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AHC-52, a Dihydropyridine Compound with Chloride Current Blocking and Cardioprotective Activities

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Key Words: AHC-52—AHC-93—Chloride current—p-Glycoprotein— Ischemia-reperfusion—Myocardium.

ABSTRACT

AHC-52 is a dihydropyridine compound known to reverse multidrug resistance through inhibition of the P-glycoprotein. In whole-cell voltage-clamped cardiomyocytes, AHC-52 had no effect on L-type calcium current but inhibited cAMP-activated chloride current. In an ischemia-reperfusion model with coronary-perfused right ventricular tissue, AHC-52 markedly enhanced the recovery of contractile force on reperfusion without any negative inotropic effect during normal perfusion and experimental ischemia, which is similar to the effects of chloride blockers such as 9-anthracenecarboxylic acid (9AC) and 4-acetamido-4′isothiocyanostilbene-2,2 disulfonic acid (SITS). Thus, AHC-52 appears to be an interesting prototype of cardioprotective drugs acting through mechanisms related to modification of anion homeostasis.

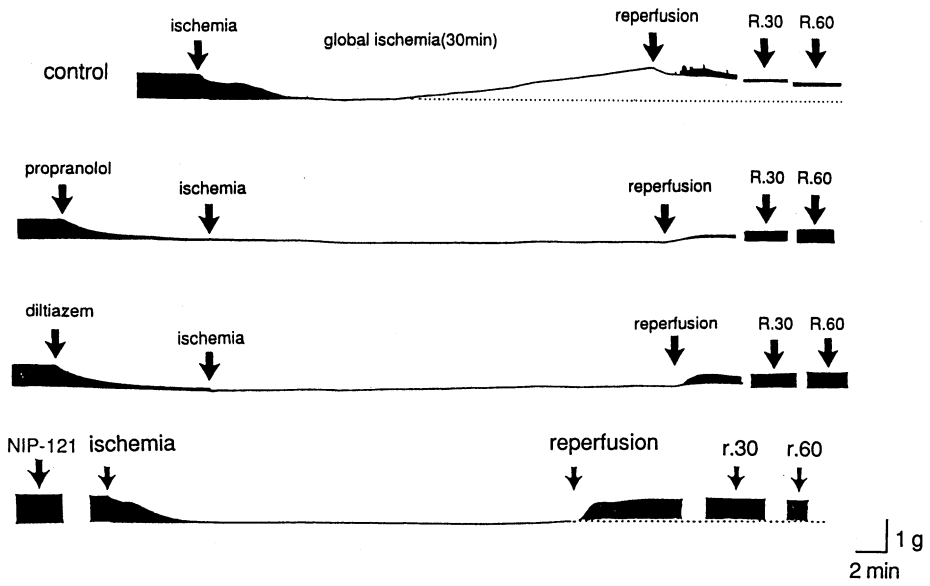

FIG. 1. Effects of propranolol, diltiazem, and NIP-121, a potent ATP-sensitive K^+ channel opener, on the force of contraction of coronary-perfused right ventricular preparations. Start of treatment with 30 μM propranolol, 10 μM diltiazem, and 0.3 μM NIP-121 are indicated with arrows. r.30 and r.60 indicate 30 and 60 min after reperfusion, respectively. From refs. 26,27.

preparation subjected to experimental ischemia were presented in Fig. 1 (26,27). The preparations were subjected to 30 min of no-flow ischemia in the presence and absence of drugs followed by 60 min of reperfusion. In untreated preparations, a decrease in contractile force and an increase in resting tension were observed during the no-flow period. On reperfusion, transient arrhythmias were observed and contractile force returned to less than 50% of preischemic values. In preparations pretreated with propranolol, diltiazem, or NIP-121*, a potent ATP-sensitive K^+ channel opener (14), the recovery of contractile force was enhanced to more than 70% of initial values. However, propranolol and diltiazem greatly decreased the contractile force under normal perfusion, and NIP-121 accelerated the decline in contractile force under experimental ischemia. Thus, although shown to be cardioprotective in various experimental models, clinical administration of drugs with negative inotropic activity may not be desirable because of their cardiosuppressive effects, especially in the case of patients with a tendency toward heart failure. Thus, it is of value to search for cardioprotective agents acting through entirely different mechanisms.

ANION HOMEOSTASIS IN THE HEART

In contrast to the extensively accumulated knowledge on myocardial cation homeostasis and its modification by pharmacological interventions, less information is available

* NIP-121 is (+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidin-1-yl)-6H-pyrano-(2,3-f)benz-2,1,3-oxadiazole.

on myocardial anion homeostasis (9). Among the negative charge carriers in the myocardium (i.e., Cl^-, organic acids, phosphate, sulfates) the major permeant anion is Cl^- (8). Ion-selective microelectrode studies have shown the intracellular Cl^- concentration (activity) of mammalian Purkinje fiber and ventricle to be 10 to 20 mM, which is higher than predicted for passive diffusion of Cl^- 4 to 6 mM (30). Several channels and transporters are known to be involved in the regulation of Cl^- concentration. The cAMP-activated Cl^- channel, a cardiac isoform of the cystic fibrosis transmembrane regulator (CFTR) (6), is activated by stimuli such as β-adrenergic stimulation and is inhibited by compounds, such as anthracene-9-carboxylic acid (9AC) (32). The Cl^-/HCO_3^- exchanger is involved also in the regulation of intracellular pH and is sensitive to 4-acetamide-4′-isothiocyanato-stilbene-2,2′-disulfonic acid (SITS) (31). The estimated intracellular Cl^- concentration places the equilibrium potential for Cl^- in the range of –65 to –45 mV under normal physiological conditions. Thus, activation of Cl^- currents would contribute to both inward and outward current depending on the membrane potential (e.g., repolarizing outward currents during the action potential plateau phase and depolarizing current near the resting membrane potential). In the case of the cAMP-dependent Cl^- current as well as some other Cl^- currents, the major physiological role may be to minimize the significant action potential prolongation associated with β-adrenergic stimulation of I_{Ca}. Arrhythmogenic and antiarrhythmic effects of certain pharmacological interventions were partly ascribed to activation and inhibition of Cl^- currents (9). Ion-sensitive microelectrode measurements revealed that intracellular Cl^- activity increases during myocardial ischemia (11). It was also reported that substitution of extracellular Cl^- with NO_2^- prevents ischemia and reperfusion-induced arrhythmia in Langendorff heart preparations (2,20). Thus, although its exact role in human cardiac pathophysiology is unclear at this time, Cl^- channels and transporters might be considered as novel potential targets for the development of antiarrhythmic and cardioprotective agents.

CARDIOPROTECTIVE EFFECTS OF Cl^- BLOCKERS

To examine whether Cl^- blockers have protective effects against myocardial ischemia-reperfusion injury, we applied chloride blockers 9AC and SITS to the above mentioned ischemia-reperfusion model (25). We found that the two agents significantly enhanced the recovery of developed tension after reperfusion (Fig. 2). A remarkable feature of both agents was that they did not show any negative inotropic effect both under normal conditions and during experimental ischemia. This is different from the above mentioned cardioprotective agents, which act through manipulation of cation movements. Simultaneous microelectrode recordings showed that the two agents slowed the decline in action potential duration during experimental ischemia, which was suggested to be due to reduced flow of outward Cl^- current. Measurement of intracellular pH with pH-sensitive fluoroprobes showed intracellular acidification during experimental ischemia was significantly attenuated in SITS-treated preparations but not in 9AC-treated preparations, whereas the effects of the two agents on the contractile force and action potential configuration were almost the same. Thus, although the precise mechanisms were not clear, protection from increases in intracellular Cl^- concentration, rather than reduction of acidification, appeared to be important for the cardioprotective effects of the two Cl^- blockers. In

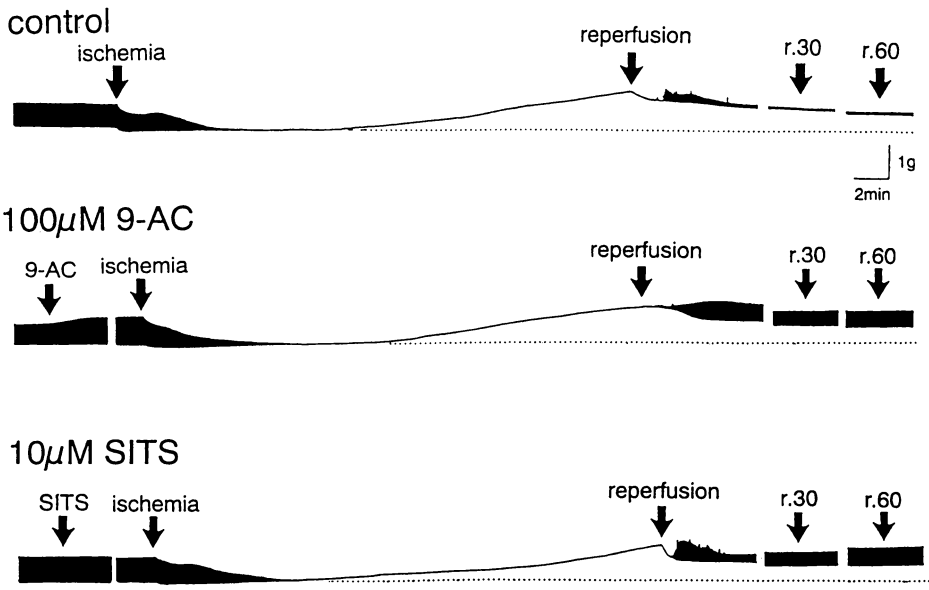

FIG. 2. Effects of 9AC and SITS on force of contraction of coronary-perfused right ventricular preparations. Start of treatment with 100 μM 9AC or 10 μM SITS are indicated as 9AC and SITS, respectively. r.30 and r.60 indicate 30 and 60 min after reperfusion, respectively. From ref. 25.

any case, these results encouraged further investigation and search for clinically applicable agents acting on Cl^- movements.

RE-EVALUATION OF DIHYDROPYRIDINE COMPOUNDS

On the other hand, dihydropyridine compounds, which are well known to inhibit the L-type Ca^{2+} channel, may also have effects on other membrane channels which could improve their therapeutic potential. For example, we have found that efonidipine, a phosphonated dihydropyridine compound with inhibitory effects on both L-type and T-type Ca^{2+} channels, shows potent bradycardic effects through a characteristic prolongation of the phase 4 depolarization of the cardiac pacemaker (12,13), which leads to minimum reflex tachycardia or to bradycardia during its experimental and clinical application (5). Thus, re-evaluation of dihydropyridine compounds might lead to the discovery of therapeutic agents which act through novel mechanisms.

In the field of anticancer chemotherapy, acquired multidrug resistance has been a major obstacle. Multidrug resistance is attributed to the expression of the MDR1 gene which encodes for a plasma membrane glycoprotein (P-glycoprotein) known to be a multidrug efflux pump (4). Multidrug resistance is reversed by a variety of compounds which inhibit P-glycoprotein function, allowing anticancer agents to accumulate in multidrug-resistant cells. The dihydropyridine compound AHC-52, methyl 2-(*N*-benzyl-*N*-methylamino)ethyl-2,6-dimethyl-4-(2-isopropyl-pyrazolo[1,5-a]pyridine-3-yl)-1,4-dihydropyridine-3,5-dicarboxylate (Fig. 3), has also been reported to reverse multidrug resistance by inhib-

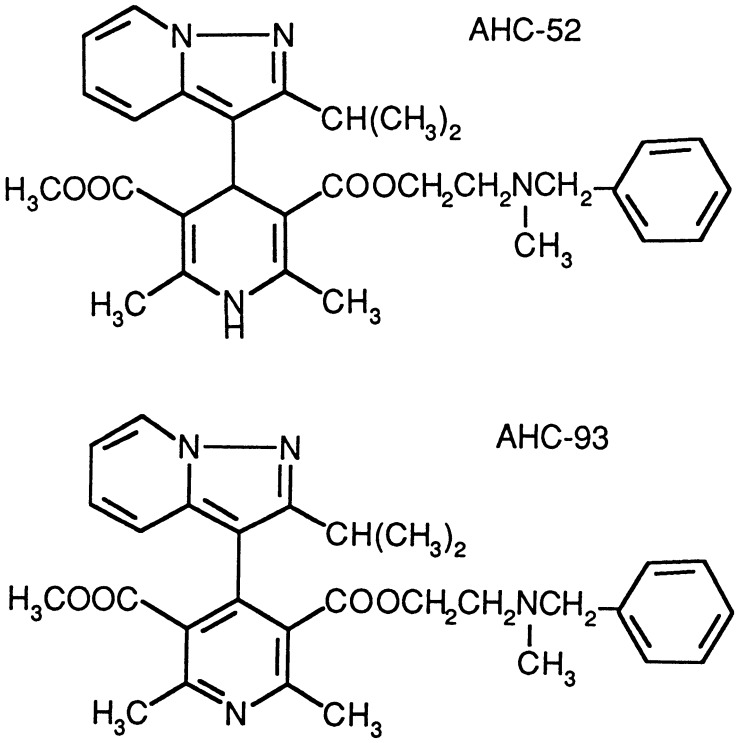

FIG. 3. Chemical structure of AHC-52 and AHC-93.

iting P-glycoprotein (10,23). As the P-glycoprotein and cAMP-activated Cl^- channel are known to have structural homology and belong to the ABC superfamily of transporters (7,9), it is possible that pharmacological agents which act on the P-glycoprotein also affect the cAMP-activated Cl^- channel and thus have protective effects against myocardial ischemia-reperfusion injury. Thus, we studied the effect of AHC-52 on the myocardium under normal conditions and during experimental ischemia (28).

CARDIOPROTECTIVE EFFECTS OF AHC-52

In coronary-perfused ventricular preparations, AHC-52 (0.1 μM) had no effect on the mechanical parameters of this preparation under normal conditions (Fig. 4A). During ischemia, AHC-52 did not affect the decline in developed tension but inhibited the rise in resting tension. Recovery of developed tension on reperfusion was improved to about 80% of preischemic values in preparations pretreated with AHC-52 (Fig. 4B). The protective effects of AHC-52, enhanced recovery of contractile force after reperfusion with no effect during normal conditions, were similar to the effects of Cl^- blockers 9AC and SITS (Fig. 2) (25). Thus, we applied AHC-52 to myocardial preparations under normoxic conditions to clarify the pharmacological profile of the drug (unpublished observations). In isolated atrial and ventricular tissue preparations of the guinea pig myocardium,

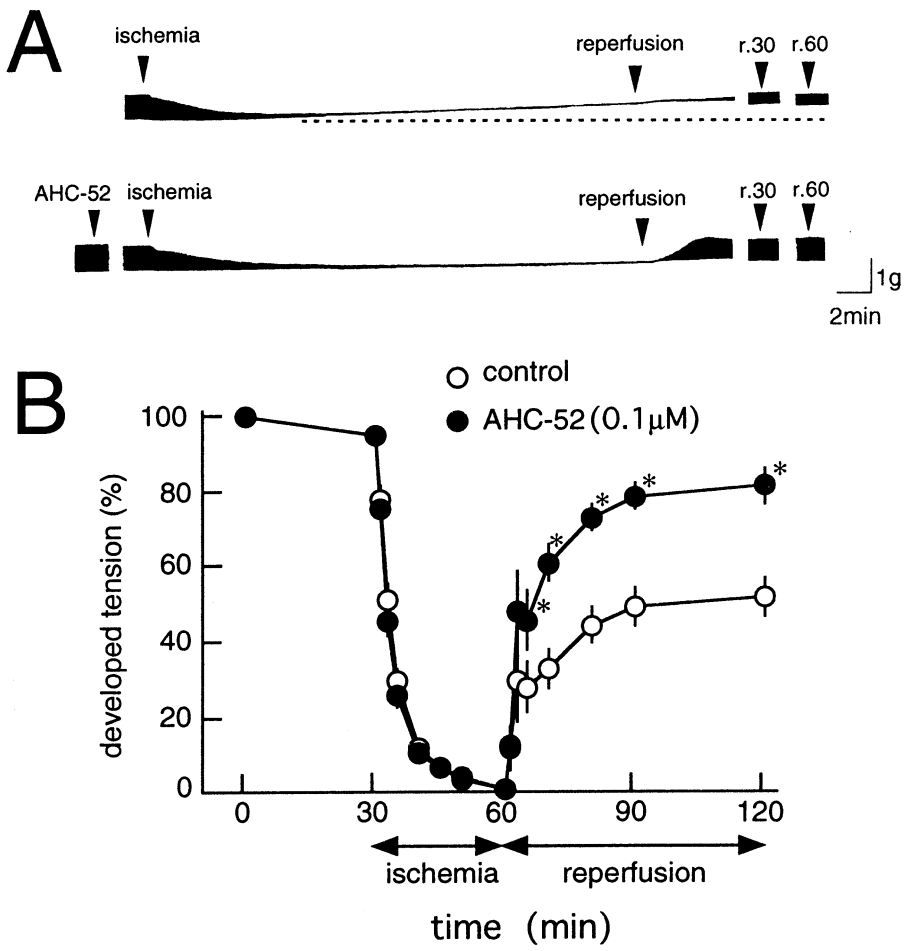

FIG. 4. Effects of AHC-52 (0.1 μM) on force of contraction of coronary-perfused right ventricular preparations. A: Typical traces. Arrowheads: AHC-52, start of treatment with AHC-52; r.30 and r.60 indicate 30 and 60 min after reperfusion, respectively. B: Summarized data. AHC-52 was added at 0 min. Each point with vertical bars represents the mean ± S.E.M. of five preparations. Asterisks indicate significant difference ($P < 0.05$) from corresponding control values as evaluated by the unpaired *t*-test. From ref. 28.

AHC-52 showed slight negative inotropic effects; decrease in contractile force by 10^{-5} M AHC-52 was less than 10% of the basal contractile force in both regions. AHC-52, at 10^{-6} M, had no effect on the concentration-response curve for the positive inotropic effect of isoproterenol on isolated papillary muscles, indicating the lack of β-adrenergic blocking activity. AHC-52, at 10^{-6} M, had no effect on the action potential configuration. In isolated aorta, AHC-52, up to 10^{-6} M, had no effect on the tension development induced by norepinephrine; relaxation was observed at 10^{-5} M. In whole cell voltage-clamped ventricular myocytes (28), 1 μM AHC-52 had no significant effect on the peak inward and steady state membrane currents (Fig. 5A). Thus, AHC-52, at 10^{-6} M, appeared to have no or minimum Ca^{2+} antagonistic activity despite its dihydropyridine structure.

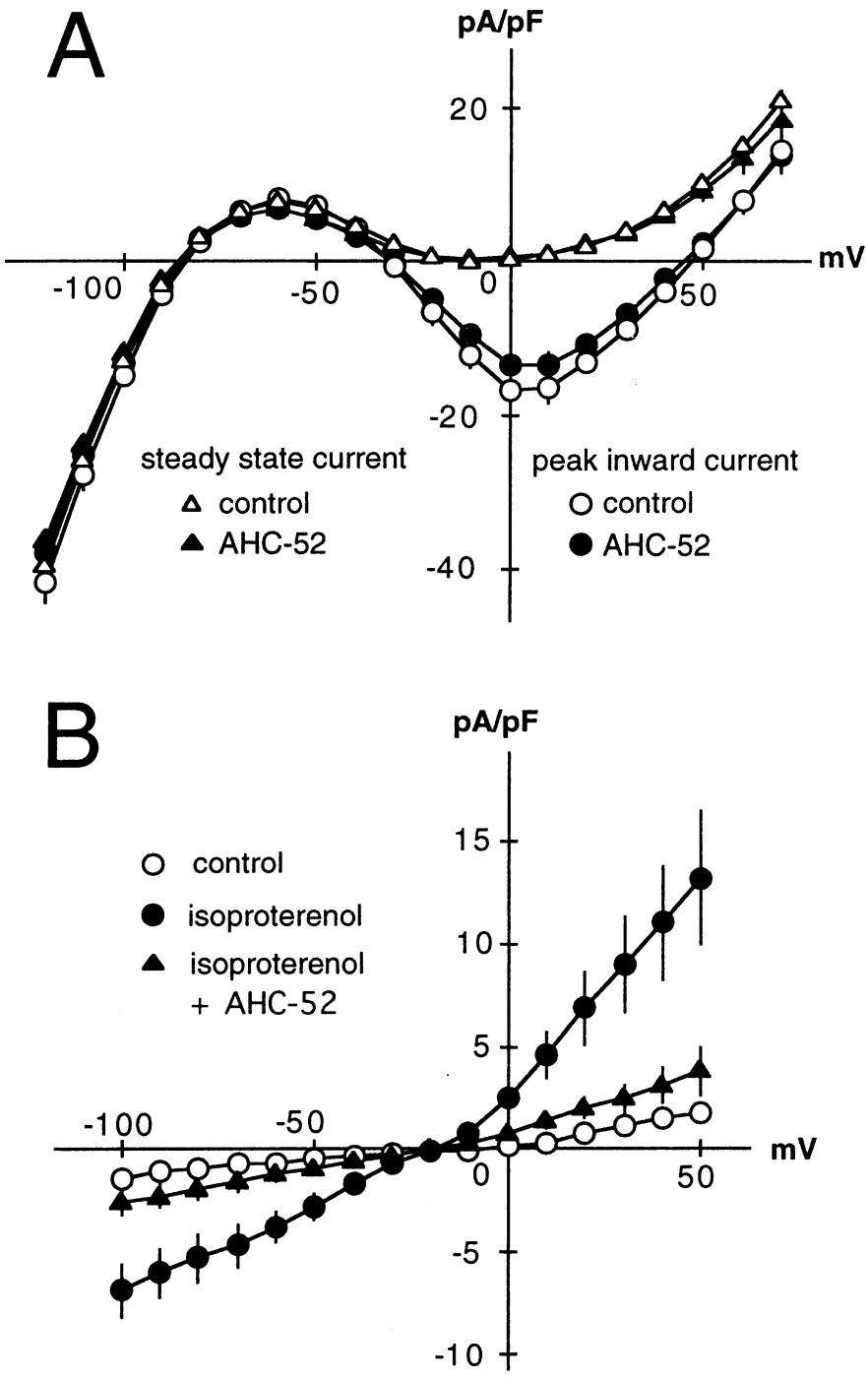

FIG. 5. Effect of AHC-52 (1 μM) on membrane currents in isolated guinea pig ventricular cells. A: Peak inward and steady-state currents elicited by 300-msec voltage-clamp pulses from a holding potential of –40 mV. B: Isoproterenol-induced chloride current at the end of 200-msec voltage-clamp pulses. Each point with vertical bars represents the mean ± S.E.M. of five preparations. From ref. 28.

Ca^{2+} antagonistic effects of AHC-52 under ischemic conditions, however, cannot be totally ruled out because the drug inhibited the rise in basal tension during experimental ischemia (Fig. 4A), an effect observed also with Ca^{2+} antagonists.

Cl^- BLOCKING EFFECTS OF AHC-52

In ventricular myocytes, isoproterenol (1 μM) induced a time-independent Cl^- current which had a slightly outward rectifying voltage dependence. AHC-52 markedly inhibited this current at all membrane potentials examined (Fig. 4B). AHC-52, at 0.1 μM and 1 μM, reversibly inhibited the isopreterenol-induced chloride current at +50 mV by about 60 and 90%, respectively, of that in the absence of the drug. Thus, the cardioprotective effect of AHC-52 against ischemia and reperfusion damage was suggested to be related to modulation of chloride homeostasis. Basically the same experiments were performed also with another structurally related compound, AHC-93, 3-[2-(*N*-benzyl-*N*-methylamino)-1-ethoxycarbonyl]-5-methoxycarbonyl-2,6-dimethyl-4-[3-(2-isopropylpyrazolo[1,5–a]pyridine (Fig. 3), and the results were almost the same as those obtained with AHC-52, both under normoxic conditions and during experimental ischemia and reperfusion (22). These two compounds appear to be pharmacologically identical so far but differ in their fluorescent profiles. AHC-52 is highly fluorescent when excited at 351 nm and, thus, may provide information on the cellular localization of the drug. AHC-93 is virtually nonfluorescent and, thus, may be used in combination with ion-sensitive fluoroprobes such as indo-I.

POSSIBLE MECHANISMS OF AHC-52 ACTION

Although it is highly possible these compounds enhance contractile force recovery after ischemia-reperfusion through modification of Cl^- homeostasis, the precise mechanism of the cardioprotective effect remains to be clarified. AHC-52 may possibly affect intracellular pH because intracellular Cl^- homeostasis is related to intracellular pH through the sarcolemmal Cl^-/HCO_3^- exchanger. In fact, in cultured YTN cells, a human natural killer cell derived cell line, AHC-52 was reported to affect intracellular pH (33); however, results with 9AC and SITS mentioned above (25) might indicate that intracellular pH itself is not the critical factor for myocardial ischemia-reperfusion damage. Confocal imaging of the distribution of AHC-52 revealed the drug is quickly taken up by the cell and is distributed throughout the cytoplasm when applied to the extracellular solution (unpublished observation). This observation raises the possibility that the drug may have an intracellular site of action. A number of anion channels have recently been identified in membranes of intracellular organelles, such as the mitochondria, sarcoplasmic reticulum, and the nuclear envelope (9), which might be related to the pharmacological effects of AHC-52 and AHC-93. There are also results suggesting AHC-93 is involved in the pH regulation of lysozomes in YTN cells (24). Furthermore, it can be speculated that agents affecting the P-glycoprotein and/or Cl^- channels might exert cardioprotective effects in vivo through effects on nonmyocardial cells. It has been suggested that heart failure may be mediated by the biological effects of immune cells and released cytokines (21). Vesnarinone, an inotropic agent with long-term beneficial effects on congestive heart failure, is considered to exert its cardioprotective effects by inhibiting natural killer cell activity and reducing cytokine production by lymphocytes (15). In this con-

nection, it is interesting that AHC-52 (10), as well as AHC-93 (24) inhibited natural killer cell-mediated cytotoxicity. Thus, although much remains to be clarified, AHC-52 and AHC-93 appear to be interesting prototypes of cardioprotective drugs which act through mechanisms related to modification of anion homeostasis.

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