after reperfusion. In fact, SEA0400 enhanced the recovery of contractile force after reperfusion, even when it was applied only during the pre-ischemic and ischemic periods and washed out on reperfusion [10]. Also, in coronary perfused rabbit [22] and rat [23] hearts, SEA0400 has been reported to enhance the recovery of left ventricular developed pressure and +dP/dt, respectively, even when applied only before ischemia. This could be attributed to the reduction of irreversible mitochondrial damage by SEA0400. It has been reported that, in cardiomyocytes, an elevated mitochondrial Ca^{2+} concentration during experimental ischemia correlated with the occurrence of cell hypercontracture after reperfusion [34]. On the other hand, enhanced recovery of contractile force was observed when SEA0400 was applied only during reperfusion [10]. On reperfusion, the washout of extracellular protons accelerates extrusion by the Na^+-H^+ exchanger of intracellular protons accumulated during ischemia. This causes an increase in intracellular Na^+ concentration, which in turn causes rapid influx of Ca^{2+} through the reverse mode NCX and leads to intracellular Ca^{2+} overload. SEA0400 applied on reperfusion could inhibit this rapid Ca^{2+} influx. Thus, the inhibition of the reverse mode NCX could be cardioprotective both during ischemia and during reperfusion.

Pharmacological agents, such as Na^+ and Ca^{2+} channel blockers [3], β-blockers [35], and K^+ channel openers [36] have cardioprotective effects against ischemia-reperfusion damage. A common feature of these agents appears to be cardiosuppression; a reduced heart rate and a reduced contractile force result in reduced oxygen requirements and the preservation of high-energy phosphonucleotides. We examined the effects of these types of drugs on guinea pig coronary-perfused right ventricular tissue preparations during experimental ischemia and reperfusion, and our results were consistent with this view [16, 37]. However, the suppression of cardiac function may be a disadvantage of these drugs. These agents, either directly or indirectly, inhibit Ca^{2+} influx through the L-type Ca^{2+} channel, which is the major trigger for Ca^{2+} release from the sarcoplasmic reticulum. In ventricular cardiomyocytes, L-type Ca^{2+} channels exist on the T-tubular membrane in close proximity to the ryanodine receptor/Ca^{2+} release channel located on the junctional sarcoplasmic reticulum [38]. Functional experiments suggest that a Ca^{2+} microdomain exists and includes the L-type Ca^{2+} channel and the ryanodine receptor/Ca^{2+} release channel [39]. On the other hand, the NCX protein exists on the T-tubular membrane but appears to be excluded from the Ca^{2+} microdomain; the efficiency of Ca^{2+} influx through the NCX to trigger Ca^{2+} release is through the

ryanodine receptor/Ca^{2+} release channel is too low to contribute to myocardial contraction. Thus, it is reasonable that inhibition of Ca^{2+} influx through the reverse mode Na^{+}-Ca^{2+} exchanger has no cardiosuppressive effects. Therefore, inhibition of the reverse mode NCX appears to be an ideal mechanism by which to attenuate Ca^{2+} overload under ischemia.

Ischemia-Reperfusion-Induced Arrhythmia

The NCX also appears to be involved in arrhythmia induced by ischemia-reperfusion. In the guinea-pig coronary perfused ischemia-reperfusion model, intermittent arrhythmia was observed after reperfusion in all of the preparations either untreated or treated with SEA0400 [10]. SEA0400 could not prevent the occurrence of arrhythmia on reperfusion, but reduced the incidence of arrhythmia during the period between 10 and 60 min after reperfusion. In the ischemia-reperfusion arrhythmia model of anesthetized rats, SEA0400 significantly reduced the incidence of ventricular fibrillation and mortality rate [23]. Direct or indirect effects of SEA0400 on myocardial refractory period might be involved in its anti-arrhythmic action. In this connection, an interesting coincidence is that enhanced recovery of APD and reduction of arrhythmic contraction both became prominent at 10 min after reperfusion [10]. Ischemia produces a decrease in tissue ATP content, which is only partially recovered after reperfusion [15]. A decrease in tissue ATP results in opening of the ATP sensitive potassium channel, shortening of the APD, and decrease in contractile force. As SEA0400 enhanced the recovery of tissue ATP after reperfusion, the most probable explanation for the antiarrhythmic effect of SEA0400 is enhanced recovery of APD and refractory period. However, as NCX is present not only on the sarcolemma but also on the mitochondrial inner membrane and that its inhibition results in augmentation of ATP production [40], the direct and indirect effects of SEA0400 on mitochondrial NCX and ATP production remain to be investigated.

Effect of NCX Inhibition on Ouabain-induced Arrhythmia

Ouabain has been considered to increase intracellular Na^{+} concentration, shift the balance of the two modes of NCX to favor the reverse mode and increase cellular Ca^{2+} load. When this Ca^{2+} load exceeds the capacity of the SR, abnormal Ca^{2+} release from the SR occurs, which in turn triggers abnormal electrical activity and arrhythmic contractions. In isolated guinea-pig ventricular preparations, the ouabain-induced

increase in basal tension and arrhythmic contractions were significantly reduced by SEA0400 [12]. Arrhythmic contractions were preceded by small oscillations in resting tension suggesting that intracellular Ca^{2+} oscillations were acting as their trigger. This provides pharmacological evidence that NCX plays a crucial role in ouabain-induced arrhythmogenesis. It was also reported that SEA0400 attenuated ouabain-induced arrhythmia in a canine *in vivo* model [41] and *in* isolated Purkinje fibers [42]. Further, long term administration of SEA0400 to Dahl salt-sensitive rats attenuated the ouabain-induced rise in intracellular Ca^{2+}, and fibrosis, which suggests that Ca^{2+} entry via reverse mode NCX induced by the endogenous digitalis-like factor is involved in the development of fibrosis and heart failure [43].

Automaticity of the Pulmonary Vein Myocardium

Pulmonary veins are considered to be involved in the initiation and maintenance of atrial fibrillation, one of the most frequent arrhythmia in clinical practice [44]. Pulmonary veins contain a myocardial layer, whose electrical activity is considered to underlie their arrhythmogenic activity [45]. The pulmonary vein myocardium has different electrophysiological properties from those of the working myocardium including lower density of I_{K1} and a less negative resting membrane potential [46]. The precise mechanisms of the pulmonary vein electrical activity as well as its pharmacological properties are now receiving attention as the basis to develop an effective therapeutic strategy against atrial fibrillation. We applied microelectrode techniques to the myocardial layer of isolated guinea-pig pulmonary veins, and examined the effects of SEA0400 and ryanodine on the spontaneous and ouabain-induced electrical activity. About 15% of the guinea-pig pulmonary vein preparations showed spontaneous activity. The incidence was increased to 95% by ouabain, which induces intracellular Ca^{2+} overload through inhibition of the Na^+/K^+ ATPase [12]. Increased cellular Ca^{2+} load can cause spontaneous Ca^{2+} release from the SR. If this occurs during the diastolic period, this would accelerate the diastolic depolarization through activation of the forward-mode NCX, and elicit spontaneous electrical activity. Our present results that both spontaneous and ouabain-induced electrical activity were inhibited by ryanodine and SEA0400 suggest that this is indeed the case in the pulmonary vein myocardium. Acceleration of late repolarization and reduction of the slope of diastolic depolarization by SEA0400 [47] supports the view that Ca^{2+} extrusion through forward mode NCX occurs during the repolarization phase to the diastolic

depolarization phase. However, functional components of the intracellular Ca^{2+}-induced depolarization such as the sarcoplasmic reticulum and NCX are present not only in the pulmonary vein, but also in the working myocardium. In fact, depolarization induced by the forward mode NCX was reported to be involved in the generation of fibrillation and torsades de pointes in the ventricular myocardium [48-50]. Thus, the pulmonary vein myocardium possibly has some characteristic feature which underlies its tendency to generate spontaneous activity.

The pulmonary vein myocardium appears to have different electrophysiological properties from those of the working myocardium. Ouabain, at a concentration of 1 μM, induced spontaneous activity in the guinea-pig pulmonary vein myocardium but not in the ventricular myocardium [12]. The resting membrane potential of the guinea-pig pulmonary vein myocardial cells was about -75.4 mV, which was less negative than that of the guinea-pig atria and ventricle (-78 to -85 mV; [51]). The current density of I_{K1}, the major membrane current responsible for the maintenance of the resting membrane potential, was reported to be smaller in the pulmonary vein cardiomyocyte [46]. The smaller contribution of I_{K1} around the resting membrane potential would allow underlying depolarizing mechanisms to cause diastolic depolarization leading to the generation of spontaneous action potentials in the pulmonary vein myocardium. On the other hand, abnormalities in intracellular Ca^{2+} homeostasis induced by factors such as increased mechanical stretch has been suggested to underlie the generation of ectopic activity in the pulmonary vein [52]. The Ca^{2+} influx through stretch-activated cation channels may load the SR above its capacity and cause spontaneous focal Ca^{2+} release from the SR. This would lead to the generation of arrhythmias through forward-mode NCX activity. The present results with SEA0400 indicate that Ca^{2+} overload-induced spontaneous activity in the pulmonary vein myocardium can be reduced by NCX inhibition. Thus, NCX inhibition may be an effective therapeutic strategy for the treatment of atrial fibrillation of pulmonary vein origin.

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