}]_o\) of 1.8 mM, taurine tended to reduce the repolarization velocity as at 3.6 mM \([Ca^{2+}]_o\), although the extent of the reduction was much smaller at 1.8 mM (data not shown).
We determined if the voltage at which the maximum repolarization occurred was also altered by taurine. Figure 3B shows the phase-plane analysis to clarify the maximally-repolarizing voltage. As can be seen in the figure, the maximally repolarizing voltage remained unchanged (ca. -60 mV), irrespective of whether or not myocytes were exposed to taurine and whether \([\text{Ca}^{2+}]_o\) was high or low: -58.9±2.6 mV in the control, -61.9±3.2 mV with taurine-treatment, and -58.8±1.6 mV after washing with the high \([\text{Ca}^{2+}]_o\) solution (N=7); -58.3±2.3 mV in the control, -59.4±2.2 mV with taurine-treatment, and -57.5±2.4 mV after washing with the low \([\text{Ca}^{2+}]_o\) solution (N=13). In other words, when taurine lengthens the AP duration by virtue of the augmented \(I_{\text{Ca}}\) at a low \([\text{Ca}^{2+}]_o\), it simultaneously ac-

|                      | High \([\text{Ca}^{2+}]_o\) | Low \([\text{Ca}^{2+}]_o\) |
|----------------------|-----------------------------|-----------------------------|
| A. Control (V/sec)   | N=7                         | N=13                        |
|                      | -2.257±0.265                | -2.119±0.240                |
| B. Taurine (V/sec)   | N=7                         | N=13                        |
|                      | -1.802±0.217                | -2.298±0.243                |
| difference           | +0.456±0.194*               | -0.179±0.051**              |
| C. Wash (V/sec)      | N=5                         | N=12                        |
|                      | -2.115±0.154                | -2.257±0.278                |
| difference           | +0.144±0.190                | +0.122±0.069                |

Values are means±S.E.M. Difference: difference from the corresponding control (V/sec). N denotes the number of observations. Statistical significance: *P<0.05, **P<0.01.
A. High [Ca\(^{2+}\)]\(_o\)
(a) Control
![Control current trace]
(b) Taurine
![Taurine current trace]
(c) Wash
![Wash current trace]

B. I - V relation

![Current-voltage relationship graph]

Fig. 4. Effect of taurine on the late outward K\(^+\) current (I\(_K\)) in a guinea pig ventricular myocyte at a high (3.6 mM) [Ca\(^{2+}\)]\(_o\).
A: Original current traces in response to depolarizing voltage steps of 300-msec length in -5 mV increments at a holding potential (V\(_H\)) of -40 mV. Before (a: from -25 to +65 mV) and during (b: from -25 to +60 mV) exposure to 10 mM taurine and after washing for 3 min (c: from -10 to +70 mV). [K\(^+\)]\(_o\)=5.4 mM, Pulse rate = 1/4 sec, C\(_m\)=101 pF, R, (after 70% compensation) = 3.4 M\(\Omega\). B: Current (I)-voltage (V) relation of I\(_K\). The amplitude of the current measured at the end of the 300-msec pulse (shown in panel A) is plotted against the step voltage between 0 and +60 mV. Symbols in the figure represent the following: ○, control; △, taurine, 10 mM; and □, washout of taurine. The smooth curves are drawn according to the equation: I(nA) = EXP(A x (V\(_m\)+B)), where values of the parameters, A and B, are given in the figure.

Late outward K\(^+\) current (I\(_K\))

The primary current responsible for the AP repolarization is the delayed rectifier K\(^+\) current. To test if taurine affected the delayed rectifier current at a high and low [Ca\(^{2+}\)]\(_o\) differently, the magnitudes of currents at the end of the step pulses of various voltage, 300–500-msec-long, were measured in 3.6 mM and 0.9 mM [Ca\(^{2+}\)]\(_o\). A representative experiment is shown in Fig. 4A (at the high [Ca\(^{2+}\)]\(_o\)) and Fig. 5A (at the low [Ca\(^{2+}\)]\(_o\)). As expected, when [Ca\(^{2+}\)]\(_o\) was high (Fig. 4A), taurine depressed the magnitudes of I\(_K\), as it did well as I\(_{Ca}\). At [Ca\(^{2+}\)]\(_o\) of 1.8 mM, taurine tended to reduce the magnitude of the current, as it did at 3.6 mM [Ca\(^{2+}\)]\(_o\), although the extent of the reductions was much smaller at 1.8 mM (data not shown). In contrast, when [Ca\(^{2+}\)]\(_o\) was low (Fig. 5A), taurine conversely increased the magnitude of both the currents.

To quantify such taurine-induced changes in I\(_K\), chord (linear) conductance values between two potentials were determined (Table 2). In the control period (Table 2A), the conductance value at high [Ca\(^{2+}\)]\(_o\) was significantly (P < 0.05, unpaired) higher than that at the low [Ca\(^{2+}\)]\(_o\). In the presence of 10 mM taurine (Table 2B), the conductance at the high [Ca\(^{2+}\)]\(_o\) decreased and that at the low [Ca\(^{2+}\)]\(_o\) conversely increased significantly (P < 0.05). Such
taurine-induced changes in conductance in either direction tended to return toward the control level after taurine removal (Table 2C).

To obtain more accurate data of taurine-modified conductance, the downward concave I-V relation was approximated by an exponential function in order to yield the slope conductance value (Figs. 4B and 5B). In such approximations, the slope conductance value (in $\mu$S) at a certain voltage ($V_m$) was given by $A \times \exp(A \times (V_m + B))$.

Figure 6 summarizes the taurine-induced changes in the slope conductance for $I_K$. Taurine decreased and increased the $K^+$ conductance for any voltage at the high and low $[Ca^{2+}]_o$, respectively. The result coincided with the repolarization data with respect to the manner of modifications by $[Ca^{2+}]_o$.

### Table 2. Taurine-induced change in chord conductance

|                | High $[Ca^{2+}]_o$ | Low $[Ca^{2+}]_o$ |
|----------------|-------------------|------------------|
| A. Control ($\mu$S) | $N=7$             | $N=13$           |
|                 | $0.00587 \pm 0.00093$ | $0.00366 \pm 0.00040$ |
| B. Taurine ($\mu$S) | $N=7$             | $N=13$           |
|                 | $0.00429 \pm 0.00053$ | $0.00460 \pm 0.00045$ |
| difference      | $-0.00159 \pm 0.00052^*$ | $+0.00094 \pm 0.00021^{**}$ |
| C. Wash ($\mu$S)  | $N=5$             | $N=12$           |
|                 | $0.00530 \pm 0.00111$ | $0.00394 \pm 0.00036$ |
| difference      | $-0.00050 \pm 0.00038$ | $+0.00028 \pm 0.00024$ |

Values are means $\pm$ S.E.M. Chord conductance was determined between $-10$ to $+30$ to $+50$ mV. Difference: difference from the corresponding control ($\mu$S). $N$ denotes the number of observations. Statistical significance: $^P < 0.05$, $^{**}P < 0.01$. 

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**Fig. 5.** Effect of taurine on the late outward $K^+$ current ($I_K$) in a guinea pig ventricular myocyte at a low (0.9 mM) $[Ca^{2+}]_o$. A: Original current traces in response to depolarizing voltage steps of 400-msec length in 3-mV increments at a holding potential ($V_H$) of $-40$ mV. Before (a: from $-27$ to $+33$ mV) and during (b: from $-27$ to $+24$ mV) exposure to 10 mM taurine and after washing for 1 min (c: from $-27$ to $+21$ mV). $[K^+]_o=5.4$ mM, Pulse rate $=1/4$ sec, $C_m=103$ pF, $R_e$ (after 60% compensation) $=3.1$ M$\Omega$. B: Current (I)-voltage (V) relation of $I_K$. The amplitude of currents measured at the end of 300-msec pulses (shown in panel A) is plotted against the step voltage between $-10$ and $+40$ mV. Symbols in the figure represent the following: $\bigcirc$, control; $\bigtriangleup$, taurine, 10 mM; and $\square$, washout of taurine. The smooth curves are drawn as in Fig. 4.
Fig. 6. Effect of taurine on the slope conductance of the late outward K⁺ current (IK). The slope conductance values, A × EXP(A × (Vm+B)), at potentials (Vm) between 0 and +50 mV were computed (cf. Figs. 4 and 5). Shown (in %) are taurine-induced changes of the slope conductance from the control values. A: Summary of 6 data at the high [Ca²⁺]₀. B: Summary of 11 data at the low [Ca²⁺]₀. Values represent means±S.E.M. Plus and minus signs in the ordinate show the decrease and increase of the outward conductance, respectively. Statistical significance: *P<0.05.

Late outward K⁺ conductance

To analyze changes in the K⁺ conductance, the magnitude of IK measured at the end of the 300–500-msec-long step pulse was used. In the absence of any channel blockers and in the external presence of Na⁺ and Ca²⁺ at the physiological level to record both AP and ionic currents under the same experimental conditions, it was possible that the residual (persistent) components of these currents may, more or less, be included in this IK. Taurine, 10 mM, decreased and increased the K⁺ conductance at the high and low [Ca²⁺]₀, respectively. Recently (1995), Satoh reported that in 3-day-old embryonic chick ventricular myocytes, taurine exerted either inhibitory or stimulatory actions on IK at high and low [Ca²⁺]₀, respectively (16). Hence, it is of interest that the effects of taurine on IK seem to be [Ca²⁺]-dependent, irrespective of the species, although the internal Ca²⁺ level is not necessarily closely coupled to [Ca²⁺]₀.

Inward rectifier current

The N-shaped rectifier current in the ramp-pulse study herein was little affected by taurine. Taurine shifted the reversal potential by a few mV. Such an apparent shift in the I-V relation was seen at the restricted voltages near the reversal potential. At present, we regard the apparent I-V antiarrhythmic efficacies in both AP parameters and ionic currents, the whole-cell v-c and c-c experiments were conducted in guinea pig ventricular myocytes. Experiments of AP and current measurements were carried out in solutions having the same ionic composition with high (3.6 mM) and low (0.9 mM) [Ca²⁺]₀ to compare changes in both the parameters. Following findings were obtained.

At the high [Ca²⁺]₀, taurine decreased the L-type Ica, but at the low [Ca²⁺]₀, taurine increased Ica. Concomitantly, at the high [Ca²⁺]₀, the AP duration was shortened and the overshoot of AP was decreased, whereas at the low [Ca²⁺]₀, the AP duration was lengthened. At both [Ca²⁺]₀, RMPs were slightly hyperpolarized. While the magnitude and chord and slope conductances for IK were decreased at high [Ca²⁺]₀, those at low [Ca²⁺]₀ were increased. In line with these changes, the rate of the terminal repolarization (−dV/dt) was decelerated at the low and accelerated at the high [Ca²⁺]₀. However, the voltage for the maximum −dV/dt, ca. –60 mV, was unchanged, regardless of [Ca²⁺]₀ or the presence of taurine. In contrast, at either [Ca²⁺]₀, the magnitude of the inward rectifier currents remained unaffected after taurine addition. The reversal potential for the inward rectifier current was shifted by a few mV in the negative potential direction. The taurine actions were essentially reversible upon washout.

Discussion

To search for taurine-induced alterations relevant to its...
A. Ramp-Pulse Study

shift as an increased K+ conductance.

Changes in AP and presumed antiarrhythmic mechanisms

The changes in AP duration induced by taurine when affected by [Ca\textsuperscript{2+}]_o was qualitatively in accordance with the changes in I_{Ca} observed at such [Ca\textsuperscript{2+}]_o. Hence, even if the change in AP duration was not necessarily derived solely from the change in I_{Ca}, the I_{Ca}-change must take part in the change in AP duration. Also, taurine-induced changes in the rate of repolarization were in line with those in I_K. Taking account both the I_{Ca} and I_K data together, it may be concluded that under a condition where I_{Ca} is intensified and, hence, AP duration is lengthened, taurine functions to curtail the prolonged AP, and vice versa. This effects may result in a stabilization of the refractory period. Although the fast Na\textsuperscript{+} channel is available at potentials negative to -60 mV, the voltage for the maximum $-dV/dt$, ca. -60 mV, remained unchanged either in the absence or presence of taurine, permitting the membrane to immediately return to RMP. Hence, even in the presence of taurine, the vulnerable period, susceptible to external stimuli, will still remain short.

Taurine slightly increased the RMP, irrespective of [Ca\textsuperscript{2+}]_o. A similar increase in RMP was reported by Dolara et al. (17) (1978) in a microelectrode study with guinea pig ventricular muscles exposed to 10 mM taurine and by Sawamura et al. (3) (1990) in guinea pig ventricular myocytes exposed to 20 mM taurine. Such an increase in RMP suggests the increase in the K\textsuperscript{+} conductance at potentials in the vicinity of RMP. The ramp-pulse study
revealed a slight negative shift of the I-V relation for the inward rectifier current after taurine addition. If this is also the case in vivo, prevention of the automatic depolarization may be anticipated. In fact, in 3-day-old embryonic chick ventricular cells showing spontaneous electrical activity, 10 mM taurine slowed the automaticity at high (pCa = 7) [Ca$^{2+}$]$_i$, although not at low (pCa = 10) [Ca$^{2+}$], (16). The present study was carried out at [Ca$^{2+}$]$_o$ of 0.9 (low), 1.8 (normal) and 3.6 mM (high). Since the low and high Ca$^{2+}$ concentrations tested herein were much lower and higher when compared with the physiological [Ca$^{2+}$], the present experimental results should not be simply extrapolated for taurine actions occurring in vivo. Whether or not taurine exerts similar Ca$^{2+}$-dependent actions when [Ca$^{2+}$]$_i$ is altered within a physiological range remains to be clarified.

In summary, the following are the presumed factors in view of the antiarrhythmic efficacy of taurine: the normalizing actions on the Ca$^{2+}$ influx and the intracellular Ca$^{2+}$ load, minimized disparity of the refractory period, and the increase in the K$^+$ conductance near RMP.

**Taurine concentration**

The physiological concentration gradient for taurine across the cardiac cell membrane provided by the active transport is 200–400 vs 1, that is, a few tens mM inside vs a few hundreds μM outside (1). Most of electrophysiological studies of taurine action have been carried at concentrations of 5–50 mM, which are much higher than the physiological plasma level. We suppose that in those studies, the taurine-concentrating mechanism, i.e., active transporter, is markedly depressed under the experimental conditions: isolated preparations or isolated cells with proteolytic enzymes, lowered temperature and lavage of the cell interior with artificial pipette solutions. Consequently, the external presence of taurine in a high concentration must be required.

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