Action Potential Shortening and Negative Inotropic Effects of a Novel Potassium Channel Opener, NIP-121, as Compared with Cromakalim in Guinea Pig Ventricular Myocardium

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ABSTRACT—The potencies of NIP-121, a new potassium channel opener, to shorten action potential duration and to decrease the contractile force was examined using isolated guinea pig right ventricular free wall and papillary muscle preparations, respectively; and they were compared with those of cromakalim. NIP-121 was about 10 times more potent than cromakalim with respect to both effects. This potency ratio in cardiac muscle was about the same as that observed in rat aorta and portal vein. These cardiac effects of both agents were antagonized by glibenclamide.

A considerable number of potassium channel openers have been developed, and intensive studies have also been performed. Although many therapeutic applications of these agents are possible, their main therapeutic use is for the treatment of hypertension. Therefore, most of the studies on these agents deal with vascular smooth muscles, and the prototypic agent cromakalim has been the most extensively investigated. It is proposed to produce hyperpolarization by increasing the potassium permeability of the cell membrane, resulting in the vasorelaxation (1-3). It is suggested that cromakalim activates the ATP-dependent potassium channel and that this channel is selectively blocked by sulfonylurea antidiabetic agents such as glibenclamide (4-6).

ATP-regulated K⁺ channels were suggested to be present in cardiac muscle (7). Therefore, theoretically, these types of agents should shorten the action potential duration (APD) and decrease the contractile force in cardiac muscle. Yanagisawa et al. (8) first demonstrated the negative inotropic and effective refractory period (ERP) shortening effects of a K⁺ channel opener, nicorandil, in cardiac muscle. The action potential shortening effect of this K⁺ channel opener was also first shown by Yanagisawa and Taira (9) and later with other K⁺ channel openers which was antagonized by glibenclamide (e.g., see ref. 10 for cromakalim). Recently, cromakalim and pinacidil, as well as nicorandil, are reported to elicit the negative inotropic effects in canine atrial muscle, which is inhibited by glibenclamide (11).

NIP-121 ((+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidin-1-yl)-6H-pyrano-(2,3-f)benz-2,1,3-oxidiazole) is a newly synthesized K channel opener that possesses potent and long-lasting hypotensive activity (12). The potency of NIP-121 was examined in vascular smooth muscle and reported to be
roughly 10 times higher than that of cromakalim (13). In this report, mainly examined were the relaxation of rat aorta, spontaneous contraction of rat portal vein, and \(^{86}\text{Rb}^+\) efflux from rat aortic cells.

In the present study, cardiac effects of NIP-121 were investigated electrophysiologically and mechanically. The aims of the present study were to determine: (i) whether NIP-121 does shorten the APD similarly to cromakalim, (ii) whether NIP-121 produces a decrease in contractile force, and, if (i) and (ii) are confirmed to be the case, (iii) how potent NIP-121 is in cardiac muscle compared to cromakalim, and (iv) whether the electrophysiological and mechanical effects of NIP-121 and cromakalim are antagonized by glibenclamide.

Male guinea pigs weighing 220–490 g were sacrificed and the hearts were quickly removed. Ventricular free wall and papillary muscle preparations were dissected out from the right ventricle.

For the electrophysiological experiments, the free wall preparations were pinned down horizontally on a silicon block placed in a 30 ml organ bath containing physiological salt solution (PSS) of the following composition: 135 mM NaCl, 5 mM KCl, 2 mM CaCl\(_2\), 1 mM MgCl\(_2\), 15 mM NaHCO\(_3\) and 5.5 mM glucose. The bathing solution was bubbled and circulated with an oxygen mixture (95% O\(_2\)–5% CO\(_2\)) at 37°C. Conventional microelectrode penetrations were made into the endocardial surface using glass microelectrodes filled with 3 M KCl. The output of a microelectrode preamplifier (Nihon Kohden, MEZ-7101) with high input impedance and capacity neutralization was fed into a dual beam cathode ray oscilloscope (Nihon Kohden, VC-9). Preparations were driven by external electrical stimulation using bipolar platinum electrodes and rectangular current pulses (5 msec duration, about \(1.5 \times \) threshold strength) at a constant frequency (1 Hz) that were generated by an electronic stimulator (Nihon Kohden, SEN-3201).

For the mechanical studies, papillary muscle preparations were placed horizontally in an organ bath containing 20 ml PSS of the same composition as described above, and the bathing solution was bubbled with the same oxygen mixture at 37°C. Isometric contractions were measured with a force displacement transducer (Nihon Kohden, TB-611T) by applying a baseline tension of about 0.5 g. They were recorded on an ink-writing oscillograph (Nihon Kohden, RM-6100). Electrical field stimulation of 5 msec duration was applied through bipolar platinum plate electrodes at a frequency of 1 Hz (about \(1.5 \times \) threshold strength).

NIP-121 and cromakalim were synthesized in the Central Research Laboratories of Nissan Chemical Industries and generously supplied to us. They were dissolved in ethanol (\(3 \times 10^{-3}\) M for NIP-121 and \(10^{-2}\) M for cromakalim), serial dilutions were made with distilled water, and small aliquots of the dilutions were added to an organ bath to give the desired final concentrations. The concentrations of ethanol did not exceed 0.5%.

Action potential configurations before and after the administration of NIP-121 or cromakalim were carefully examined, and these agents were found to mainly produce the shortening of APD without substantially affecting other action potential parameters (typical tracings are shown in Fig. 1A). From the experiments on the concentration-response relation for these agents, it was found that \(3 \times 10^{-6}\) M NIP-121 and \(3 \times 10^{-5}\) M cromakalim produced about the same degree of shortening. Thus, the time course of the decrease in APD produced by these concentrations of both agents were examined (Fig. 1B). As shown, the time course of the action potential shortening produced by \(3 \times 10^{-6}\) M NIP-121 and \(3 \times 10^{-5}\) M cromakalim are quite similar. Sanguinetti et al. (10) have also reported about the same degree of action potential shortening by \(3 \times 10^{-3}\) M cromakalim, with about the same time course. Glibenclamide dose-dependently inhibited the action potential shortening by both agents. In Fig. 1B is shown the antagonism by \(3 \times 10^{-7}\) M glibenclamide. The extent of inhibition seems
to be different for NIP-121 and cromakalim; however, the reason for this is unknown at present and further studies are needed. In rat portal vein and aorta, glibenclamide exhibited about the same degree of inhibition to NIP-121 and cromakalim (13).

In the mechanical studies using guinea pig papillary muscles, both cromakalim and NIP-121 showed marked negative inotropic effects. Dose-response curves for the negative inotropic actions of both agents are shown in Fig. 2. As shown, with respect to this effect, NIP-121 was again about 10 times more potent than cromakalim. The negative inotropic effects by both agents are also antagonized by glibenclamide dose-dependently. In Fig. 2 is shown the antagonism by 10 times higher concentration of glibenclamide than that shown in Fig. 1B. Thus, the inhibitory effect of glibenclamide on the negative inotropic effects of both agents appeared to be smaller than that on the action potential shortening effects of both agents. The reason for this is unknown at present. In this regard, Sanguinetti et al. (10) made a similar observation with cromakalim and raised the possibility that cromakalim may have other actions in addition to activation of ATP-dependent potassium channels, such as blockade of calcium channels.

The present study clearly demonstrated that

![Fig. 1. Action potential shortening effects of NIP-121 and cromakalim. A: Typical tracing showing the APD shortening effects of NIP-121 and cromakalim. Upper row: control action potential before the addition of agents. Lower row: action potential recorded from the same cell 30 min after the addition of $3 \times 10^{-6}$ M NIP-121 and $3 \times 10^{-5}$ M cromakalim. B: Time course of the shortening in APD produced by $3 \times 10^{-6}$ M NIP-121 (solid line) and $3 \times 10^{-5}$ M cromakalim (dotted line) in the absence (open symbol) or presence (closed symbol) of $3 \times 10^{-5}$ M glibenclamide. Ordinate: Time required to achieve 50% (APD 50) and 90% (APD 90) repolarization as expressed by the percentage of the value immediately before the addition of NIP-121 or cromakalim (shown by arrow 2). Glibenclamide was added at arrow 1. Abscissa: time (min) after the addition of each K+ channel opener. Each point with vertical bar represents the mean ± S.E.M from 10–12 experiments; data adopted were only those from experiments in which electrode penetrations were successfully maintained before and after the addition of drugs.](image-url)
NIP-121, a novel potassium channel opener, was about 10 times more potent than cromakalim with respect to cardiac action, i.e., action potential shortening and negative inotropism. This potency ratio agrees well with that obtained in the experiments with vascular smooth muscle, in which were studied the relaxation of the aorta, $^{86}$Rb$^+$ efflux in rat aorta, and inhibition of the spontaneous contraction of rat portal vein (13). Therefore, it may be concluded that the potency ratio of both agents as potassium channel openers is not different in vascular smooth muscles and cardiac muscles.

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Fig. 2. Dose-response curves for the negative inotropic effects of cromakalim (A) and NIP-121 in the absence (open symbol) and presence (closed symbol) of $3 \times 10^{-6}$ M glibenclamide in guinea pig papillary muscles driven at 1 Hz. Ordinate: contractile force expressed as percentage of the force before the addition of each K$^+$ channel opener. Each point with vertical bar represents the mean ± S.E.M. from 10–12 experiments.
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