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Effect of bile acid on electrophysiological properties of rabbit sino-atrial node *in vitro*

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1 In order to examine the action of bile acid on cardiac pacemaker activity, the effect of sodium taurocholate (NTC) was studied on the membrane potential and current of rabbit sino-atrial node preparations by means of a double microelectrode voltage clamp technique.

2 In spontaneously beating sino-atrial node preparations, NTC (above 30 μM) decreased the maximum rate of rise of the action potential. Above 100 μM, the compound also exerted a bradycardiac effect and decreased the rate of diastolic depolarization significantly.

3 On the current systems, NTC produced dose-related decreases in the slow inward Ca^{2+} current and the time-dependent outward K^+ current.

4 It is concluded that NTC depresses the spontaneous discharge of the sino-atrial node through decreases in both inward and outward current systems.

Introduction

Bile acids have been shown to exert a direct depressant effect on cardiac function (Wakim *et al.*, 1940; Joubert, 1978; Bogin *et al.*, 1983; Green *et al.*, 1984). Furthermore, Song *et al.* (1983) demonstrated that sinus bradycardia is induced by obstructive jaundice. A recent *in vitro* study (Binah *et al.*, 1987) showed that bile acids exert a negative inotropic effect mainly by a depression of Ca^{2+} influx of the cell membrane. Since Ca^{2+} conductance is predominantly responsible for the depolarization in sino-atrial node (Irisawa, 1978), a decrease in Ca^{2+} influx would alter the pacemaker spontaneous discharge. However, it is not obvious whether bile acids have a direct chronotropic action on the cardiac pacemaker cells. Hence, in the present study, we examined the effects of a bile acid (sodium taurocholate) on the membrane potential and the membrane current of rabbit sino-atrial node by means of a double microelectrode voltage clamp method.

Methods

Rabbits, weighing 1.5–2.0 kg, were stunned by a blow behind the neck, and the heart was quickly removed. The right atrium, including the sino-atrial node region, was dissected in Tyrode solution. A small sino-atrial node preparation of about 0.3 × 0.3 mm was prepared according to the method of Noma & Irisawa (1976). The preparation was usually smaller than one half of the length constant of the rabbit sino-atrial node preparation described by Bonke (1973) and Seyama (1976). In the experiments, we used the central area of the node, termed the compact area, as previously described by Bleeker *et al.* (1980) and Brown (1982).

The two-microelectrode voltage clamp method, one electrode to measure the membrane potential and the other to supply current intracellularly, was used. Glass microelectrodes were filled with 3 M KCl and the resistance was 10–30 MΩ. Membrane potential and current were monitored on an oscilloscope (Kikusui Denshi, 5516 ST) and were recorded with a penrecticorder (Nihon Kohden, RJG-4100). The feedback circuit was the same as that described by Noma and colleagues (1980), and the ground amplifier was essentially the same as that used by New & Trautwein (1972). When both microelectrodes recorded identical action potentials the preparation was clamped to −40 mV (holding potential), where the holding current was nearly zero (Noma *et al.*, 1979).

The composition of the normal Tyrode solution was (in mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, Na_2HPO_4 0.6 and the pH was adjusted to 7.4 by adding Na_2HPO_4. Sodium taurocholate (NTC) was of highest purity and was obtained from Sigma Chemical Co. The temperature of the solution was maintained at 36–37°C throughout the experiments.

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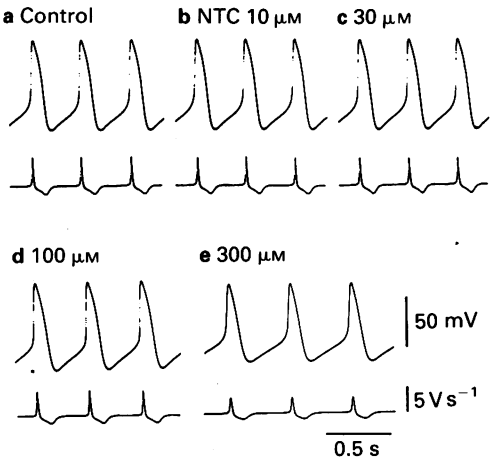

Figure 1 Effect of sodium taurocholate (NTC) (b–e) on spontaneous action potentials in rabbit sino-atrial node. In each panel, upper trace shows action potentials and lower shows their first derivative.

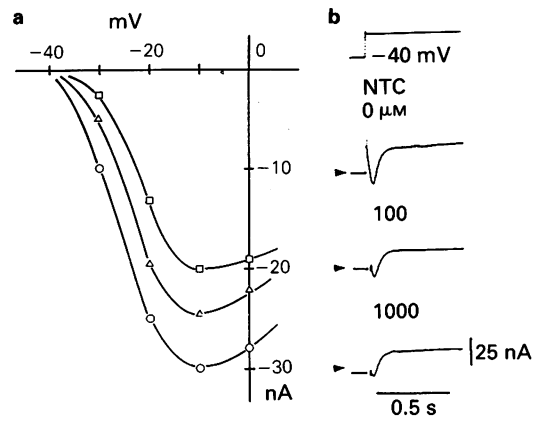

Figure 2 Voltage-dependence of sodium taurocholate (NTC)-induced decrease in the slow inward current (I_{si}). (a) Current-voltage relationship for I_{si}. (b) Voltage clamp recordings. In (b) (▶) shows the holding current before exposure to NTC. I_{si} was measured before (○) and after an application of 100 μM (△) and 1000 μM (□) NTC.

Electrophysiological measurements were made 15 min after changing to a new solution.

For the various parameters of the action potentials and membrane currents, the values are presented as mean ± s.d. Statistical analysis was performed by use of Student's t test, and P values less than 0.05 were considered significant.

Results

Effects on the transmembrane action potentials

In seven spontaneously beating sino-atrial node preparations, the effect of sodium taurocholate (NTC), a bile acid, on membrane potentials was examined by means of a conventional microelectrode method.

Figure 1 shows the action potential traces during increasing concentrations of NTC. Although 10 μM NTC did not produce any significant changes in the action potential, the compound reduced the maximum rate of depolarization ($\dot{V}_{max}$) at above 30 μM. After an application of a NTC concentration higher than 100 μM, the sinus rate and the rate of diastolic depolarization (RDD) were also significantly decreased. These changes in the action potential parameters are summarized in Table 1. The action potential amplitude (APA) tended to be decreased by the drug, but this change was not statistically significant. NTC 1000 μM induced sinus arrest in 3 out of 7 sino-atrial node preparations; this is not shown in the figure. These results indicate that the NTC induced a negative chronotropic effect associated with the reduction of $\dot{V}_{max}$ and RDD, which may reflect a decrease in Ca^{2+} influx through the cell membrane.

Table 1 Effect of sodium taurocholate (NTC) on various characteristics of sino-atrial node action potentials

	SCL (ms)	$\dot{V}_{max}$ ($V s^{-1}$)	*APA* (mV)	APD_{50} (ms)	*RDD* ($mV s^{-1}$)
Control	397.9 ± 56.7	6.6 ± 1.1	93.4 ± 6.5	85.6 ± 7.8	105.3 ± 10.2
NTC					
10 μM	411.6 ± 61.0	6.1 ± 1.3	92.6 ± 6.7	86.1 ± 8.4	102.5 ± 14.0
30 μM	401.1 ± 74.0	5.9 ± 1.1*	93.5 ± 5.9	86.7 ± 8.7	101.5 ± 12.6
100 μM	440.9 ± 70.0*	5.1 ± 1.2**	92.7 ± 5.8	86.7 ± 8.5	92.7 ± 16.4*
300 μM	459.0 ± 79.7*	4.2 ± 1.4**	88.1 ± 8.9	86.4 ± 10.3	86.7 ± 20.4*

Data show means ± s.d., $n = 7$. SCL: spontaneous cycle length; $\dot{V}_{max}$: maximum rate of rise; APA: action potential amplitude; APD_{50}: action potential duration at half-amplitude; RDD: rate of diastolic depolarization. * $P < 0.05$, ** $P < 0.01$ with respect to control values.

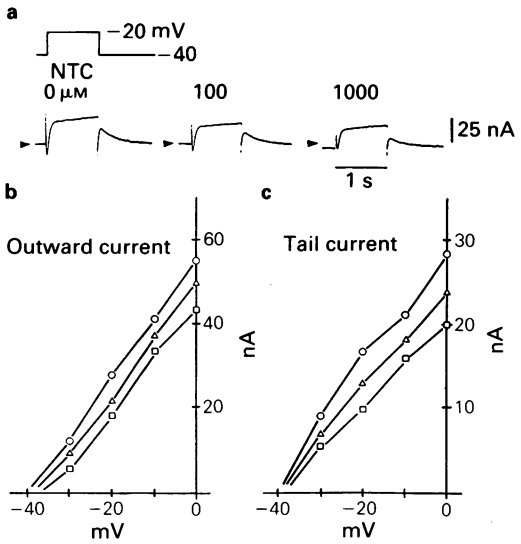

Figure 3 Voltage-dependence of sodium-taurocholate (NTC)-induced decrease in the outward K^+ current (I_K). (a) Voltage clamp recordings. Current-voltage relationships for outward current measured at 1 s after the test pulse (b) and for tail current (c). Outward current amplitude was measured before (○) and after exposure to 100 μM (△) and 1000 μM (□) NTC. In (a) (▶) shows the holding current before NTC treatment.

Effects on the membrane current systems

To investigate the ionic mechanism of the NTC-induced bradycardia, voltage clamp experiments were performed.

Figure 2 shows the effect of NTC on the slow inward current (I_{si}) in sino-atrial node preparations. The membrane potential was held at −40 mV, and depolarizing test pulses were applied with 10 mV increments. These pulses were usually applied at 0.1 Hz. The amplitude of I_{si} was measured as the difference between the peak inward current and the current at 100 ms after the onset of the clamp pulse (McDonald & Trautwein, 1978; Satoh & Hashimoto, 1986), and a current-voltage relationship for I_{si} was obtained before and after exposure to NTC (Figure 2a). NTC obviously depressed I_{si} in a dose-dependent fashion. At a concentration of 100 μM, I_{si} was decreased by a factor of 0.79 ± 0.09 ($P < 0.01$) during depolarization to −10 mV ($n = 4$).

In Figure 3, the effect of NTC on the time-dependent outward K^+ current (I_K) was examined. I_K in sino-atrial node cell is equivalent to I_x in Purkinje fibre (Irisawa, 1978). A depolarizing test pulse elicited a slowly developing outward current following the inactivation of I_{si}, while a gradually decaying tail current was observed after repolarization (Figure 3a). The former shows activation of I_K and the latter shows deactivation of I_K. Current-voltage relationships for the outward current amplitude during depolarization and for the tail current amplitude were plotted in the absence and presence of NTC (Figure 3b,c). NTC clearly decreased both current amplitudes dose-dependently, implying a depression of I_K. NTC (100 μM) decreased the outward current amplitude measured at 1 s after the onset of the depolarizing test pulse to −10 mV by a factor of 0.81 ± 0.14 ($P < 0.01$, $n = 4$).

Discussion

The present study has demonstrated that sodium taurocholate (NTC), a bile acid, decreased the sinus rate associated with decreases in $\dot{V}_{max}$ and RDD. Under the voltage clamp experiments, NTC depressed both I_{si} and I_K in a dose-related manner.

Above 100 μM, NTC produced a slight but significant decrease in the rate of phase 4 depolarization (RDD), wihch mainly contributed to the reduced heart rate. There are several hypotheses for the pacemaker current of sino-atrial node cells (Noble, 1984), but no clear-cut mechanism has at yet emerged. In the present experiments, a decrease in I_{si} seems to be the most important factor for a reduction of RDD and a negative chronotropic effect induced by NTC. In fact, we have already shown that drugs which reduce Ca^{2+} influx through the cell membrane decrease the rate of phase 4 depolarization (Kotake *et al.*, 1986; 1987; 1988). Furthermore, decreases in I_K would reduce the maximum diastolic potential, which might also inactivate pacemaker currents.

On the other hand, the action potential duration at half-amplitude (APD_{50}) was unaffected after an application of NTC. Changes in APD of the sino-atrial node are generally due to changes in I_{si} and I_K (Senami & Irisawa, 1981). A decrease in I_K would prolong APD, whereas a depression of I_{si} would shorten it. As a result of such a combined effect, APD_{50} was not significantly affected after exposure to NTC.

The circulating plasma concentration of all the bile acids is usually less than 1 μg ml^{-1}, which is equivalent to about 2 μM. However, obstruction of bile duct results in higher circulating concentrations of bile acids, as high as 1000 μg ml^{-1} (Makino *et al.*, 1969; Pennington *et al.*, 1977; Song *et al.*, 1983). In rats whose bile ducts have been ligated, the total bile acid concentration rises from 0.2 μg ml^{-1} to about 200 μg ml^{-1} (Bogin *et al.*, 1983; Hardison *et al.*, 1983). A previous electrophysiological study by Binah *et al.* (1987) demonstrated that NTC significantly reduced the developed tension of rat isolated papillary muscle at concentrations between 0.001

and 1.0 μM, which are almost within the physiological range. They also showed that 1 μM NTC depressed I_{si} of the rat ventricular myocyte. In our study, NTC failed to modify the electrical activity of rabbit sino-atrial node at the physiological concentration range, but it exerted significantly depressant membrane actions at concentrations above 100 μM. However, such a high concentration of bile acid is found to occur in obstructive jaundice, as mentioned above. Although the threshold concentration of NTC for inducing a negative chronotropic action is much higher than that for inducing a negative inotropism, sinus bradycardia could occur in severe jaundice through its direct membrane action. These findings might also support the observation by Song *et al.* (1983) in which obstructive jaundice could cause sinus bradycardia. It is difficult to relate clinical plasma concentrations to those of the compound perfusing isolated cardiac tissues and there may be species differences in pacemaker sensitivity to NTC. Nevertheless, our experimental results indicate that high concentrations of bile acid induce an inhibitory effect on the electrophysiological properties of sino-atrial node pacemaker cells and that severe jaundice might modify the spontaneous discharge of the heart.

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