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Short Communication

Cardiac Ca^{2+} Channel-Blocking Effects of the Cyproheptadine Derivative AH-1058 in Isolated Guinea Pig Cardiomyocytes

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Abstract. The Ca^{2+} channel-blocking efficacy of the cyproheptadine derivative AH-1058 (4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1-[(*E*)-3-(3-methoxy-2-nitro)phenyl-2-propenyl]piperidine hydrochloride) was quantitatively assessed using isolated guinea pig cardiomyocytes. AH-1058 (0.001 – 10 μM) and its mother compound cyproheptadine (1 – 100 μM) reduced the Ca^{2+} currents elicited from the holding potential of −80 or −40 mV. The IC_{50} values for cyproheptadine at holding potentials of −80 and −40 mV were 42.44 and 7.75 μM, respectively, whereas those for AH-1058 were 4.91 and 0.32 μM, respectively, whose potency was equivalent to those of the typical Ca^{2+} channel blocker verapamil. These results suggest that the introduction of the cinnamil structure to cyproheptadine can generate a potent L-type Ca^{2+} channel-blocking compound as potent as verapamil.

Keywords: AH-1058, cyproheptadine, L-type Ca^{2+} current

AH-1058, 4-(5*H*-dibenzo[*a,d*]cyclohepten-5-ylidene)-1-[(*E*)-3-(3-methoxy-2-nitro)phenyl-2-propenyl]piperidine hydrochloride (Fig. 1), is a novel Ca^{2+} channel blocker, whose chemical structure is quite different from typical Ca^{2+} channel blockers, including verapamil, diltiazem, and nifedipine (1 – 3). AH-1058 has been characterized as a cardioselective Ca^{2+} channel blocker based on its cardiovascular profiles that the drug predominantly exerts suppressive effects on cardiac functions rather than vasodilator actions assessed using isolated preparations and in vivo canine models (1, 4 – 8). Our previous electrophysiological assessment using the guinea pig cardiomyocytes has shown that AH-1058 can suppress cardiac L-type Ca^{2+} currents with both tonic- and use-dependent blocking properties in contrast to typical Ca^{2+} channel blockers like verapamil, diltiazem, and nicardipine (2). However, since the inhibitory effects of AH-1058 on the L-type Ca^{2+} channels were analyzed using a single concentration of the drug (2, 3), its Ca^{2+} channel-blocking potency is still undetermined.

The present study was designed to assess the effects of AH-1058 on L-type Ca^{2+} currents in the isolated guinea pig cardiomyocytes using a whole cell patch-clamp technique to clarify the efficacy of the drug on the cardiac L-type Ca^{2+} channels. In addition, we compared the inhibitory effects of an anti-allergic drug cyproheptadine (Fig. 1) and AH-1058 on the currents to obtain guidance to make chemical designs for new types of Ca^{2+} channel blockers since AH-1058 has been chemically synthesized based on earlier information that cyproheptadine possessed a Ca^{2+} channel-blocking action in isolated cardiac and vascular tissues (9).

All experiments were conducted according to the Animal Ethics Committee of Ajinomoto (Kawasaki).

Myocyte preparation: Single ventricular myocytes were isolated from the hearts of adult male guinea pigs (Charles River Japan, Yokohama) as previously described (2, 10). The heart was removed from the open-chest guinea pig anesthetized with pentobarbital sodium, and mounted on a modified Langendorff perfusion system for retrograde perfusion of coronary circulation with a normal HEPES-Tyrode solution. The perfused medium was then changed to a nominally Ca^{2+}-free HEPES-Tyrode solution and then to a solution containing type I collagenase (0.4 μg/ml). After digestion, the heart was perfused with a high K^+, low Cl^- solution (KB solution). The ventricular tissue was cut into small

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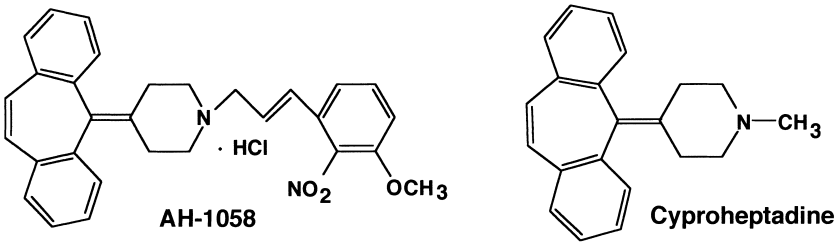

Fig. 1. Chemical structures of AH-1058 and cyproheptadine.

pieces in the KB solution and the cell suspension was stored in a refrigerator (4°C) for later use.

Solutions: The composition of external solution for the measurement of Ca^{2+} channel currents was: 144 mM tetraethylammonium chloride (TEACl), 4 mM CsCl, 0.53 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.5 mM glucose, and 5 mM HEPES-Tris-OH buffer (pH 7.4). The conventional patch-pipette solution contained: 140 mM CsCl, 5 mM $MgCl_2$, 0.28 mM $CaCl_2$, 2 mM ATP-Na_2, 5 mM EGTA, and 10 mM HEPES-KOH buffer (pH 7.2). The composition of the HEPES-Tyrode solution was: 143 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.33 mM NaH_2PO_4, 5.5 mM glucose, and 5 mM HEPES-Tris-OH buffer (pH 7.4). The composition of KB solution was: 70 mM L-glutamic acid, 30 mM KCl, 15 mM taurine, 10 mM KH_2PO_4, 0.5 mM $MgCl_2$, 11 mM glucose, 0.5 mM EGTA, and 10 mM HEPES-KOH buffer (pH 7.4).

Electrical measurements: Electrical measurements were performed using a conventional patch-clamp technique (2, 10). The pipette was pulled from 1.5 mm capillary glass (Narishige, Tokyo) in two stages of a vertical pipette puller (PB-7, Narishige). The pipette tip was fire-polished before use. The resistance between the recording electrode filled with pipette solution and the reference electrode in external solution was 2 – 4 MΩ. The current was measured with a patch-clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo), monitored on both a storage oscilloscope (DS-9121; Iwatsu, Tokyo) and a pen recorder (RECTI-HORIZ-8K; Sanei, Tokyo), and stored on video tapes after digitization with a PCM processor (RD-120TE; TEAC, Tokyo). When the series resistance was measured by a circuit for the series resistance compensation system in the amplifier, the values ranged from 6 to 10 MΩ. The value of compensation of the series resistance was 70 – 85%. The current signals were filtrated at 1 kHz (NF Instruments, Yokohama) and acquired by the use of pClamp software (Axon Instruments, Foster City, CA, USA) by a 386 IBM compatible computer (Deskpro; Compaq, Houston, TX, USA).

Drugs: AH-1058 was synthesized at the Pharmaceutical Research Laboratories of Ajinomoto Co., Inc. (Kawasaki), while cyproheptadine hydrochloride, verapamil hydrochloride, diltiazem hydrochloride, nicardipine hydrochloride, and type I collagenase were purchased from Sigma (St. Louis, MO, USA). Test drugs were dissolved in dimethylsulfoxide at 10^{-2} M and diluted by each external solution before use.

Data analysis: Data are expressed as the mean ± S.E.M. For the evaluation of the half-maximal inhibitory concentration (IC_{50}) of the concentration-inhibition curve, the data were fitted to the following equation using a least-square fitting procedure, as previously reported (10): $I/I_{max} = 1 - C^n / (C^n + IC_{50}{}^n)$, where I is current, I_{max} is the maximum response, n is Hill coefficient, and C is the concentration of the antagonist.

Typical tracings showing the effects of AH-1058 on Ca^{2+} currents are depicted in Fig. 2A. To isolate the cardiac L-type Ca^{2+} channel currents, the dissociated cardiomyocyte was held at a holding potential of −40 or −80 mV and depolarized to +20 mV. Four minutes after the treatment with AH-1058 in a concentration of 0.3 μM, the L-type Ca^{2+} channel currents elicited by depolarizing pulses from −80 mV were reduced by 15.9%, while those elicited from −40 mV were more effectively suppressed (by 55.3%). As shown in Fig. 2B, pretreatment with AH-1058 or cyproheptadine for 4 min reduced Ca^{2+} channel currents elicited by depolarizing pulses from the holding potential of −80 or −40 mV to +20 mV in a concentration-dependent manner. The slope of the concentration-$I_{Ca^{2+}}$ curves for AH-1058 was less steep than those for cyproheptadine, which may be associated with the different accessibility to their binding site between the two drugs since concentration-response curve for the displacement of a Ca^{2+} channel ligand by AH-1058 was not less steep when assessed in the brain membrane preparation (1). The effects of both drugs on the magnitude of Ca^{2+} channel currents were dependent on the holding potential; the IC_{50} values for AH-1058 and cyproheptadine at −80 mV were 4.91 ± 1.02 and 42.22 ± 16.29 μM, respectively, whereas those at −40 mV were 0.32 ± 0.24 and 7.75 ± 1.28 μM, respectively. The effects of verapamil, diltiazem and

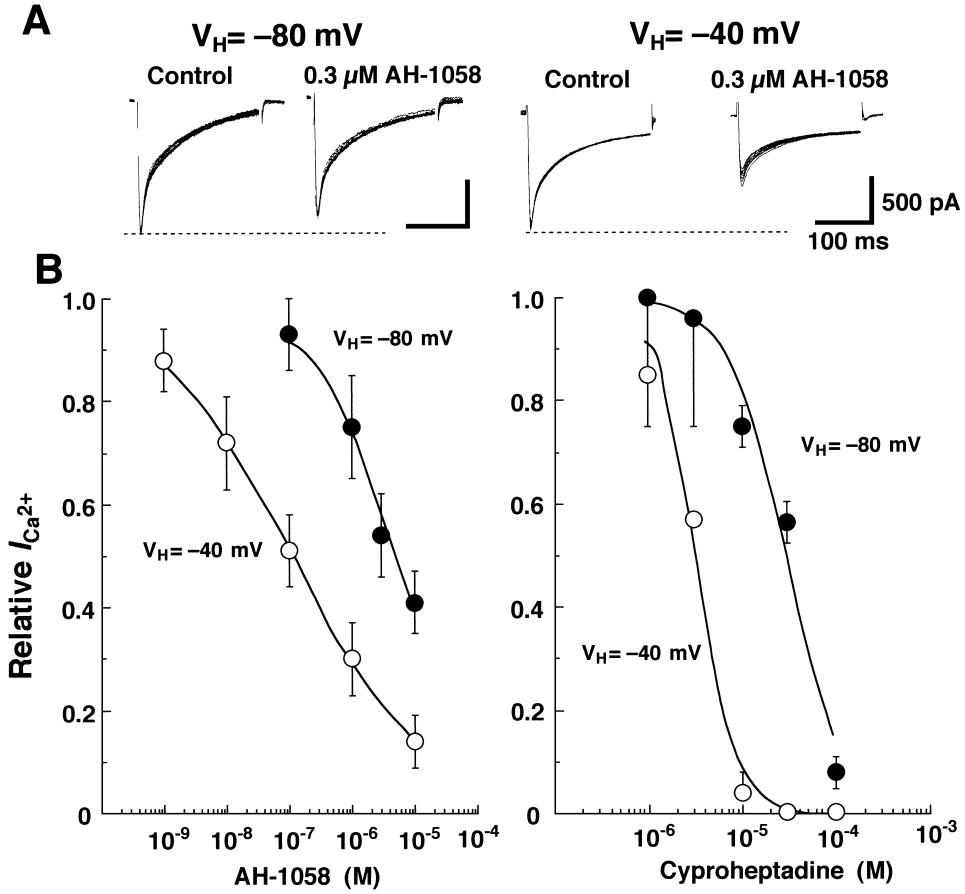

Fig. 2. Effects of AH-1058 and cyproheptadine on Ca^{2+} currents ($I_{Ca^{2+}}$) in guinea pig cardiomyocytes. A: Typical traces showing the voltage-dependent effects of AH-1058 on Ca^{2+} currents. Ten depolarizing pulses were applied to the cardiomyocyte for 200 ms from each holding potential (V_H; –80 and –40 mV) to +20 mV every 10 s. Four minutes after the application of AH-1058 (0.3 μM), the same depolarizing pulses were reapplied to the cardiomyocyte. All recordings before and after the drug treatment were obtained from the same cardiomyocyte. B: Concentration-dependent suppression of Ca^{2+} currents by AH-1058 and cyproheptadine. Each concentration of the drug was applied to the cardiomyocyte 4 min before subsequent 10 depolarizing pulses were applied every 10 s. Every 10th record of peak Ca^{2+} current was plotted. All responses were normalized to the peak current amplitude in the absence of each drug. Data are expressed as the mean ± S.E.M. of 4 experiments.

Table 1. Effects of AH-1058, cyproheptadine and typical Ca^{2+} channel blockers on Ca^{2+} currents at holding potentials of –80 and –40 mV in guinea pig cardiomyocytes

Drugs	IC_{50} (μM)		Ratio
	V_H = –80 mV	V_H = –40 mV	(–80 mV/–40 mV)
AH-1058	4.91 ± 1.02	0.32 ± 0.24	15.3
Cyproheptadine	42.22 ± 16.29	7.75 ± 1.28	5.4
Verapamil	5.60 ± 1.87	0.34 ± 0.14	16.5
Diltiazem	58.08 ± 22.27	0.45 ± 0.11	129.1
Nicardipine	0.45 ± 0.15	0.0028 ± 0.0004	160.7

Data are average of 4 experiments. IC_{50}, 50% inhibition of drug concentration; V_H, holding potential.

nicardipine on L-type Ca^{2+} channel currents were also examined in the same manner, and their IC_{50} values at holding potentials of –80 and –40 mV and the ratio of IC_{50} values at –80 mV to those at –40 mV are summarized in Table 1.

In the present study, we clearly showed that AH-1058 suppressed the cardiac L-type Ca^{2+} channel currents at holding potentials of –40 and –80 mV, whose potency

was equivalent to that of a well-established Ca^{2+} channel blocker verapamil. Its mother compound cyproheptadine also suppressed the current, and its efficacy was 14- and 33-fold less than that of AH-1058 at holding potentials of −40 and −80 mV, respectively. These results indicate that introduction of the cinnamil structure to piperidine in cyproheptadine, as shown in Fig. 1, can generate novel L-type Ca^{2+} channel-blocking compounds as potent as verapamil, which is in accordance with previous knowledge that some drugs having a cinnamil structure like flunarizine, cinnarizine, and cilnidipine possess a potent Ca^{2+} channel-blocking action (11). In addition, the efficacy of AH-1058 and cyproheptadine as expressed as the IC_{50} values roughly correlates with the previous in vivo evidences that AH-1058 (0.1 mg /kg, i.v.) and cyproheptadine (3 mg/kg, i.v.) effectively inhibited the Ca^{2+} channel-associated ventricular arrhythmias in the canine epinephrine-arrhythmia model (4, 12, 13), which may suggest that complete inhibition of Ca^{2+} channel currents by drugs is not necessary to suppress the in vivo ventricular arrhythmias.

Another important observation in this study was the voltage-dependent effect on Ca^{2+} currents; namely, the ratio of AH-1058 dividing IC_{50} value at a holding potential of −80 mV by that at −40 mV was smaller than that of nicardipine, as shown in Table 1. Since the membrane potential in vascular smooth muscle cells is definitely less negative than the diastolic membrane potential of working cardiac muscle cells, Ca^{2+} channel blockers having larger ratio values like nicardipine can theoretically suppress vascular rather than cardiac functions (14, 15). Thus, the cardioselective action of AH-1058 (4 – 8) may be partly associated with the electrophysiological property of the drug. However, systematical analysis using vascular as well as cardiac preparations will be required to fully clarify the mechanisms of cardiovascular selectivity of AH-1058.

In conclusion, AH-1058 can suppress cardiac L-type Ca^{2+} channels, whose potency was equal to that of verapamil in guinea pig cardiomyocytes. Since chemical modification of cyproheptadine can generate more potent Ca^{2+} channel blockers like AH-1058, the present results will become essential information regarding chemical designs for new Ca^{2+} channel blockers.

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