

Full Paper

Electropharmacology of Taurine on the Hyperpolarization-Activated Inward Current and the Sustained Inward Current in Spontaneously Beating Rat Sino-Atrial Nodal Cells

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Abstract. Modulation by taurine of the pacemaking activity and the underlying ionic currents, especially a hyperpolarization-activated inward current (I_f) and a sustained inward current (I_{ST}), in rat sino-atrial (SA) nodal cells was investigated at different pCa levels using a patch-clamp technique. Increasing pCa levels from 10 to 6 stimulated the spontaneous activity and simultaneously increased the I_f . Application of taurine depressed more strongly the spontaneous activity at higher pCa levels. At all pCa levels, however, taurine (20 mM) increased the I_f by $60.1 \pm 1.7\%$ ($n = 8$, $P < 0.001$) at pCa 10 and by $48.0 \pm 1.4\%$ ($n = 8$, $P < 0.01$) at pCa 7. At pCa 7, taurine (10 and 20 mM) decreased the sustained inward current (I_{ST}) by $13.3 \pm 1.1\%$ ($n = 5$, $P < 0.05$) and by $38.1 \pm 2.4\%$ ($n = 5$, $P < 0.01$), respectively. Taurine (20 mM) inhibited the L-type Ca^{2+} current (I_{CaL}) by $35.8 \pm 2.5\%$ ($n = 8$, $P < 0.01$), whereas taurine enhanced the T-type Ca^{2+} current (I_{CaT}) by $29.3 \pm 2.9\%$ ($n = 8$, $P < 0.05$). Also, taurine at pCa 7 decreased the delayed rectifier K^+ current; taurine at 20 mM inhibited the rapidly activated K^+ current (I_{Kr}) by $55.6 \pm 3.3\%$ ($n = 6$, $P < 0.001$), but not the slowly activated K^+ current (I_{Ks}). Taurine often elicited dysrhythmias, dependent on taurine's concentrations and pCa levels. These results indicate that taurine causes a negative chronotropic effect due to the inhibitions of the pacemaking ionic currents such as I_{CaL} , I_{Kr} and I_{ST} , and suggest that the I_f and I_{CaT} currents make a minor contribution to pacemaking activity in rat SA nodal cells.

Keywords: taurine, pacemaker current, spontaneous action potential, ionic current, rat sino-atrial nodal cell

Introduction

Lots of taurine's actions on the ionic channel currents of cardiac cells have already been reported. Taurine is abundant in cardiac muscles (1–3), and is considered to play an important role for maintenance of physiological functions (4–6). Our recent studies have also shown the beneficial effects of taurine on cardiac functions to maintain the $[Ca^{2+}]_i$ (7–9). Taurine at high pCa inhibits

the I_{CaL} and I_K , whereas taurine at low pCa enhances both I_{CaL} and I_K (8, 10–13). Thus, the action of taurine on the channel currents is strongly dependent on the pCa levels.

I have already reported the modulation by taurine of the automaticities in embryonic chick cardiomyocytes and in rabbit SA nodal cells (11, 14, 15). In the pacemaker cells of the SA node, the spontaneous action potential is exerted by the contribution of many ionic currents (15, 16). Not only I_{CaL} and I_f but also I_{Kr} and I_{ST} currents play an important role for the generating of spontaneous activity. Our recent studies on the pacemaking mechanism of rat SA nodal cells have demon-

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Abbreviations used are (in alphabetical order): ANOVA: analysis of variance, APA: action potential amplitude, APD_{50} : action potential duration at 50% repolarization, $[Ca^{2+}]_i$: cellular Ca^{2+} concentration, CL: cycle length, I_{CaL} : L-type Ca^{2+} current, I_{CaT} : T-type Ca^{2+} current, I_f : hyperpolarization-activated inward current, I_K : delayed rectifier K^+ current, I_{Kr} : rapidly activated K^+ current, I_{Ks} : slowly activated K^+ current, I_{ST} : sustained inward current, KB solution: Kraftbrühe solution, MDP: maximum diastolic potential, SA: sino-atrial, Slow I_{Na} : slow Na^+ current

strated that it is somewhat different from that of other species (17). Now, unknown mechanisms for taurine's actions on the ionic channels and the spontaneous activity in isolated rat SA nodal pacemaker cells still remain. The aim of the present experiments was to examine the modulation of the chronotropic effect of taurine at different pCa levels in spontaneously beating rat SA nodal cells. Also, the alterations of the spontaneous action potentials and the underlying ionic currents (especially the I_f current) were examined by the patch-clamp technique.

Materials and Methods

All experiments were carried out according to the guidelines laid down by the Nara Medical University Animal Welfare Committee and also under the terms of the Declaration of Helsinki.

Cell preparation

Wistar rats, weighing 200–300 g, were anesthetized with sodium pentobarbital (30 mg/kg, i.p.). Rat SA nodal cells can not be isolated successfully with enzyme solution using the Langendorff apparatus. Therefore, a new dissociation technique has recently been developed by Noma's group, as previously described (17). Under artificial respiration, the chest cavity was opened. An injection needle, connected to a perfusion line, was inserted through the right atrial wall, and Tyrode solution was directly infused into the atrial cavity at a rate of approximately 10 ml/min with a hydrostatic pressure of approximately 70 cmH₂O. To avoid mixing of the perfusate with venous return, and also to expand the atrial cavity by using the perfusion pressure, the superior and inferior venae were ligated. Then, the inferior vena cava was cut distal to the ligature to allow drainage of perfusate, which passed through the pulmonary and then the systemic circulation. Within several minutes, the drained perfusate became largely blood-free. The spontaneous heart beat was stopped by switching the perfusate from normal Tyrode to a nominally Ca²⁺-free Tyrode solution. Then, Ca²⁺-free solution containing 0.4 mg/ml trypsin (Wako Pure Chemical Industries, Osaka) was applied for 5–6 min to remove the endocardial endothelium. Ca²⁺-free solution containing 0.85 mg/ml collagenase (Wako) was perfused for approximately 5 min. Then, the heart was dissected out into fresh collagenase solution. The right atrium was opened by cutting along the atrial septum and also by cutting the ventral wall of the superior vena cava. The atrial tissue including the SA node was dissected out and was gently shaken in the collagenase plus elastase (0.1 mg/ml) solution (Boehringer Mannheim GmbH,

Mannheim, Germany). The enzyme treatment lasted for 20–25 min, depending on the extent of tissue digestion seen under a dissection microscope. Finally, the digested tissue was put in the modified KB solution and trimmed by scissors into a small SA node fragment of approximately 1 mm in width and 3–4 mm in length. The major bundle of atrial cells running through the crista terminalis was discarded. The small SA node tissue was placed in a 35-mm plastic petri dish with a fresh KB solution and SA node cells were dissociated by gentle puffing with KB solution to the tissue. The dissociated cells were stored in the same solution at 4°C for experimentation.

Whole-cell voltage- and current-clamp experiments

Whole-cell voltage-clamp recordings were performed using an Axopatch patch-clamp amplifier (Axon Instruments, Burlingame, CA, USA) and standard techniques. Patch pipettes from borosilicate glass capillaries were fabricated using a two-stage puller; they had a resistance of 5–7 MΩ. The series resistance was less than 10 mV, and no compensation was used. The liquid junction potential between the pipette and the external solutions (less than 10 mV) was corrected for all membrane potential recordings. For the action potential parameters, the action potential amplitude was measured as the difference between the peak of the action potential and the maximum diastolic potential, the action potential duration as the duration at 50% repolarization, and the cycle length as the interval between each peak action potential. The I_{CaL} and I_{CaT} was measured as the difference between the peak current and the zero current, and the I_f was the difference between the current at the end of a 1-s test pulse and the zero current. The I_K (I_{Kr} and I_{Ks}) was measured the peak of outward tail current.

Experiments were carried out at 36°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 2 kHz for plotting. All the values are given as means ± S.E.M. The differences between the mean values were analyzed by ANOVA and Student's *t*-test for paired data, and a *P* value less than 0.05 was considered significant.

Experimental solutions

The composition of the modified Tyrode solution was as follows: 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 1.0 mM MgCl₂, 0.3 mM NaH₂PO₄, 5.0 mM glucose, and 5.0 mM HEPES [N-(2-hydroxyethyl)piperazine-*N'*-2-ethansulfonic acid] (Wako). The pH was adjusted to 7.4 with NaOH. The pipette (intracellular) solution contained: 110 mM K-aspartate, 20 mM KCl, 1 mM MgCl₂,

5 mM EGTA, 5 mM Mg-ATP, 5 mM creatine phosphate, and 5 mM HEPES (pH 7.2). For measurement of I_{CaL} and I_{ST} , K^+ in the pipette solution was replaced by Cs^+ , and for I_{ST} , low Ca^{2+} (0.9 mM) was used. For I_{Kr} and I_{Ks} , nicardipine (1 μ M) was added to the bath solution to avoid other currents. For the T-type Ca^{2+} current (I_{CaT}), tetrodotoxin (10 μ M) was added to the bath solution.

The concentrations of free Ca^{2+} in the internal solution were calculated on the basis of the apparent stability constants for EGTA, Ca^{2+} -ATP, Ca^{2+} -creatine phosphate, Mg^{2+} -EGTA, Mg^{2+} -ATP, and Mg^{2+} -creatine phosphate, according to the calculation of Fabiato and Fabiato (18) and the correction of Tsien and Rink (19).

Results

Effects on the spontaneous action potentials at different pCa levels

In normal Tyrode solution, the spontaneous beating was stronger at higher pCa levels (but not significantly). In comparison with the action potential parameters from pCa 10 to pCa 7, the APD_{50} decreased by $14.6 \pm 1.8\%$ ($n = 8$, $P < 0.05$). The CL was shortened with an increase in pCa levels (10 to 7), but not to a significant amount (by approximately +11.0%). At pCa 6, the CL rather increased. No change in the effects on the other parameters occurred. These changes in the parameters at different pCa levels are summarized in Table 1.

At all the pCa levels, taurine application had inhibitory actions on the spontaneous activity, and the

stronger inhibition was produced at higher pCa levels. At 10 mM, taurine did not cause any significant effects at pCa 10 to 7, but decreased the sinus rate at pCa 6 (Fig. 1: A and B). At 20 mM, taurine also affected the parameters at pCa 10 to 7 significantly. The APA and MDP decreased. At pCa 6, taurine prolonged the CL. These percentage effects are also summarized in Table 1.

Taurine (10 and 20 mM) often elicited some dysrhythmias, as shown in Fig. 1C. In some cells, an arrest (with small oscillations) occurred. The incidence is summarized in Table 2. The occurrence was dependent on taurine concentrations and was more frequently at higher pCa levels. The dysrhythmias lasted for 10–15 min even during washout of taurine. However, the regular rhythm as the control was irreversible in almost SA nodal cells.

In quiescent SA nodal cells, the resting potential was -41 ± 1 mV ($n = 8$). The resting potential was not modified by application of taurine and not even by increasing the concentrations from 5 to 20 mM, as shown in Fig. 1D.

Effect on the hyperpolarization-activated inward currents at different pCa levels

The I_f was activated to a greater extent at higher pCa levels (Fig. 2: A and B). The averaged values at different pCa levels are summarized in Fig. 2C. The cell capacitance was 48.3 ± 1.2 pF ($n = 15$).

The I_f at both pCa 10 and 7 increased after application

Table 1. Effects of taurine on the spontaneous action potentials in rat SA nodal cells

	n	APA (mV)	APD_{50} (ms)	MDP (mV)	CL (ms)
pCa 10					
Control	8	81.1 ± 1.2	116.2 ± 2.2	-58.3 ± 2.5	363.5 ± 12
Taurine (10 mM)	8	-5.2 ± 2.3	-6.1 ± 2.1	-1.1 ± 2.1	$+2.4 \pm 1.7$
Taurine (20 mM)	8	-9.3 ± 1.7	-11.8 ± 2.3	-2.5 ± 1.6	$+14.6 \pm 3.1$
pCa 8					
Control	8	80.4 ± 1.4	110.5 ± 2.3	-58.0 ± 3.8	340.8 ± 13
Taurine (10 mM)	8	-4.8 ± 2.7	-5.1 ± 2.3	-1.7 ± 2.0	$+3.2 \pm 2.6$
Taurine (20 mM)	8	-10.7 ± 2.1	-8.5 ± 1.8	-4.6 ± 2.2	$+15.7 \pm 3.0^a$
pCa 7					
Control	10	78.3 ± 3.2	98.9 ± 2.6	-59.1 ± 2.4	323.4 ± 10
Taurine (10 mM)	10	-4.2 ± 2.4	-3.0 ± 2.0	-5.2 ± 2.2	$+6.1 \pm 2.3$
Taurine (20 mM)	10	-10.1 ± 2.0	-4.6 ± 2.4	-6.3 ± 2.5	$+12.8 \pm 3.4^a$
pCa 6					
Control	8	72.0 ± 2.8	91.8 ± 2.7	-59.2 ± 2.7	481.3 ± 11
Taurine (10 mM)	8	-3.0 ± 1.7	$+1.3 \pm 2.4$	-6.1 ± 2.1	$+9.4 \pm 2.8$
Taurine (20 mM)	8	-8.1 ± 2.6	$+7.5 \pm 3.2$	-7.8 ± 3.3	$+31.7 \pm 4.1^b$

Values (%) are represented as the control \pm S.E.M. ^a $P < 0.05$, ^b $P < 0.01$, with respect to the control value.

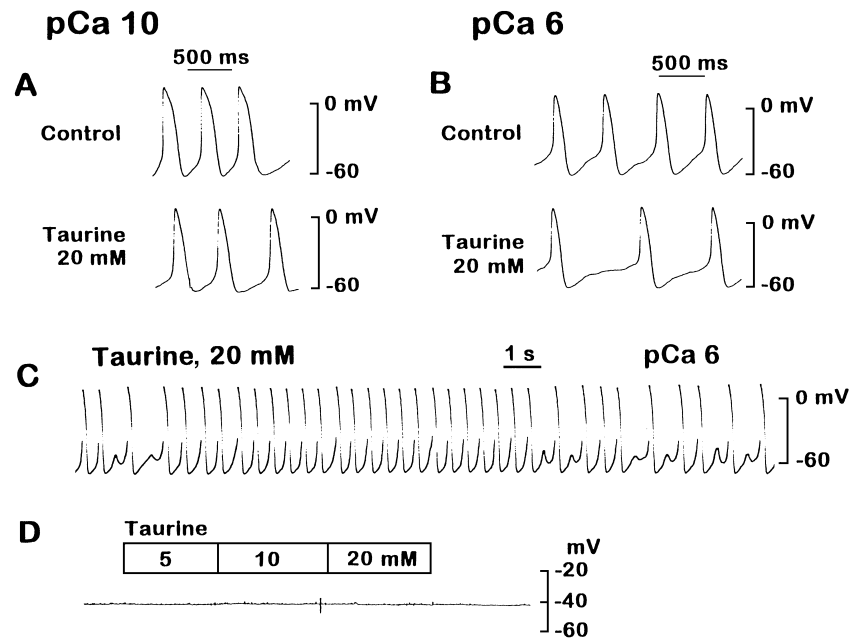


Fig. 1. Effect of taurine on a spontaneously beating rat sino-atrial (SA) nodal cell. A: Spontaneous action potentials at pCa 10 in the control and 20 mM taurine. B: Applications of taurine (20 mM) at pCa 6. C: Taurine-induced dysrhythmias approximately 2 min after application. D: Resting membrane potential recording during exposure to taurine (5–20 mM) in quiescent SA nodal cell.

Table 2. Incidence of occurrence of dysrhythmias and arrest in rat SA nodal cells

	pCa 10	pCa 8	pCa 7	pCa 6
Taurine (10 mM)	0/17	0/16	4/16	8/15
Taurine (20 mM)	0/14	3/17	7/16	12/16

Values are represented as [the number of occurrences / the number of experiments].

of taurine (Figs. 3 and 4). At pCa 10 to 6, the percentage increase in I_f in the presence of taurine (10 and 20 mM) are summarized in Fig. 5. Taurine at 10 mM enhanced the I_f in a pCa-dependent fashion, by $24.5 \pm 3.1\%$ ($n = 8$, $P < 0.001$) at pCa 10, and by $36.1 \pm 2.9\%$ ($n = 8$, $P < 0.01$) at pCa 6. On the other hand, taurine at 20 mM also enhanced I_f , but the enhancement decreased with an increase in pCa levels, by $60.1 \pm 1.7\%$ ($n = 8$, $P < 0.001$) at pCa 10, and $48.0 \pm 1.4\%$ ($n = 8$, $P < 0.01$) at pCa 7. The I_f recovered to approximately 40–50% of the control values after 10-min washout.

Effect on the I_{ST}

The I_{ST} has been considered to be specific and be one of the pacemaking currents to generate the diastolic depolarization of SA nodal cells (17, 20). The I_{ST} was sensitive to nicardipine, independent of pCa levels. In Fig. 6 (A and B), the test pulse was applied to -10 mV from -80 mV of a holding potential. Low Ca^{2+} concentration (0.9 mM) in Tyrode solution was used (17).

Taurine markedly inhibited I_{ST} by $13.3 \pm 1.1\%$ ($n = 5$, $P < 0.05$) at 10 mM and by $38.1 \pm 2.4\%$ ($n = 5$, $P < 0.01$) at 20 mM (Fig. 6C). The percentage inhibitions by taurine are summarized in Fig. 6D.

Effects on the other currents

Taurine markedly inhibited the I_{CaL} at both pCa 10 and 6 (Fig. 3: D and 4). At pCa 10, the average values were $-13.7 \pm 1.8\%$ ($n = 7$, $P < 0.05$) at 10 mM and $-35.3 \pm 2.4\%$ ($n = 7$, $P < 0.01$) at 20 mM of taurine. At pCa 7, taurine had a more potent inhibitory effect on I_{CaL} by $35.8 \pm 2.5\%$ ($n = 8$, $P < 0.01$) at 10 mM and by $-56.6 \pm 2.7\%$ ($n = 8$, $P < 0.001$) at 20 mM. After 15-min washout, the I_{CaL} current was recovered to 50–60% of the control value. I_{CaT} was activated by a depolarized pulse from -80 mV of a holding potential. The I_{CaT} was enhanced by $15.1 \pm 2.3\%$ ($n = 8$, $P < 0.05$) at 10 mM and by $29.3 \pm 2.9\%$ ($n = 8$, $P < 0.05$) at 20 mM taurine, as shown in Fig. 7. The current amplitude recovered to 80 to 90% of the control level after 15- to 20-min washout.

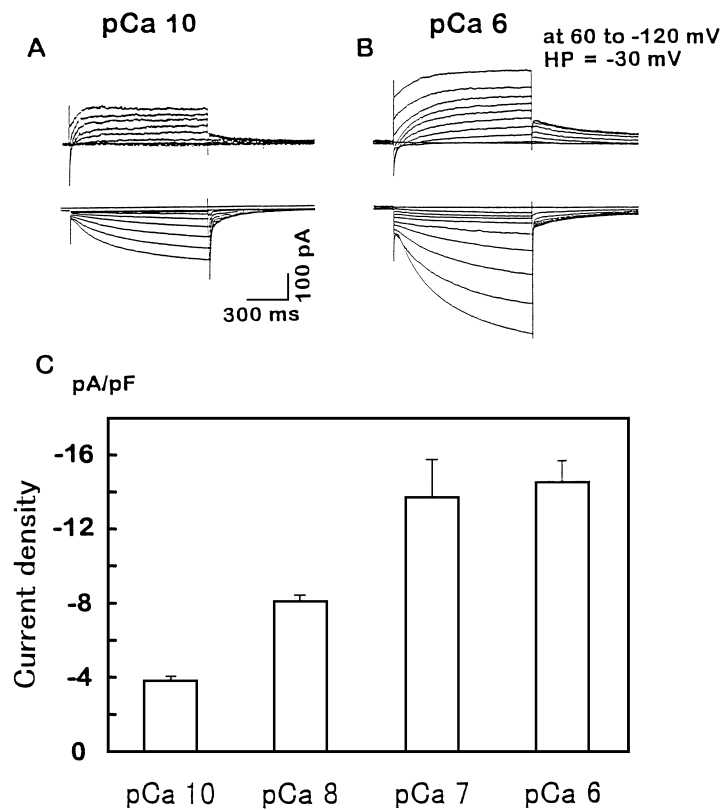


Fig. 2. pCa-dependent activation of the hyperpolarization-activated inward current (I_f) (pacemaker current). A and B: Current traces at pCa 10 and 6. Test pulses (for 1 s) were applied to -20 to 60 mV and -30 to -120 mV from a holding potential of -40 mV. The horizontal line indicates the zero current level. C: Activation of the pacemaker current I_f at different pCa levels.

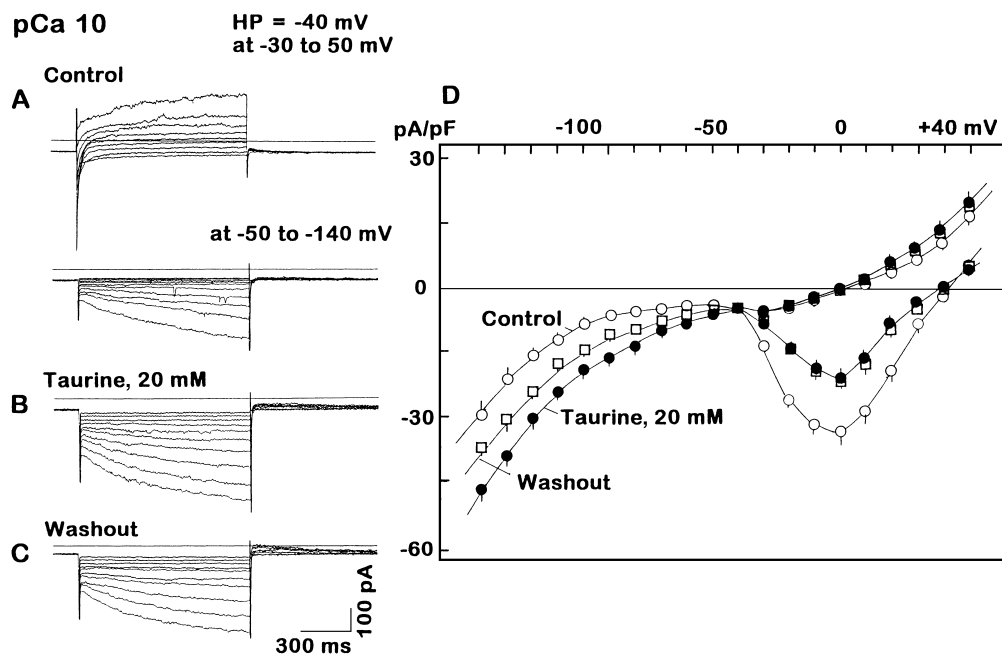


Fig. 3. Changes in the hyperpolarization-activated inward current (I_f) at pCa 10. A: Current traces in the control. Test pulse for 1 s was applied to -30 to 50 mV and -50 to -140 from a holding potential -40 mV. B: I_f at 20 mM taurine. C: Current traces after 7 min-washout. A test pulse for 1 s was applied to -50 to -140 from a holding potential -40 mV. The horizontal line indicates the zero current level. D: I-V curves in the control, in 20 mM taurine, and after washout. The values are represented as the mean \pm S.E.M. ($n = 8$).

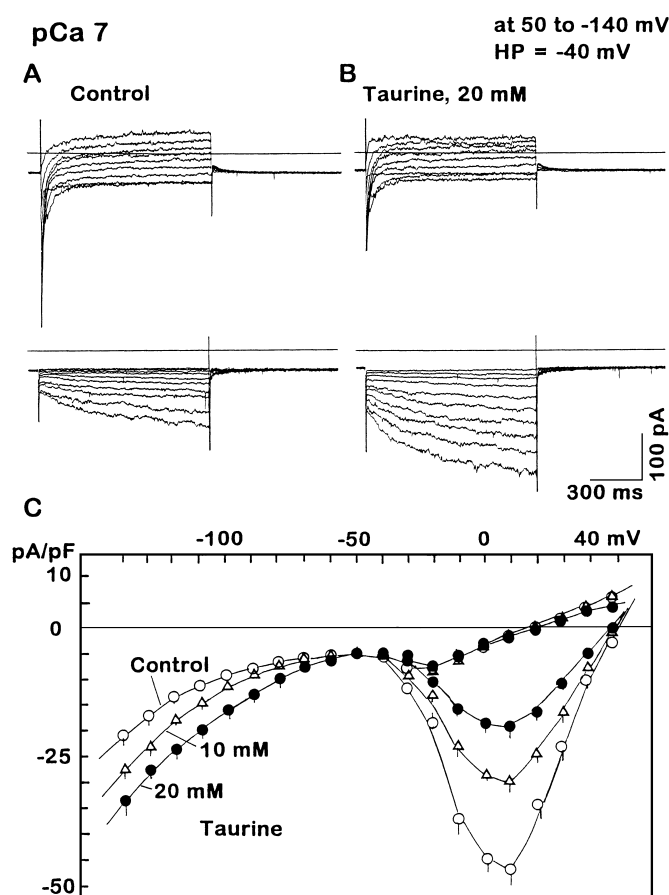


Fig. 4. Modulation by taurine at pCa 7 in rat SA nodal cells. A: Control. B: Taurine (20 mM). The horizontal line indicates the zero current level. The holding potential was -40 mV. Test pulses for 1 s were applied to -30 to 50 mV and to -50 to -140 mV. The horizontal line indicates the zero current level. C: I-V curves for the hyperpolarization-activated inward current and the Ca^{2+} current. The values are represented as the mean \pm S.E.M. ($n = 8$).

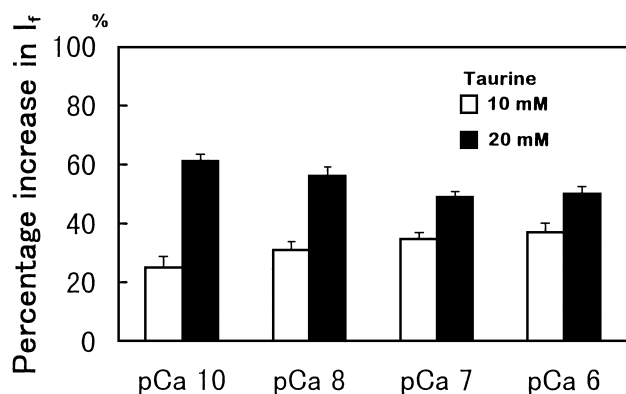


Fig. 5. Increase in I_f current by taurine at different pCa levels. The percentage values are represented as the mean \pm S.E.M. ($n = 8$).

Taurine at pCa 7 significantly decreased the I_K . The I_K is composed of an I_{Kr} and a I_{Ks} . Taurine inhibited I_{Kr} by $23.3 \pm 2.3\%$ ($n = 6$, $P < 0.05$) at 10 mM and by $55.6 \pm 3.3\%$ ($n = 6$, $P < 0.001$) at 20 mM of taurine, as

shown in Fig. 8. On the other hand, taurine (10 and 20 mM) in the presence of $5 \mu\text{M}$ E-4031 did not affect I_{Ks} to any significant extent. The I_{Ks} was sensitive to 293B ($30 \mu\text{M}$), similar to our previous reports (13, 17).

Discussion

Taurine is present plentifully in heart muscles, but stress-loading depletes the content (1–3). Under disease conditions, thus, an application of taurine may be expected to protect against injuries and damages of cardiac cells (4, 6, 9). The present study shows that a) increasing pCa levels enhanced the I_f , but did not stimulate the spontaneous activity; b) taurine application enhanced the I_f at all the pCa levels; c) with an increase in pCa levels, an enhancement induced by 10 mM taurine increased further, whereas an enhancement induced by 20 mM decreased; d) taurine decreased I_{CaL} , but stimulated I_{CaT} ; e) taurine inhibited I_{Kr} but not I_{Ks} ; f) taurine inhibited I_{ST} ; and g) dysrhythmias often

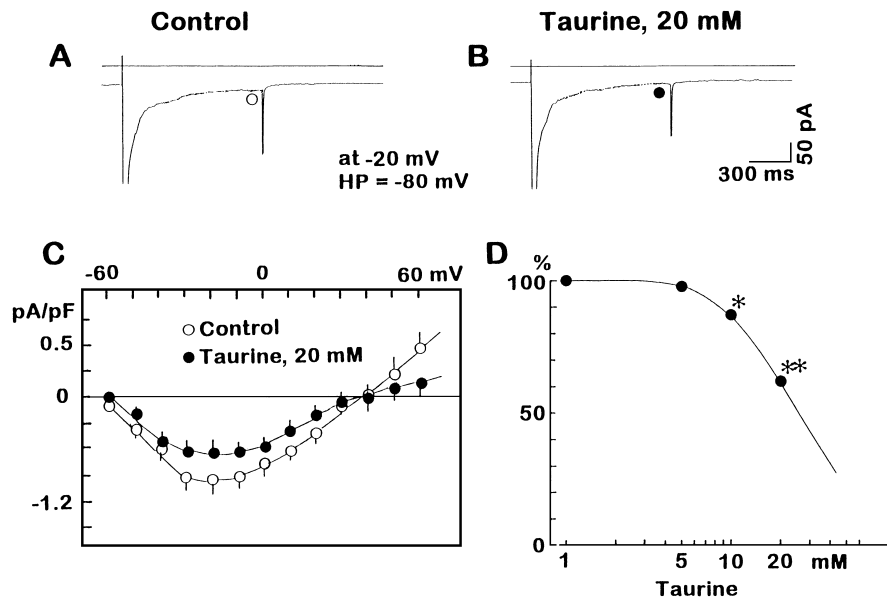


Fig. 6. Modulation by taurine of the sustained inward current at pCa 7 in rat SA nodal cells. A: Control. B: Taurine (20 mM). The holding potential was held at -80 mV. Test pulses were applied to -20 mV for 1 s. The horizontal line indicates the zero current level. C: I-V curves for the sustained inward current. The values are represented as the mean \pm S.E.M. ($n = 5$). D: Summary of the percentage inhibition induced by taurine. Vertical bars of S.E.M. ($n = 5$) are included in the symbols. * $P < 0.05$, ** $P < 0.01$, with respect to control value.

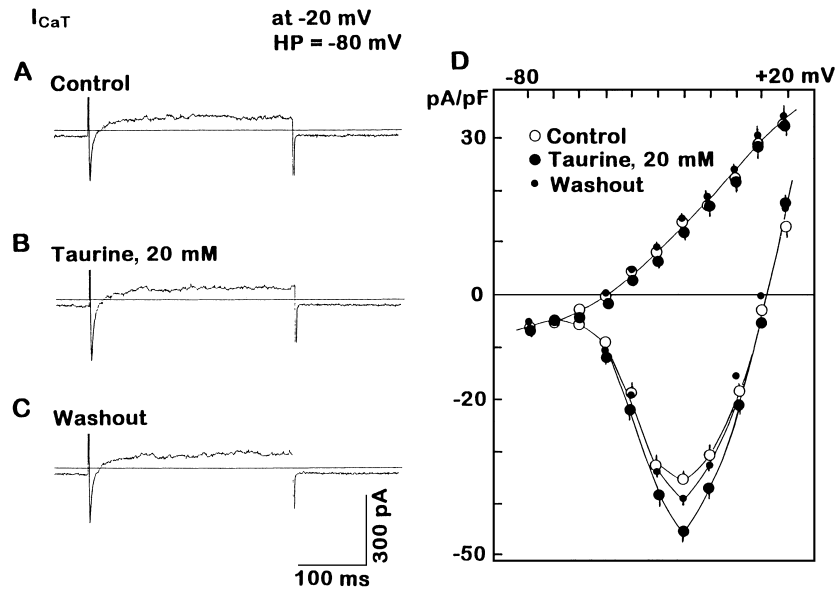


Fig. 7. Enhancement by taurine of the T-type Ca^{2+} current. A: Control. B: Taurine 20 mM. C: Washout (10 min). The holding potential was -80 mV. Test pulses for 1 s were applied to -20 mV. The horizontal line indicates the zero current level. D: I-V curves. The values are represented as the mean \pm S.E.M. ($n = 8$).

occurred. The incidence was concentration-dependent and was more frequently at higher pCa levels. Finally, h) a washout of taurine recovered the spontaneous action potentials and the ionic currents to approximately 50–60% of the control values.

Taurine does not modify the intracellular signaling cascades: cAMP, PI, and cGMP mediated through receptors (21, 22). No pharmacological studies have yet demonstrated the presence of a specific taurine receptor. Thus, taurine appears to exert the direct actions

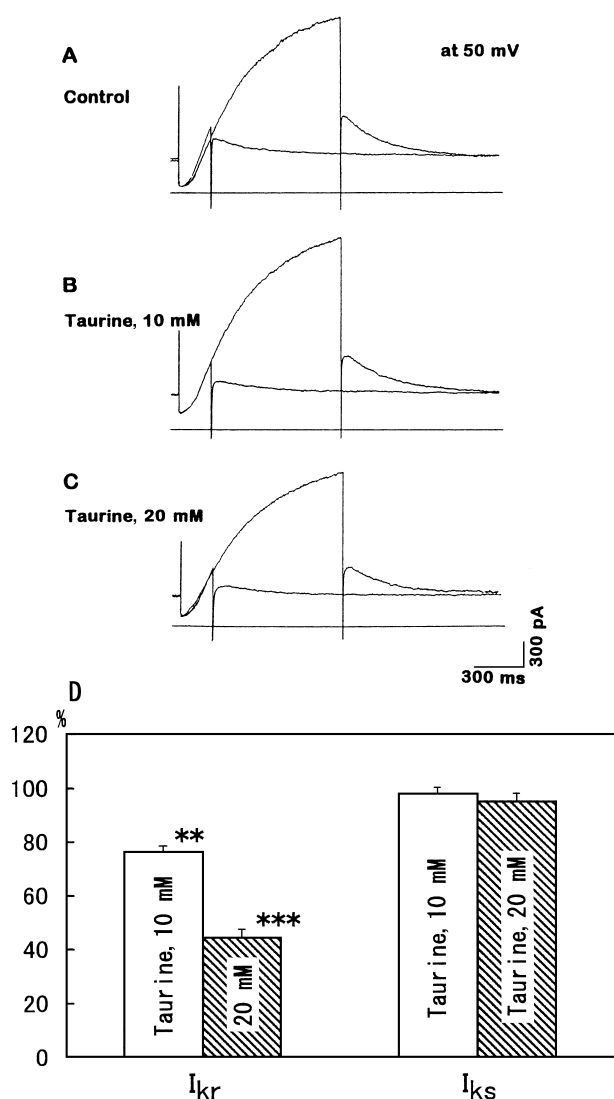


Fig. 8. Modulation of the delayed rectifier K^+ current (I_K) by taurine. A: Control. B and C: Taurine 10 and 20 mM, respectively. Test pulses were applied to 60 mV from a holding potential of -40 mV and the durations of 200 ms and 1 s. The horizontal line indicates the zero current level. D: Effects of taurine on rapidly activated K^+ current (I_{Kr}) and slowly activated K^+ current (I_{Ks}). The values are represented as the mean \pm S.E.M. ($n = 6$).

on the ionic channels and the ionic transports (4, 9). In my taurine experiments for heart, the responses to taurine were reversible incompletely, which may show that it has the properties of a long acting drug (13, 15).

The negative chronotropic effect of taurine would result mainly from the decrease in the rate of the slow diastolic potential (phase 4 depolarization) of SA nodal action potentials. In spontaneously beating embryonic chick cardiomyocytes, taurine caused a positive chronotropic effect at low pCa and caused a negative chronotropic effect at high pCa (11). In the present experiments, however, taurine caused only a negative chronotropic

effect, consistent with the results in rabbit SA nodal cells (14). No positive chronotropic effect of taurine occurred at both low and high pCa levels. The stronger negative effect was produced at higher pCa levels. The discrepancy is now not explained clearly yet, but several possibilities may exist. In embryonic heart cells, a I_{Na} is activated in compensation for the ionic functions of premature cells (9). Taurine increases the slow I_{Na} , resulting in stimulation of spontaneous activity. Also, under these conditions, the taurine- Na^+ cotransport system might be related directly and indirectly to the automaticity.

The regulation of spontaneously beating SA nodal cells has been considered to be due to a) I_{CaL} , b) decline of I_K conductance, and c) I_f (16, 23). Taurine decreased the I_{CaL} and I_K and increased the I_f at high and low pCa levels in this study. Furthermore, I_{CaT} has also been reported to contribute to the early part of the pacemaker potential (16). The I_{CaT} was stimulated by taurine, consistent with our previous reports (8, 9). However, the recent studies have revealed less or no contribution of I_{CaT} to pacemaker depolarization (24, 25). The I_{CaT} would regulate the Ca^{2+} release from sarcoplasmic reticulum and the rate of change in $[Ca^{2+}]_i$ levels for the contraction (26).

The I_f is specific in the pacemaking nodal cells and is activated pCa-dependently, consistent with the findings by Hagiwara et al. (24). Taurine enhanced the I_f at all the pCa levels. In rat SA nodal cells, however, the I_f did not always appear even with spontaneous beating (17). The I_f activation needs higher hyperpolarization (over -70 mV) and a stimulation pulse of longer duration (over 1 s). Thus, it seems unlikely that the I_f contributes to the pacemaking activity under the normal conditions. Therefore, it is concluded that the enhancement by taurine of the I_f would cause a minor effect on the spontaneous activity in rat SA nodal cells. If the I_f in rat SA nodal cells was dependent on Cl^- permeability (27), the I_f might be enhanced by taurine. Taurine stimulates the Cl^- channel current in neurons (28). Anyway, the enhancement of both I_{CaT} and I_f induced by taurine would make less or no contribution to the spontaneous activity.

Most recently, Noma's group has also demonstrated that the pacemaker mechanism in the SA nodal cells is involved with the I_{Kr} and the I_{ST} (17, 20). In either complete blockade of the I_{Kr} by E-4031 or the I_{ST} by nicardipine, the spontaneous activity was depressed and then sinus arrest often occurred. In the present experiments, taurine selectively affected the I_{Kr} , but not I_{Ks} , quite consistent with my previous reports in guinea pig ventricular cardiomyocytes (13). In addition, taurine potentially inhibited the I_{ST} (by approximately 38% at 20 mM). These potent inhibitory actions on the currents

would be closely related to the generation of the pacemaker potentials.

The pacemaker (slow diastolic) potential is not regulated by only one current, but by an interaction with many ionic currents. Therefore, the modulation of many currents by taurine application would pronouncedly contribute to regulation of SA nodal pacemaking activity (29, 30). At all the pCa levels, taurine had the stimulatory actions on I_f and I_{CaT} and had the inhibitory actions on I_{CaL} , I_{Kr} , and I_{ST} . Thus, these modulations that cause a negative chronotropic effect would lead to the cardio-protective actions of taurine.

Taurine often elicited dysrhythmias in rats, like in rabbit SA nodal cells (14, 15). The incidence was much higher at higher pCa levels. Also, the incidence by taurine was produced in a concentration-dependent fashion: in the presence of 20 mM, approximately 18% at pCa 8 and 75% at pCa 6. Even after 20-min washout, the regular rhythm was not recovered fully in almost all SA nodal cells, similar to our previous results (8, 13).

Thus, taurine may possess an arrhythmogenic action, although taurine possesses many inhibitory (or protective) actions on the ionic channel currents under disease conditions (9). The excess Ca^{2+} elevation may cause the negative chronotropic effect and simultaneously elicit some dysrhythmias. Under conditions of calcium overload such as hypoxia, ischemia, and cardiac failure, a triggered activity, which is a trigger to cause arrhythmias, easily occurs (29–31). However, it is certain that taurine exerts many beneficial actions on cardiac cells. Further experiments are needed to clarify the complex mechanisms in detail.

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