A Xanthine Derivative Denbufylline Inhibits Negative Inotropic Response to Verapamil in Guinea Pig Ventricular Papillary Muscles, Independent of Its Phosphodiesterase Inhibitory Activity

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ABSTRACT—A phosphodiesterase (PDE) III inhibitor, amrinone, inhibited both the negative inotropic actions of verapamil and nifedipine in guinea pig ventricular papillary muscle; this effect was canceled by the protein kinase A inhibitor H-89. The PDE IV inhibitor 1,3-di-*n*-butyl-7-(2'-oxopropyl)xanthine (denbufylline), which elicited a negative inotropic action by itself, attenuated the action of verapamil up to 10 μ M, without any interaction with nifedipine. The attenuation by denbufylline was not influenced by H-89. This suggests that in the ventricular papillary muscle, denbufylline acts on some verapamil-sensitive site(s) in the membrane and interferes with the calcium channel function without involvement of its PDE inhibitory activity.

Keywords: Denbufylline, Negative inotropy, Calcium channel

Transmembrane influx of extracellular calcium through specific calcium channels plays an important role in the excitation-contraction coupling of cardiac muscles. It has been reported that the function of calcium channels is regulated by cyclic AMP-dependent phosphorylation (1). Phosphorylation of calcium channels by cyclic AMP-dependent protein kinase (PK-A) leads to an increase in the mean probability of channel opening (2), resulting in the positive inotropy. Both phosphodiesterase (PDE) III and IV isoenzymes are responsible for the hydrolyzing of cyclic AMP in guinea pig cardiac cells (3), and inhibition of these isoenzymes should induce positive inotropy through PK-A activation. Indeed, the positive inotropic actions of a nonselective PDE inhibitor, 3-*n*-isobutyl-1-methylxanthine (IBMX), and a PDE III inhibitor, amrinone, were abolished by treatment with a PK-A inhibitor, *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide (H-89) (4). However, we found that denbufylline, a xanthine derivative having selective PDE IV inhibitory activity (5), decreased the contractile force of the ventricular papillary muscle of guinea pigs, whereas a nonxanthine PDE IV inhibitor, Ro 20-1724, had no inotropic effect (4). In this study, we investigated the effect of denbufyl-

line on the inotropic actions of L-type calcium channel inhibitors on guinea pig ventricular papillary muscle.

Male Hartley guinea pigs, weighing 300–500 g, were killed by stunning and then bled. The heart was rapidly removed and transferred to a dissection bath filled with oxygenated warm Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.55 mM CaCl₂, 1.18 mM MgSO₄, 24.88 mM NaHCO₃, 1.18 mM KH₂PO₄ and 11.1 mM glucose, pH 7.4). The right ventricular papillary muscles were dissected from the heart carefully. The muscle was mounted under 0.5 g of resting tension in an organ bath (30°C) containing 10 ml of Krebs-Henseleit solution aerated with a gas of 95% O₂–5% CO₂. The muscle was stimulated by rectangular pulses of 1-Hz frequency, 1 msec in duration and 1.5 times the threshold voltage, delivered by bipolar platinum stimulating electrodes connected to an electronic stimulator (SEN-7203; Nihon Kohden, Tokyo). Isometric tension developed in the preparation was measured with a force transducer (T7-30-240; Orientec, Tokyo) and recorded on a pen recorder (SR6211; Graphtec, Tokyo). The preparations were allowed to equilibrate for at least 60 min before the experiments were begun.

Amrinone (Sigma Chemical Co., St. Louis, MO, USA) and denbufylline, synthesized at Hokuriku University, at

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a) Verapamil

b) Nicardipine

Fig. 1. Effects of denbufylline and amrinone and/or H-89 on negative inotropic actions of verapamil and nicardipine in ventricular papillary muscles. Verapamil (a) and nicardipine (b) were applied in a cumulative manner 15 min after treatment with (●) or without (○) 10 μ M denbufylline or amrinone. Application of 1 μ M H-89 (△) was done 15 min before the administration of denbufylline or amrinone. The bars are the S.E. of at least three experiments. * $P < 0.05$, compared with the group treated with verapamil or nicardipine alone by Student's *t*-test.

10 μ M produced direct inotropic changes to $107.0 \pm 2.1\%$ ($n=3$) and $94.8 \pm 0.9\%$ ($n=7$) of the initial force, respectively. As shown in Fig. 1, treatment with 10 μ M amrinone shifted to the right the concentration-response curves of both verapamil (Wako Pure Chemicals, Osaka) and nicardipine (Sigma Chemical) and significantly changed the EC_{20} values for the negative inotropic actions of verapamil and nicardipine from 8.9 ± 2.6 nM ($n=5$) and 23.0 ± 4.4 nM ($n=5$) to 54.3 ± 9.0 nM ($n=3$) and 60.5 ± 7.5 nM ($n=3$), respectively. These inhibitory effects of amrinone were completely canceled by treatment with the PK-A inhibitor H-89 (6) (Seikagaku Kogyo Co., Tokyo) at 1 μ M for 15 min, which decreased the contractile force by 10% or less. The non-selective PDE inhibitor IBMX also inhibited the negative inotropic actions of these calcium channel inhibitors, and the inhibitions were also abolished by H-89 (data not shown).

On the other hand, treatment with 10 μ M denbufylline

inhibited only the negative inotropic action of verapamil to 64.8 ± 11.2 nM ($n=3$). However, the inhibition by denbufylline was not influenced by H-89. Denbufylline hardly affected the negative response to nicardipine (Fig. 1). Ro 20-1724 had little or no effect on the response of verapamil and nicardipine (data not shown). These results indicate that the inhibition of the response to verapamil by denbufylline was mediated by mechanisms other than PDE IV inhibition.

Figure 2 shows the negative inotropic action of denbufylline and the effect of denbufylline on the negative inotropic response to verapamil of the papillary muscle. Verapamil (100 nM) decreased the contractile force to $60.9 \pm 5.3\%$ ($n=5$) of the initial contractile force, and the negative inotropic action was inhibited by treatment with denbufylline in the range of 0.1–10 μ M, which had little or no effect on the contractile force. The inhibition by denbufylline, however, gradually became weak at con-

Fig. 2. Effects of denbufylline on the negative response to 100 nM verapamil in the contractile force of the ventricular papillary muscle. Fifteen minutes after treatment with the indicated concentrations of denbufylline, 100 nM verapamil was applied to the papillary muscle. a: The points represent the % of initial tension induced by denbufylline alone (○) and denbufylline plus verapamil (●). b: The change in verapamil-induced tension in combination with denbufylline (□) is expressed as the percentage response of the muscle, when the contractile force after treatment with denbufylline was defined as 100%. The bars are the S.E. of three experiments. * $P < 0.05$, compared with the control by Student's *t*-test.

centrations over 30 μM , and the combination of 100 μM denbufylline with 100 nM verapamil additionally decreased the contractile force of the muscle.

It has been reported that dihydropyridines bind to their receptor site from the extracellular surface of the Ca^{2+} channel in guinea pig ventricular myocytes (7), but phenylalkylamines act from the intracellular surface of the cardiac cell membrane (8). Denbufylline may act on some verapamil-sensitive site(s) in the membrane, followed by interference with the calcium channel function. This report indicates that the action of denbufylline is cyclic AMP-independent and quite different from those of other xanthine derivatives such as IBMX.

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