

## Electrophysiological Actions of Taurine on Spontaneously Beating Rabbit Sino-Atrial Nodal Cells

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*Received July 25, 1994 Accepted October 19, 1994*

**ABSTRACT**—Effects of taurine on the spontaneous action potentials in rabbit sino-atrial nodal cells were examined at different extracellular  $\text{Ca}^{2+}$  concentrations ( $[\text{Ca}]_o$ ). Experiments were performed at  $36^\circ\text{C}$ . The firing rate of spontaneous activity was  $132.5 \pm 12.1$  beats/min ( $n=18$ ) in normal Tyrode's solution ( $[\text{Ca}]_o = 1.8$  mM). Increasing  $[\text{Ca}]_o$  level from 0.9 to 10.8 mM significantly changed the maximum rate of depolarization. Other parameters of the action potentials were unaffected. When  $[\text{Ca}]_o$  was 0.9 mM, application of taurine (1 to 20 mM) tended to cause a positive chronotropic effect and hyperpolarized the maximum diastolic potential. In the normal solution (at 1.8 mM  $[\text{Ca}]_o$ ), taurine significantly enhanced only the maximum rate of depolarization. In contrast, under high  $[\text{Ca}]_o$  (5.4 and 10.8 mM), taurine at 1 and 5 mM had a negative chronotropic effect, but 20 mM taurine had a positive chronotropic effect. Also, taurine shortened the action potential duration and hyperpolarized the maximum diastolic potential. The maximum rate of depolarization was inhibited. In 10.8 mM  $[\text{Ca}]_o$  solution, irregular spontaneous activity (dysrhythmias) occurred in 4 of 6 preparations, and addition of taurine (1 to 20 mM) abolished it. These results indicate that taurine modulates the action potential configuration in the sino-atrial nodal cells dependent on  $[\text{Ca}]_o$ .

**Keywords:** Taurine, Automaticity,  $\text{Ca}^{2+}$  concentration, Antiarrhythmic effect, Sino-atrial nodal cell (rabbit)

Taurine, a sulfur-containing amino acid, is abundant in cardiac cells (over 10 mM), but only present at low concentration in the plasma (1). Many biological roles of taurine have recently been reported. Electrophysiological studies show that taurine modulates ionic currents across the cell membranes (1, 2). Satoh and Sperelakis (3–5) have recently reported that, in embryonic chick cardiomyocytes, taurine has dual actions on ionic currents dependent on intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}]_i$ ); the L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) and the delayed rectifying  $\text{K}^+$  current ( $I_{\text{K}}$ ) are inhibited at pCa 7, whereas both currents are enhanced at pCa 10. Taurine inhibits the TTX-sensitive  $\text{Na}^+$  current ( $I_{\text{Na}}$ ), independent of  $[\text{Ca}]_i$  level (3). These results resemble the results of some other investigators (1, 6, 7). The actions of taurine may regulate  $\text{Ca}^{2+}$  homeostasis and membrane stabilization. Ischemia and hypoxia produce a significant reduction of taurine content in the myocardium (8, 9). Thus, the external administration of taurine could protect against the damages induced by ischemia (or  $\text{Ca}^{2+}$  overload).

There is still considerable interest in the physiological actions of taurine. Several ionic currents would contribute to generation of the spontaneous action potentials in

rabbit sino-atrial (SA) nodal cells. Since the maximum diastolic potential (MDP) of the action potentials is  $-60$  to  $-70$  mV, the  $I_{\text{Ca}}$  and  $I_{\text{K}}$  currents would play a major role in regulation of the spontaneous action potentials (10, 11). The automaticity is strongly dependent on  $[\text{Ca}]_i$  or extracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}]_o$ ). Actually, in young embryonic chick cells with spontaneous beating, taurine modulates the automaticity by regulation of the ionic currents dependent on  $[\text{Ca}]_i$  level (12, 13). In the present experiments, the modulations by taurine of the action potentials in the spontaneously beating rabbit sino-atrial (SA) nodal cells were investigated at different  $[\text{Ca}]_o$  levels.

### MATERIALS AND METHODS

Rabbits of either sex, weighing 1.5–2.0 kg, were used. The preparations were made by the same methods as described previously (10, 11, 14). The rabbits were anesthetized with pentobarbital sodium (30 mg/kg) and exsanguinated. The chest was opened, the heart was quickly removed, and the right atrium with the SA node region left intact was dissected in the bath solution. The small SA nodal preparations cut perpendicularly to the crista

terminalis were made smaller by dissection to the size of 1.0–0.5 mm. The preparations were superfused with the bath solution oxygenated by 100% O<sub>2</sub> at 36°C and were left spontaneously beating.

Conventional glass microelectrodes filled with 3 M KCl were used, and their resistances were 20–30 M $\Omega$ . The recordings were displayed on an oscilloscope (Nihon Kohden VC-11, Tokyo) and with a thermal array recorder (Nihon Kohden, RTA-1200M). The composition of the modified Tyrode solution was: 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1.0 mM MgCl<sub>2</sub>, 0.3 mM NaH<sub>2</sub>PO<sub>4</sub>, 5.0 mM glucose and 5.0 mM HEPES. The pH was adjusted to 7.4 with NaOH. The [Ca]<sub>o</sub> level was changed from the normal Tyrode solution ([Ca]<sub>o</sub> = 1.8 mM) to the solutions containing the desired [Ca]<sub>o</sub> levels. Taurine (Sigma Chemical Co., St. Louis, MO, USA) was dissolved to the desired concentrations directly in the bath solution. Taurine was administered after the external solution was switched to one with different [Ca]<sub>o</sub>.

Data were taken about 3 to 5 min after application of taurine. When the dysrhythmias occurred, the values were obtained before the inhibition of dysrhythmias or from the action potentials of the cells beating regular rhythm during a rest of dysrhythmia (the dysrhythmias were intermittently elicited in almost all preparations). Values are given as the mean  $\pm$  S.E.M. The differences of the mean values were analyzed by Student's *t*-test for paired data, and a *P* value less than 0.05 was considered significant.

## RESULTS

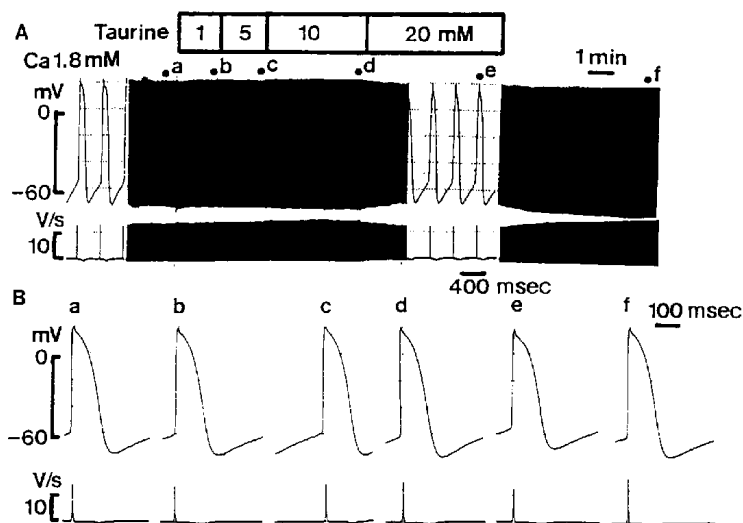
### *Effects of different [Ca]<sub>o</sub> on the spontaneous action potentials*

Changes of [Ca]<sub>o</sub> ranging from 0.9 to 10.8 mM were performed, and the effects on the action potential configuration were examined. In normal Tyrode solution, the cycle length (CL) in the spontaneously beating cells was 453  $\pm$  9 msec (*n* = 9). The percentage changes are summarized in Table 1. At 0.9 and 5.4 mM [Ca]<sub>o</sub>, the maximum rate of depolarization ( $\dot{V}_{\max}$ ) was enhanced, but at 10.8 mM, it was significantly inhibited. The action potential duration at 50% repolarization (APD) was shortened at 10.8 mM [Ca]<sub>o</sub>. However, other parameters were not sig-

**Table 1.** Effects of changes in [Ca]<sub>o</sub> on the action potential parameters in spontaneously beating rabbit sino-atrial nodal cells

	[Ca] <sub>o</sub> (mM)			
	0.9	1.8	5.4	10.8
<i>n</i>	7	9	8	6
APA (mV)	96 $\pm$ 2	94 $\pm$ 3	93 $\pm$ 3	86 $\pm$ 3
APD (msec)	123 $\pm$ 2	113 $\pm$ 4	107 $\pm$ 3	102 $\pm$ 5*
MDP (mV)	-75 $\pm$ 2	-73 $\pm$ 2	-70 $\pm$ 2	-69 $\pm$ 2
$\dot{V}_{\max}$ (V/sec)	25 $\pm$ 5***	14 $\pm$ 2	16 $\pm$ 3*	7 $\pm$ 3***
CL (msec)	481 $\pm$ 10	453 $\pm$ 9	433 $\pm$ 9	424 $\pm$ 8

Values represent means  $\pm$  S.E.M. *n*: Number of experiments. APA: Action potential amplitude, APD: Action potential duration at 50% repolarization, MDP: Maximum diastolic potential,  $\dot{V}_{\max}$ : Maximum rate of depolarization, CL: Cycle length. \*: *P* < 0.05, \*\*\*: *P* < 0.001, with respect to the control value (at 1.8 mM [Ca]<sub>o</sub>).



**Fig. 1.** Effects of taurine on the spontaneously beating rabbit sino-atrial nodal cells in normal Tyrode's solution ([Ca]<sub>o</sub> = 1.8 mM). A: The action potentials and the maximum rate of depolarization. B: High-speed traces at dots (a–f) above the action potential recordings in panel A.

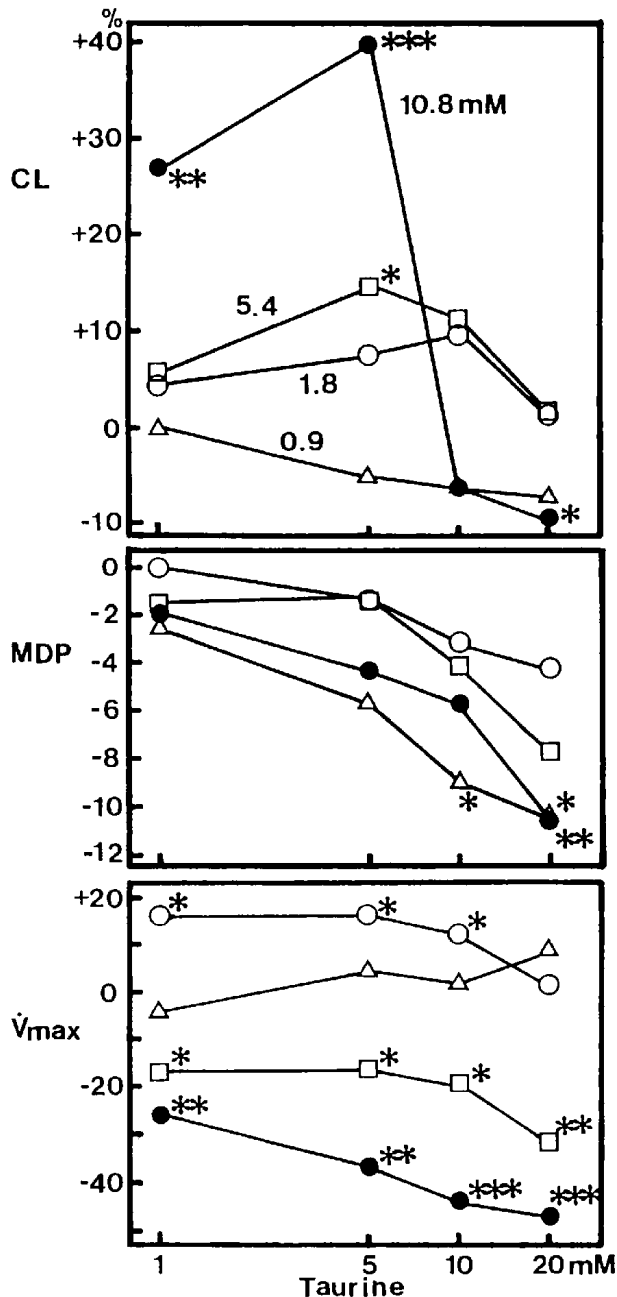
nificantly affected. Low  $[Ca]_o$  tended to cause negative chronotropic effects, hyperpolarize the maximum diastolic potential (MDP), and enhance the action potential amplitude (APA). In contrast, elevating  $[Ca]_o$  from 1.8 mM

tended to produce the opposite effects on the action potential parameters.

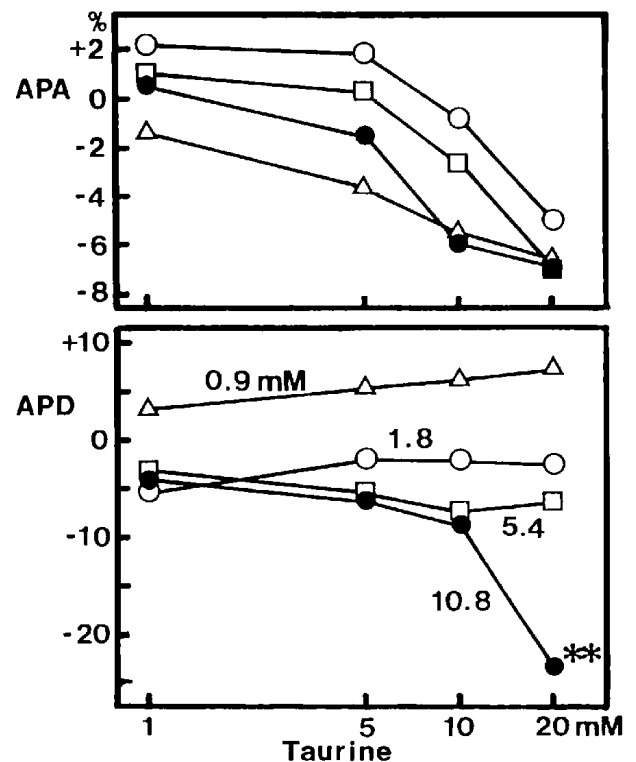
#### Effects of taurine on the action potentials at different $[Ca]_o$ levels

Effects of taurine on the action potentials of spontaneously beating rabbit SA nodal cells were examined. Taurine at 1 to 20 mM was cumulatively administered to the bath solution. At 1.8 mM  $[Ca]_o$ , increasing the concentration of taurine (1 to 10 mM) enhanced  $\dot{V}_{max}$  and slightly hyperpolarized the MDP, but at 20 mM taurine inhibited  $\dot{V}_{max}$  and depolarized the MDP, as shown in Fig. 1. These percentage changes in the parameters are summarized in Figs. 2 and 3. Taurine (1 to 10 mM) increased the  $\dot{V}_{max}$  by 13.5 to 16.2% ( $n=7$  to 9,  $P<0.01$ ), but did not affect the other parameters to any significant extent.

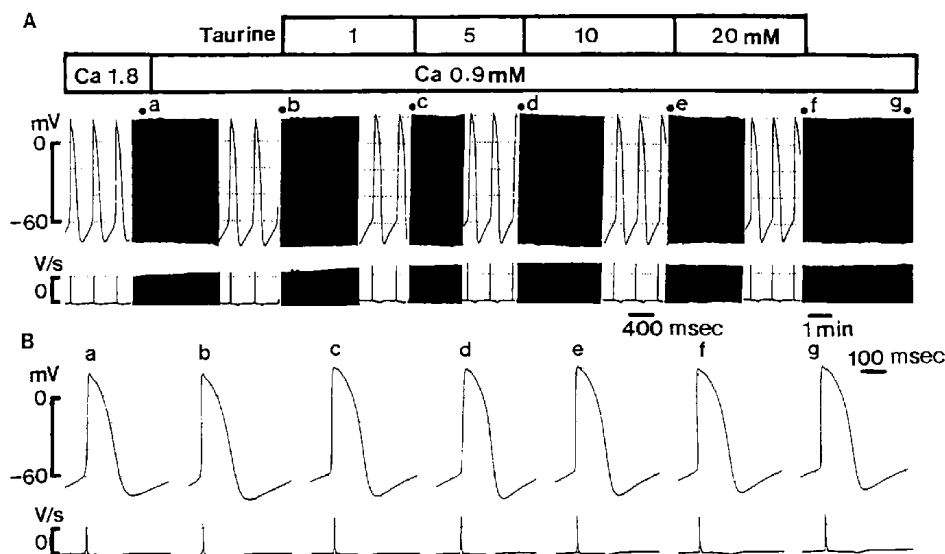
In low  $[Ca]_o$  (0.9 mM) solution, application of taurine (10 and 20 mM) enhanced  $\dot{V}_{max}$  and hyperpolarized MDP (Fig. 4). However, at 20 mM, taurine did not depress  $\dot{V}_{max}$  and depolarize MDP, different from the effects at 1.8 mM  $[Ca]_o$ . As shown in Fig. 2, taurine caused a positive



**Fig. 2.** Percentage changes in the action potential parameters by taurine in spontaneously beating rabbit sino-atrial node at different  $[Ca]_o$ . Effects on the cycle length (CL), the maximum rate of depolarization ( $\dot{V}_{max}$ ) and the maximum diastolic potential (MDP) are represented. Symbols used show  $[Ca]_o$  levels of 0.9 mM (triangles), 1.8 mM (open circles), 5.4 mM (squares) and 10.8 mM (filled circles). Values represent means  $\pm$  S.E.M. ( $n=5$  to 7). The S.E.M. bars are less than the thickness of the symbols. \*:  $P<0.05$ , \*\*:  $P<0.01$ , \*\*\*:  $P<0.001$ , with respect to the control value.



**Fig. 3.** Percentage changes in the action potential amplitude (APA) and the action potential duration at 50% repolarization (APD) by taurine in spontaneously beating rabbit sino-atrial node at different  $[Ca]_o$ . Symbols used show  $[Ca]_o$  levels of 0.9 mM (triangles), 1.8 mM (open circles), 5.4 mM (squares) and 10.8 mM (filled circles). Values represent means  $\pm$  S.E.M. ( $n=5$  to 7). The S.E.M. bars are less than the thickness of the symbols. \*\*:  $P<0.01$ , with respect to the control value.

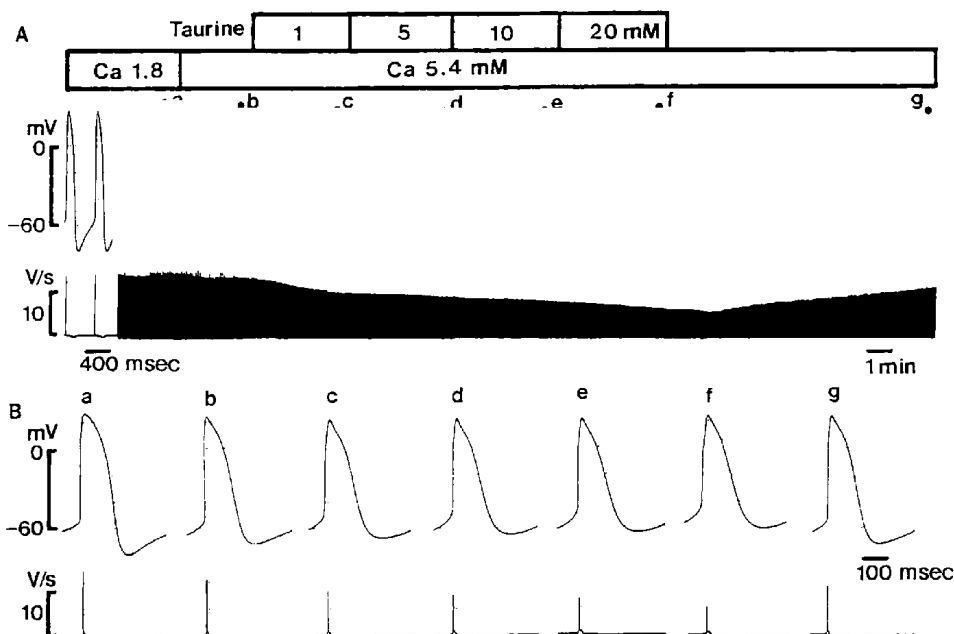


**Fig. 4.** Effects of taurine on the spontaneously beating rabbit sino-atrial nodal cells in low  $\text{Ca}^{2+}$  solution ( $[\text{Ca}]_o = 0.9 \text{ mM}$ ). A: The action potentials and the maximum rate of depolarization. B: High-speed traces at dots (a–g) above the action potential recordings in panel A.

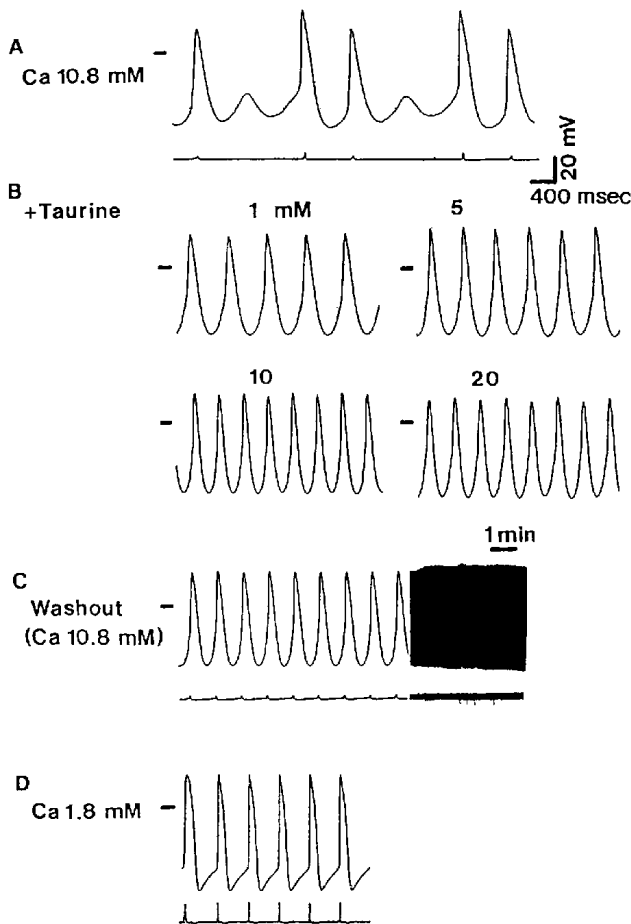
chronotropic effect, but not significantly. Taurine hyperpolarized the MDP by  $8.9 \pm 2.0\%$  ( $n=7$ ,  $P<0.05$ ) at 10 mM and by  $10.5 \pm 2.2\%$  ( $n=7$ ,  $P<0.05$ ) at 20 mM. The APA and the  $\dot{V}_{\max}$  were unaffected (Figs. 2 and 3).

On the other hand, in 5.4 mM  $[\text{Ca}]_o$  solution, taurine (1 to 20 mM) depolarized the MDP and inhibited  $\dot{V}_{\max}$

(Fig. 5). As shown in Fig. 2, at high  $[\text{Ca}]_o$  (5.4 and 10.8 mM), taurine at 1 and 5 mM had a significant negative chronotropic effect, but taurine at 20 mM had a positive chronotropic effect at 10.8 mM  $[\text{Ca}]_o$ . The  $\dot{V}_{\max}$  was inhibited by 26.3 to 44.2% ( $n=5$  to 6,  $P<0.001$ ). Taurine at 20 mM shortened the APD and hyperpolarized the



**Fig. 5.** Effects of taurine on the spontaneously beating rabbit sino-atrial nodal cells in high  $\text{Ca}^{2+}$  solution ( $[\text{Ca}]_o = 5.4 \text{ mM}$ ). A: The action potentials and the maximum rate of depolarization. B: High-speed traces at dots (a–g) above the action potential recordings in panel A.



**Fig. 6.** Antiarrhythmic effects of taurine in rabbit sino-atrial nodal cells. A: The action potentials and the maximum rate of depolarization with irregular rhythms at 10.8 mM  $[Ca]_o$ . B: Abolishment of the irregular rhythms by addition of taurine (1 to 20 mM). C: Wash-out of taurine in high  $[Ca]_o$  solution (10.8 mM). D: Return to normal Tyrode's solution. The short line at the left of the action potential recordings represents the zero mV level.

MDP. In addition, in 10.8 mM  $[Ca]_o$  solution, dysrhythmias occurred in 4 of 6 preparations, and addition of taurine (1 to 20 mM) abolished it (Figs. 6, A and B). After wash-out of the taurine, the dysrhythmias did not appear again even in 10.8 mM  $[Ca]_o$  solution (Fig. 6C) and then also in 1.8 mM  $[Ca]_o$  solution (Fig. 6D). No dysrhythmias occurred in the  $[Ca]_o$  solutions less than 5.4 mM or lower. These responses to taurine in all the experiments were reversible by 20- to 30-min wash out.

## DISCUSSION

The spontaneous firing rate is dependent on the  $[Ca]_i$  or/and  $[Ca]_o$ . In this study, the firing rate was approximately 133 beats/min at 1.8 mM  $[Ca]_o$ . Taurine application caused a positive chronotropic effect at 0.9 mM  $[Ca]_o$

and a negative chronotropic effect at 5.4 and 10.8 mM  $[Ca]_o$ , but not significantly. These are consistent with the effects on the embryonic chick cells in which the modulation of the chronotropic effects by taurine is due to changes in the ionic currents (13). However, taurine at 10 and 20 mM caused a positive chronotropic effect at high  $[Ca]_o$ , resulting from blockade of the dysrhythmias by taurine.

There are at least three currents known to contribute to the pacemaker potential (phase 4 depolarization): (a)  $I_{Ca}$  (15), (b)  $I_K$  decay (16, 17), and (c) hyperpolarization-activated inward current ( $I_f$ ) (18). In the SA nodal cells, however, the  $I_f$  current only makes a minor contribution to the generation of the pacemaker potential, because the  $I_f$  current requires longer (than 3 sec) and higher (than  $-80$  mV) hyperpolarization. In this study, the CL was less 500 msec, and the MDP was less  $-75$  mV. Thus, the membrane depolarization to the threshold potential (pacemaker potential) would be generated mainly by a combination of the other two currents (10, 11, 19, 20). During the pacemaker potential, the T-type  $Ca^{2+}$  current contributes to the early phase during diastole (19, 20), which is stimulated by taurine (4).

The MDP is one of the factors that regulate automaticity. The hyperpolarization results in prolongation of CL and the depolarization, in shortening of CL. Taurine at 20 mM hyperpolarized the MDP. The MDP is regulated by the inward rectifier ( $I_{K1}$ ) and the background currents. In guinea-pig ventricular cardiomyocytes, taurine decreased  $I_{K1}$  (H. Satoh, unpublished data), suggesting that taurine modulates these currents.

Effects of taurine on the APD were not uniform in different hearts. In this study, taurine (20 mM) shortened the APD at 10.8 mM  $[Ca]_o$ . Generally, low  $[Ca]_o$  prolonged the APD and high  $[Ca]_o$  shortened it. Taurine potentiated the  $Ca^{2+}$ -induced effects on the APD, consistent with the result in guinea pig cells (21). However, it is different from the result in embryonic chick cardiomyocytes (13, 22, 23). At plateau, the inward and outward currents are practically in balance (24), and a small change in one of the currents will greatly affect the course of the potential. The difference shows that the regulation of APD is independent of only the  $I_K$ . In guinea pig heart and rabbit SA nodal cells, taurine prolonged APD at low  $[Ca]_o$  and shortened it at high  $[Ca]_o$ . The discrepancy might be due to the difference between adult and embryonic cells.

Taurine enhanced  $I_{Ca}$  and  $I_K$  at low  $[Ca]_i$ , whereas it inhibited them at high  $[Ca]_i$  (4, 22, 23). These results indicate that taurine acts on  $I_{Ca}$  in a manner to keep the  $[Ca]_i$  level relatively constant and thereby plays an important role in maintaining cell functions. Recent studies have shown that taurine possesses potent cardioprotective ac-

tions in embryonic chick cardiomyocytes and guinea pig ventricular myocytes (3, 4, 7, 13, 21, 25). Actually, taurine abolished the arrhythmias under the  $\text{Ca}^{2+}$  overload condition (see Fig. 6B) and also in guinea pig heart (21). Therefore, these results indicate that the  $\text{Ca}^{2+}$ -induced effects on cardiac functions (or cell damages) are antagonized by taurine application; that is, taurine has a cardioprotective action (22, 23, 26). Although multiple taurine actions on the heart still remain unclear, extensive studies should reveal the possible therapeutic use of this taurine effect.

#### Acknowledgment

This work was in part supported by Terumo Life Science Foundation, Japan.

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