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Negative Chronotropic Effects of Class I Antiarrhythmic Drugs on Guinea Pig Right Atria: Correlation with L-Type Ca^{2+} Channel Blockade

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Abstract: The negative chronotropic effects of eight Vaughan Williams Class I antiarrhythmic drugs were examined in guinea pig right atrial tissue preparations. The drugs decreased the spontaneous beating rate at concentrations overlapping with their therapeutic blood levels. Cibenzoline, aprindine, flecainide, and propafenone showed stronger effects; 10 μM of each drug decreased the beating rate to about 75% of initial values. Disopyramide, mexiletine, pilsicainide, and ranolazine showed weaker effects; 10 μM of each drug decreased the beating rate to about 90% of initial values. The potency of drugs correlated with the reported IC_{50} values to block the L-type Ca^{2+} channel current rather than the Na^+ and K^+ channel currents. The reported IC_{50} values for the blockade of the hyperpolarization-activated inward current (If) and the Na^+-Ca^{2+} exchanger current were much higher than those for the blockade of the L-type Ca^{2+} channel current. These results indicate that the negative chronotropic effects of Class I antiarrhythmic drugs can be largely explained by their blockade of the L-type Ca^{2+} channel.

Keywords: class I antiarrhythmic drugs; cardiosuppression; chronotropy; L-type Ca^{2+} channel

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1. Introduction

Vaughan Williams Class I antiarrhythmic drugs, which block the voltage-dependent Na^+ channel, exert their antiarrhythmic effects mainly through inhibition of the propagation of ectopic excitation through the myocardium [1–3]. They are used variously depending on their mode of action on the voltage-dependent Na^+ channel, and on their pharmacokinetic properties. Class I antiarrhythmic drugs are moderately effective against various types of arrhythmia. However, the potential negative inotropic and chronotropic effects that might cause decreases in cardiac output limit their use in patients with reduced cardiac function. On the other hand, the development and clinical use of Class I antiarrhythmic drugs with novel modes of action on the Na^+ channels are now in progress [2–5]. To optimize the clinical usage of Class I antiarrhythmic drugs and to develop new antiarrhythmic drugs with higher efficacy and safety, understanding the mechanism of their cardiosuppressive effects is essential.

The action potential of the myocardium is formed by several major ion channel currents. In the working myocardium (atrial and ventricular myocardium; Figure 1A), the inwardly rectifying K^+ current (I_{K1}) maintains the resting membrane potential. The inward Na^+ and Ca^{2+} channel currents (I_{Na} and I_{Ca}) cause the rapid depolarization and the following plateau phase, respectively. The delayed rectifier potassium current (I_K) flowing outward causes repolarization. In the sinus node (Figure 1B), several inward currents, including the hyperpolarization-activated cation current (If) and the Ca^{2+} channel current, causes the diastolic depolarization. This is followed by the action potential upstroke that is caused mainly by the Ca^{2+} channel current (I_{Ca}). The delayed rectifier potassium current (I_K) is the major current for repolarization but its declination also contributes to the earlier half of diastolic depolarization. It is established that Class I antiarrhythmic drugs block the

Na^+ channel and reduce the upstroke of the action potential in the working myocardium. The resulting slowing of action potential propagation contributes to their antiarrhythmic effects. On the other hand, the ion channels that are responsible for the negative inotropic and chronotropic effects of Class I antiarrhythmic drugs are less understood.

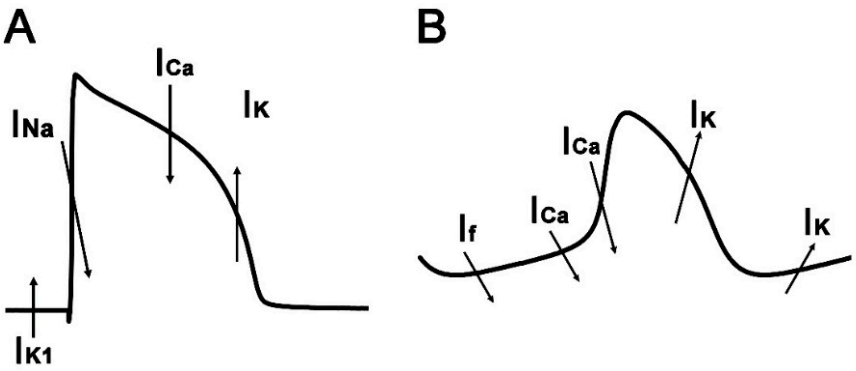

Figure 1. The action potential waveform of the working myocardium (**A**) and the sinus node (**B**). The ion channel currents responsible for each segment are indicated by arrows. The upward and downward arrows indicate outward and inward currents, respectively.

In our previous study, we examined the negative inotropic effects of Class I antiarrhythmic drugs on isolated guinea pig ventricular myocardium and demonstrated that the negative inotropic effects correlate with the L-type Ca^{2+} channel blockade rather than the Na^+ channel blockade [6]. In the present study, we examined the negative chronotropic effects of eight Class I antiarrhythmic drugs using isolated guinea pig right atria to obtain a birds-eye overview concerning the factors underlying their negative chronotropic effects. The results showed that negative chronotropic effects are observed in the therapeutic blood concentration range of these drugs. We analyzed the correlation between the decrease in the beating rate and blockade of Na^+, Ca^{2+}, and K^+ channels, and concluded that the negative chronotropy could be best explained by blockade of the L-type Ca^{2+} channel.

2. Materials and Methods

2.1. Measurement of Beating Rate

The negative chronotropic effects of eight Class I antiarrhythmic drugs were examined in the isolated right atria from Hartley strain male guinea pigs. The procedures were the same as those in our previous studies [6–8]. The right atria were mounted in an organ bath that was filled with a physiological salt solution of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 24.9 mM $NaHCO_3$, and 11 mM glucose (pH = 7.4, 37 °C), gassed with 95% O_2-5% CO_2 and maintained at 36 ± 0.5 °C. The beating rate was analyzed by Power Lab System (AD Instruments, Dunedin, New Zealand).

2.2. Drugs and Chemicals

Pilsicainide was purchased from Alomone Labs (Jerusalem, Israel), disopyramide from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan), cibenzoline and aprindine from Cosmo Bio Co., Ltd. (Tokyo, Japan), flecainide and mexiletine from Sigma Aldrich (St. Louis, MO, USA), propafenone from LKT Laboratories, Inc. (St. Paul, MN, USA), and ranolazine from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Disopyramide, flecainide, propafenone, and ranolazine were dissolved in dimethyl sulfoxide and other chemicals in distilled water. Small aliquots of the drug solutions were added to the solution in the organ bath to obtain the desired final concentrations.

2.3. Statistics

All data were expressed as mean ± standard error of the mean (S.E.M). IC_{50} values were calculated by non-linear regression analysis using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). Pearson's correlation coefficients (r) were used for evaluating correlations between the IC_{50} for the negative chronotropy and the IC_{50} for Na^+, Ca^{2+}, or K^+ channel blockade. An r value closer to 1 was considered a strong correlation. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). A *p*-value less than 0.05 was considered significant.

3. Results

The spontaneous beating rate of the guinea pig right atrial preparations were well maintained; the beating rate before the addition of drugs was 214.3 ± 2.6 bpm (n = 40). Class I antiarrhythmic drugs decreased the spontaneous beating rate of the right atria (Figure 2). The effects were dependent on the concentration of the drugs ranging from 0.1 μM to 30 μM; this concentration range includes or largely overlaps with the therapeutic plasma concentration of the drugs (Table 1). There was a difference in the potency among the drugs. Cibenzoline, aprindine, flecainide, and propafenone showed stronger effects; the beating rate in the presence of 10 μM of each drug was 74.3 ± 3.1% (n = 5) for cibenzoline, 75.0 ± 2.4% (n = 5) for aprindine, 78.4 ± 1.4% (n = 5) for flecainide, and 74.8 ± 3.4% (n = 5) for propafenone. Disopyramide, mexiletine, pilsicainide, and ranolazine showed weaker effects; the beating rate in the presence of 10 μM of each drug was 89.8 ± 4.4% (n = 5) for disopyramide, 94.8 ± 1.0% (n = 5) for mexiletine, 92.3 ± 1.0% (n = 5) for pilsicainide, and 92.1 ± 1.1% (n = 5) for ranolazine.

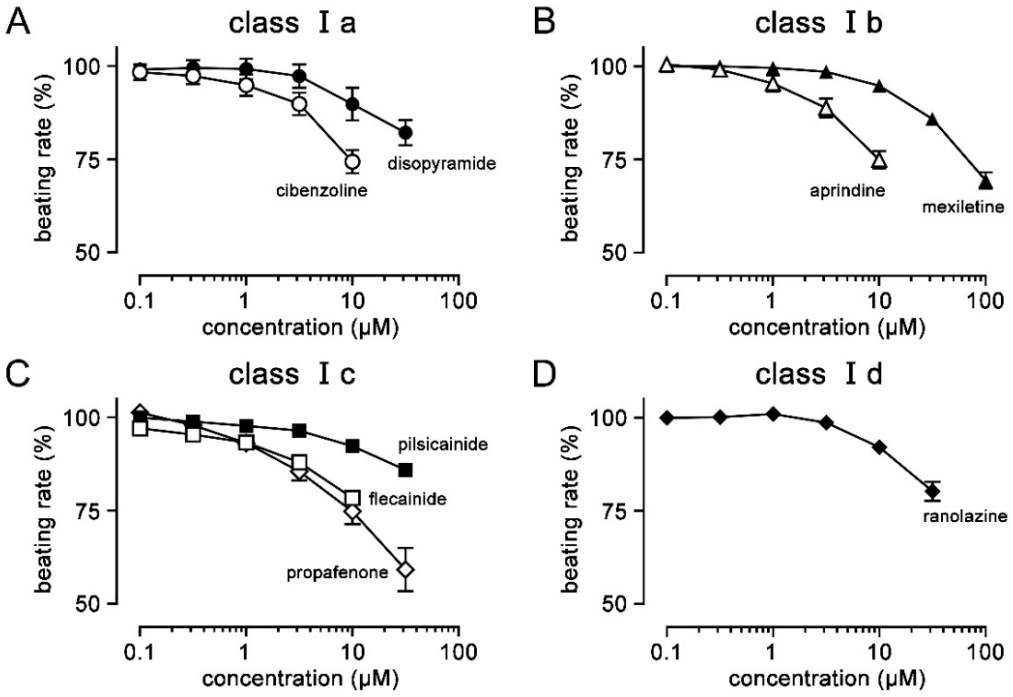

Figure 2. Negative chronotropic effects of Class I antiarrhythmic drugs. The effects of drugs belonging to the Vaughn Williams classification Ia, Ib, Ic, and Id were shown in panels (**A**–**D**), respectively. The beating rate was expressed as a percentage of the values before application. Symbols and vertical bars indicate the mean ± S.E.M. (n = 5).

To clarify whether the negative chronotropic effects of Class I antiarrhythmic drugs correlate with their Na^+ channel-blocking effects, we analyzed our data using the reported potency of drugs against the Na^+ channels (Table 1). The concentration of each drug was expressed as a ratio against its IC_{50} value for the Na^+ channel current (Table 1) and was plotted on the ordinate. If the negative chronotropic effects were the result of Na^+ channel blockade, the plot would converge on a single concentration-response curve. The result was actually different where the concentration-response curves were scattered, indicating that there was more than a 100-fold difference in the potency among the drugs (Figure 3A). Additionally, to examine the correlation by another method, virtual IC_{50} values for the negative chronotropy with each drug were calculated assuming that the beating rate reaches zero at the bottom of the concentration-response curve. The IC_{50} values for the negative chronotropy did not correlate with the IC_{50} values for the Na^+ channel blockade (Figure 3B; r = 0.213, p = 0.613).

Table 1. Therapeutic concentration range and IC_{50} values (μM) of Class I antiarrhythmic drugs.

		Therapeutic Concentration Range		IC_{50} for Negative Chronotropy *	IC_{50} for Na^+ Channel		IC_{50} for Ca^{2+} Channel		IC_{50} for K^+ Channel	
Ia	Cibenzoline	0.8–3.0	[9]	28.3	7.8	[10]	14	[10]	3.7	[11]
	Disopyramide	5.9–14.7	[12]	132.2	32	[13]	100	[13]	7.3	[14]
Ib	Aprindine	0.4–2.1	[9]	28.8	0.74	[10]	10	[10]	0.23	[15]
	Mexiletine	2.8–11.1	[12]	217.6	30	[16]	85.74	[17]	3.7	[18]
Ic	Flecainide	0.48–2.4	[12]	32.0	10.4	[19]	27.1	[20]	1.5	[21]
	Pilsicainide	0.73–3.67	[9]	174.5	0.88	[16]	>100	[22]	20.4	[23]
	Propafenone	0.27–9.58	[24]	37.2	1	[25]	5	[26]	0.55	[27]
Id	Ranolazine	2–8	[28]	128.0	428	[25]	50	[29]	11.5	[29]

* The IC_{50} values for negative chronotropy were calculated by non-linear regression analysis.

In contrast, when the concentration of each drug was expressed as a ratio against its IC_{50} value for the Ca^{2+} channel blockade (Table 1), the negative chronotropic effects of all drugs tended to converge on a single concentration-response curve; the difference in potency among drugs was decreased to less than 10-fold (Figure 3C). There was a strong correlation between the virtual IC_{50} for negative chronotropy and the IC_{50} for the L-type Ca^{2+} channel current (Figure 3D; r = 0.893, p = 0.0028).

To clarify whether the negative chronotropic effects of Class I antiarrhythmic drugs can be explained by their K^+ channel-blocking effects, we analyzed our data using the reported potency of drugs against the hERG channel (Table 1). When the concentration of each drug was expressed as a ratio against the IC_{50} value for the hERG channel current, there was more than a 100-fold difference in the potency among the drugs (Figure 3E). The virtual IC_{50} for the negative chronotropy correlated relatively weakly with the IC_{50} for hERG channel blockade (Figure 3F; r = 0.610, p = 0.108).

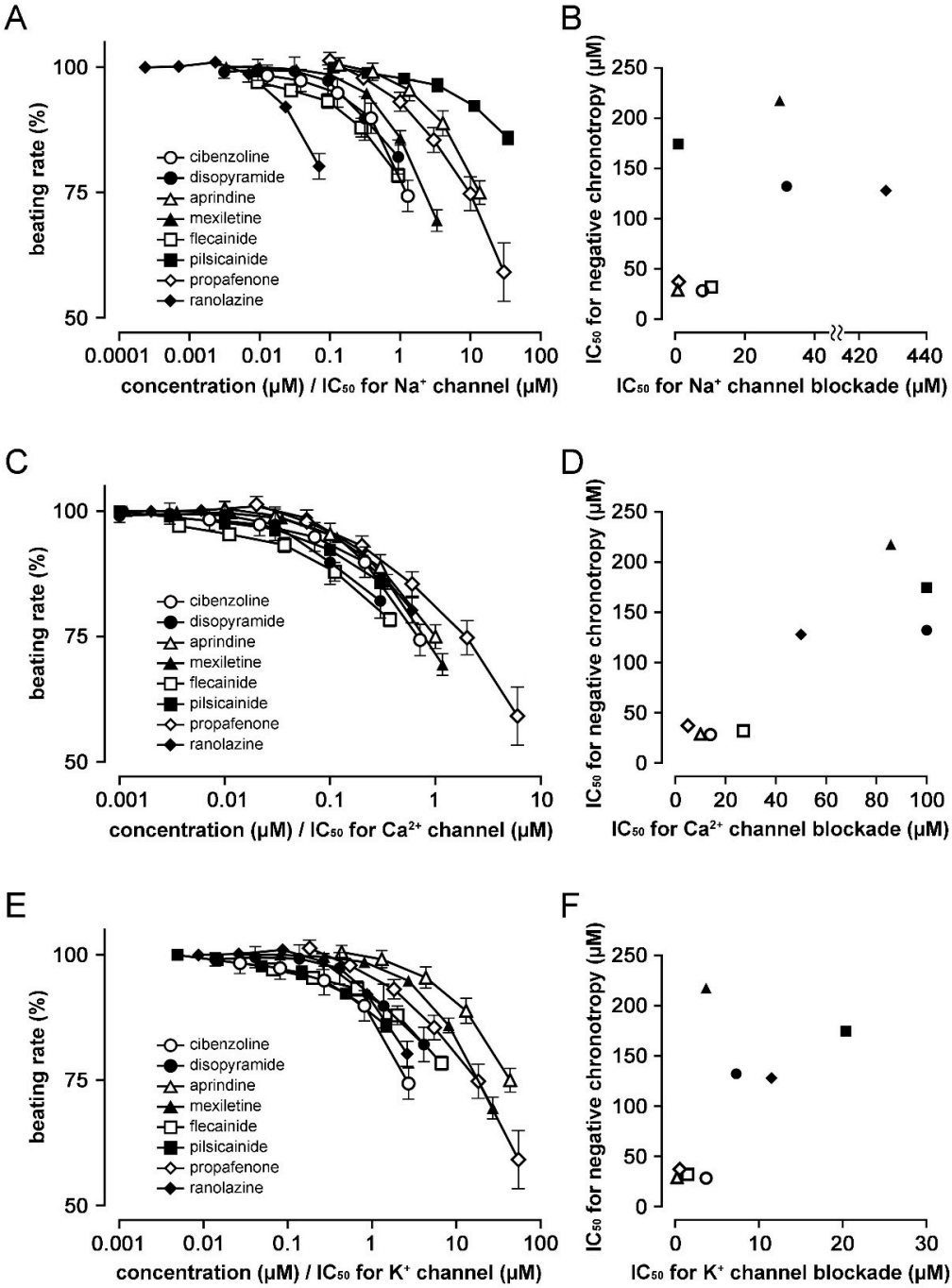

Figure 3. Correlation between the negative chronotropic effects of Class I antiarrhythmic drugs and blockade of Na^+, Ca^{2+}, and K^+ channels. The values for the negative chronotropic effects shown in Figure 2 were re-plotted against the concentration of each agent normalized as a ratio against the IC_{50} values (μM) of drugs for Na^+ (**A**), Ca^{2+} (**C**), and K^+ (**E**) channels. The correlation of IC_{50} values for negative chronotropy (ordinate) and IC_{50} values (abscissa) for the Na^+ (**B**), Ca^{2+} (**D**), and K^+ (**F**) channel blockade was plotted. IC_{50} values for each drug are shown in Table 1. The symbols used were open circles for cibenzoline, closed circles for disopyramide, open triangles for aprindine, closed triangles for mexiletine, open squares for flecainide, closed squares for pilsicainide, open diamonds for propafenone, and closed diamonds for ranolazine.

4. Discussion

In the present study, we examined the negative chronotropic effects of eight Class I antiarrhythmic drugs in isolated guinea pig right atria under identical experimental

conditions to obtain a birds-eye overview concerning the factor(s) underlying their cardiosuppressive effects (Figure 2). The results revealed that Class I antiarrhythmic drugs cause negative chronotropy in the concentration range overlapping with their therapeutic plasma concentration range (Table 1). In addition, differences in the negative chronotropic effects among drugs were observed; cibenzoline, aprindine, flecainide, and propafenone were more potent than disopyramide, mexiletine, pilsicainide, and ranolazine (Figure 2; Table 1).

Class I antiarrhythmic drugs are sub-classified based on their effects on the Na^+ channel and action potential duration [2]. Class Ia antiarrhythmic drugs block the Na^+ channel with intermediate potency; they also block K^+ channels and prolong the action potential duration. Among the Class Ia drugs examined, cibenzoline showed stronger negative chronotropic effects than disopyramide. Class Ib antiarrhythmic drugs rapidly dissociate from the Na^+ channel. Among the Class Ib drugs that were examined, aprindine showed stronger negative chronotropic effects than mexiletine. Class Ic antiarrhythmic drugs dissociate very slowly from the inactivated Na^+ channel. Among the Class Ic drugs that were examined, flecainide and propafenone showed stronger negative chronotropic effects than pilsicainide. Class Id is a new subclass that is defined as inhibitors of the persistent Na^+ current (Late I_{Na}). Ranolazine, which belongs to this subclass, showed a weak negative chronotropic effect, but this is unlikely to be the result of Na^+ channel blockade, as discussed below. Thus, the negative chronotropic effect of Class I antiarrhythmic drugs does not correlate with the Vaughn Williams subclassification based on their Na^+ channel-blocking properties. Thus, their effects on ion channels other than the Na^+ channel had to be considered.

The action potential of the sinus node, the cardiac pacemaker, has a characteristic waveform that is different from that of the working myocardium [30]. In atrial and ventricular cardiomyocytes (Figure 1A), the inward rectifier K^+ channel current (I_{K1}) repolarizes the membrane potential towards the K^+ equilibrium potential to maintain a resting membrane potential of around -80 mV. The rapid opening of the voltage-dependent Na^+ channel results in a rapid depolarization forming the action potential upstroke. Class I antiarrhythmic drugs inhibit the propagation of excitation through inhibition of this rapid depolarization. In contrast, in the sinus node cardiomyocytes which lack I_{K1} (Figure 1B), the membrane potential oscillates in a more depolarized membrane potential range. The maximum diastolic potential of the sinus node is -55 to -65 mV, at which the voltage-dependent Na^+ channels are mostly inactivated. Thus, the contribution of the Na^+ channel current to the sinus node action potential is very small, if any. In fact, tetrodotoxin, which blocks both the transient and persistent components of the Na^+ channel current, has no effect or only a small effect on the sinus node action potential waveform and firing rate [31–33]. Moreover, the negative chronotropic effects of Class I antiarrhythmic drugs did not correlate with their Na^+ channel-blocking effects (Figure 3). These results and considerations confirm that factors other than the Na^+ channel blockade are the cause for negative chronotropy.

In the sinus node, the action potential upstroke is formed by the L-type Ca^{2+} channel current. This is preceded by a diastolic depolarization phase which is formed by several ionic mechanisms including the L-type Ca^{2+} channel current [30]. Pharmacological inhibition of the diastolic depolarization prolongs the time until the next rapid depolarization and causes negative chronotropy. The L-type Ca^{2+} channel current is the most important pacemaking current because the membrane potential range for diastolic depolarization overlaps with the threshold voltage range for the activation of the L-type Ca^{2+} channel. Ca^{2+} antagonists including Class IV antiarrhythmic drugs are reported to block the L-type Ca^{2+} channel and decrease the slope of the diastolic depolarization, as well as the rapid depolarization, resulting in negative chronotropy [34]. In the present study, both the concentration-response relationship analysis (Figure 3C) and the correlation analysis with IC_{50} values (Figure 3D) showed that the chronotropic effects of Class I antiarrhythmic drugs strongly correlate with their L-type Ca^{2+} channel blocking potencies (Figure 3). At concentrations corresponding to their estimated IC_{50} value for the L-type Ca^{2+} channel blockade, the beating rate of the right atria was decreased to about 80% of the initial rate.

This was the same as the case with the Ca^{2+} antagonist, nifedipine [35,36]. These results indicated that the negative chronotropic effects of Class I antiarrhythmic drugs are mediated by the L-type Ca^{2+} channel blockade.

Other ionic mechanisms that are involved in the diastolic depolarization are unlikely to be the major mechanism mediating the negative chronotropic effect of Class I antiarrhythmic drugs. The time-dependent delayed rectifier K^+ channel current (I_K) increases during the action potential and causes repolarization towards the maximum diastolic potential. A gradual decline in this current also contributes to the early phase of diastolic depolarization. Blockade of the delayed rectifier K^+ channel current appears to result in negative chronotropy only at high concentrations of the blocker. E-4031, a selective blocker of the delayed rectifier K^+ channel current, only decreased the beating rate of the guinea pig right atria to 80% of the initial value at 100 nM, even though its IC_{50} value was 7.7 nM [37,38]; this data point for E-4031 would be located on the upper right position in Figure 3E. This means that the negative chronotropic effects of most Class I antiarrhythmic drugs are stronger than those that are attributable to K^+ channel blockade, and implies that mechanisms other than the K^+ channel blockade are the determinants of negative chronotropic potency. The observation that the correlation between negative chronotropy and delayed rectifier K^+ channel blockade was relatively weak also supports this view (Figure 3E,F).

The hyperpolarization-activated cation current (If) has been postulated to play a major role for pacemaking of the sinus node [39], although a controversy still remains concerning its degree of contribution [40]. Ivabradine, a blocker of this channel, decreases beating rate and is clinically used as a bradycardiac agent, but the drug was reported to also block the delayed rectifier K^+ channel current [41]. Concerning the Class I antiarrhythmic drugs that were examined, aprindine, cibenzoline, and propafenone were reported to block this current with IC_{50} values of 43.7 μM, 46.8 μM, and 14.3 μM, respectively [12]. Other drugs have very weak or no effect on this channel. This suggests that blocking the hyperpolarization-activated cation current might also play a role in negative chronotropy, particularly for propafenone. However, blockade of the hyperpolarization-activated cation current cannot be the major determinant of the negative chronotropic effects among Class I antiarrhythmic drugs as the blocking effect of other drugs is either very weak or absent.

The Na^+-Ca^{2+} exchanger generates a net inward current when it pumps out one Ca^{2+} in exchange for the influx of three Na^+. This current has been postulated to contribute to the diastolic depolarization of the sinus node [33], but concerning the guinea pig sinus node, experimental [8] and computational [42] evidence against the contribution of this current has also been reported. Among the Class I antiarrhythmic drugs that were examined in the present study, only aprindine and cibenzoline were reported to inhibit this current with IC_{50} values of 84 μM and 52 μM, respectively [43–45].

The T-type Ca^{2+} channel current was reported to be involved in the diastolic depolarization of the sinus node in various animal species; the contribution of this current appears to be larger in animals of smaller size, during early developmental stages, and under pathological conditions [46,47]. A selective T-type Ca^{2+} channel blocker, *R*(-)-efonidipine, caused negative chronotropy in the guinea pig right atria, but the effect was small [48]. The sustained inward current is an inward current that is activated in the membrane potential range of the diastolic depolarization of the sinus node [49], whose molecular identity was reported to be a subtype of the voltage-dependent L-type Ca^{2+} channel, CaV1.3 [50]. To the best of our knowledge, blockade of the T-type Ca^{2+} channel or the CaV1.3 L-type Ca^{2+} channel has not been reported with the antiarrhythmic drugs that were examined in the present study.

The present results and the discussion above led us to the conclusion that the major common mechanism for the negative chronotropic effects of Class I antiarrhythmic drugs is the blockade of the L-type Ca^{2+} channel. At concentrations corresponding to their reported IC_{50} values for the L-type Ca^{2+} channel blockade, the beating rate of the right atria was decreased to about 80% of the initial value. This is different from the case with negative inotropy, in which the contractile force was decreased to about 50% of the initial value

at concentrations corresponding to their reported IC_{50} value for the L-type Ca^{2+} channel blockade [6]. In the case of myocardial contraction, the intracellular Ca^{2+} activating the myofilaments is supplied either by the transsarcolemmal influx through the L-type Ca^{2+} channel or by the subsequent Ca^{2+}-induced-Ca^{2+} release from the sarcoplasmic reticulum. Thus, the L-type Ca^{2+} channel is involved in the contractile mechanism, and it is reasonable that the extent of Ca^{2+} channel blockade directly correlates with negative inotropy. In contrast, in the case of sinus node pacemaking, the L-type Ca^{2+} channel and other ionic mechanisms mentioned above work additively to form the diastolic depolarization. Thus, the role of the L-type Ca^{2+} channel blockade in the negative chronotropy induced by class I antiarrhythmic drugs is partial.

Our present and previous studies indicated that the Na^+ channel blockade itself plays only a minor role in the negative chronotropic and inotropic effects of Class I antiarrhythmic drugs [6]. This implies that modifying the mode of action of Class I antiarrhythmic drugs on the Na^+ channel may increase their therapeutic potential without increasing the risk of cardiosuppression. A promising trend is the development of blockers selective for the persistent component of the Na^+ channel current, late I_{Na} [51]. Ranolazine, a late I_{Na} blocker with multiple sites of action on the sarcolemma and sarcoplasmic reticulum, and also affects energy metabolism [29,52,53], showed a weak negative chronotropic effect (Figure 2). More selective blockers of late I_{Na}, GS-458967 [54] and NCC-3902 [7], were reported to inhibit the automaticity of the pulmonary vein myocardium, which is attracting attention as the ectopic pacemaker that is responsible for atrial fibrillation [55,56]. In canine rapid atrial pacing models, NCC-3902 prolonged the effective refractory period and intra-atrial conduction time in a dose-dependent manner and suppressed the induction of atrial fibrillation [57]. These compounds appear to have no cardiosuppressive or arrhythmogenic effects so far, and their development as antiarrhythmic drugs are highly anticipated.

In conclusion, the present study showed that the negative chronotropic effects of Class I antiarrhythmic drugs correlate with the blockade of Ca^{2+} channels, but not with the Na^+ and K^+ channels. Thus, novel blockers of the Na^+ channel with minimum cardiosuppression can probably be developed, provided that they have no Ca^{2+} channel-blocking activity.

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Institutional Review Board Statement: All experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and the Guide for the Care and Use of Laboratory Animals at the Faculty of Pharmaceutical Sciences, Toho University (22-41-507).

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