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Original Article

Extracellular Potassium Dependent Negative Dromotropic Action of Nicorandil in Guinea Pig Myocardium

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Abstract Although the antiarrhythmic action of nicorandil is drawing an increasing attention, dromotropic effect of this agent is unclear. Therefore, this was investigated by microelectrode technique to the superfused guinea pig papillary muscle to record the action potential and extracellular potential during conduction. The correlation of myocardial internal longitudinal resistance (r_i) assumed to reflect the global gap junctional resistance, maximum rate of rise of the action potential upstroke ($\dot{V}_{max}$), and conduction velocity was examined under the alterations of external potassium concentrations ($[K^+]_e$; ranging from 3.0 to 12.0 mM) in the presence or absence of 100 μM nicorandil. In the minimum $[K^+]_e$, nicorandil caused significant ($p < 0.05$) hyperpolarization and reduction in $\dot{V}_{max}$. Negative dromotropic action of nicorandil was slight but significant ($p < 0.05$) in low (3.0 mM) $[K^+]_e$ but was not evident in physiologic (5.4 mM) or elevated (9.0 to 12.0 mM) $[K^+]_e$. In conclusion, nicorandil exerts negative dromotropic action as $[K^+]_e$ decreased, which was accounted for by the cable analysis and may contribute to the prevention of low $[K^+]_e$-induced arrhythmia.

Key words: Cable theory, Conduction, Myocardium, Nicorandil, Potassium

Introduction

Myocardial electrical propagation is modulated by various antiarrhythmic agents in a complicated manner. As a representative example, class I antiarrhythmic agents slow the conduction by suppressing the fast inward sodium current (I_{Na}) in a rate- and voltage-dependent manner[1)~3)]. Individual class III agent shows the complicated dromotropic actions[4)]. A representative class IV agent, verapamil, exerts negative dromotropic action by inhibiting I_{Na} at relatively higher concentrations (> 1.0 μM) in aerobic condition, whereas this agent shows positive dromotropism by restoring cell-to-cell uncoupling at a concentration of 1.0 μM under the anaerobic condition[5)]. Ouabain, not classified by the Vaughan-Williams antiarrhythmic drugs classification[6)], also exerts negative dromotropism possibly by elevating intracellular calcium concentration leading to cell-to-cell uncoupling[7)]. Contrary to such a line of evidence with respect to the drug-induced dromotropic alterations based on the cable theory including cell-to-cell coupling concept, the dromotropic action of nicorandil, N-(2-hydroxyethyl)-nicotinamide nitrate, is unknown. Nicorandil shows hybrid prop-

erties as a nitrate and an adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channel opener[8)9)]. Therefore, this study was designed to investigate the dromotropic actions of nicorandil using guinea pig papillary muscles in which cable theory is apparently applicable[5)10)].

Materials and Methods

Heart preparations

Experimental designs and procedures of this study compiled strictly with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Physiological Society and experimental methods have been introduced elsewhere in detail[5)10)]. In brief, guinea pig weighing 300 to 350 g (Kyudo Co. Ltd., Yoshitomi, Japan) was anesthetized with an intraperitoneal injection of sodium pentobarbital (30-40 mg / kg ; Abbott Laboratory, Chicago, IL). Beating heart was excised immediately after thoracotomy and then rinsed in ice-cold normal Tyrode's solution. This solution was of the following composition (in mM / L) : NaCl, 140.0 ; KCl, 5.4 ; $CaCl_2$, 1.8 ; $MgCl_2$, 0.5 ; NaH_2PO_4, 0.33 ; glucose, 5.5. The external K^+ concentration ($[K^+]_e$) was elevated up to 12.0 mM or lowered to 3.0 mM, depending on the experimental protocol. In the case of $[K^+]_e$ alteration, NaCl was substituted for by KCl or *vice versa* to keep the total ionic strength of all kinds of solution constant. Solution was oxygenated with 100 % O_2 and the temperature was maintained at 36.0 ± 1.0°C. The pH was adjusted to 7.4 using 10.0 mM N-2- hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES ; Sigma Co. Ltd., St Louis, MO) with NaOH titration (~0.5 g). Partial oxygen pressure of the perfusate estimated by a blood gas analyzer (ABL 620 ; Radiometer, Copenhagen, Denmark) was 800 mmHg approximately. Excised right ventricular papillary muscle was mounted on a tissue chamber and covered by nylon mesh to produce the thin layer of perfusate around the preparation on which two external glass electrodes were positioned in the same longitudinal axis to record the extracellular potential during electrical propagation. A pair of tungsten wire electrodes was positioned at the cut end of the preparation for stimulation and a conventional 3M KCl-filled glass microelectrode was applied to record the propagating action potential. Preparation was superfused by oxygenated Tyrode's solution at a flow rate of 3.0 ml / min and paced with 2 msec duration and twice the end-diastolic threshold intensity at constant basic cycle length (BCL) of 1.0 sec. Extracellular potential, action potential and its first time derivative were monitored by a pen-writing recticorder (Nihon-Kohden, Tokyo, Japan) and stored on digital audiotapes using a data recorder (RD-101T PCM, TEAC, Tokyo) for off-line analyses.

Theory

The following electrophysiologic parameters were analyzed under the $[K^+]_e$ alterations, i. e., resting membrane potential (RMP), the maximum rate of rise of the action potential upstroke ($\dot{V}_{max}$), action potential duration at 90 % repolarization (APD), interelectrode conduction time (CT), amplitudes of extracellular and intracellular potentials (V_e, V_i) generated by the instantaneous potential gradient between the excited and resting myocardium at a moment of electrical propagation. Here, the amplitude of intracellular action potential recorded in the bathed preparation is the sum of V_i and V_e[10)]. Conduction velocity (θ) of the action potential propagation was

calculated by dividing the interelectrode distance by CT.

Fig. 1 (upper) illustrates schematically the local circuit current (I) generated by the voltage gradient in the extracellular and intracellular domains during electrical propagation. Relation of V_e, V_i and I follows Ohmic equation;

$$V_i = I \cdot r_i \quad [1]$$
$$V_e = I \cdot r_e \quad [2]$$

where r_i and r_e are the internal and external longitudinal tissue resistance per unit length. Since I is constant in the instantaneous local circuit according to the charge conservation theory[11], dividing equation [1] by equation [2] yields,

$$r_i / r_e = V_i / V_e \quad [3]$$

Total longitudinal tissue resistance (r_{total}) was measured by applying subthreshold constant current to the extracellular space through the platinum wires placed at the opposite ends of the preparation. Since r_{total} is the parallel combination of r_i and r_e according to the equilibrium circuit indicated in Fig. 1 (lower), the following equation is established,

$$1 / r_{total} = 1 / r_i + 1 / r_e \quad [4]$$

Based on these equations [3] and [4], the following equation is obtained[10)11)],

$$r_i = r_{total} (1 + V_i / V_e) \quad [5]$$

Major component of r_i is the gap junctional but not cytoplasmic resistance, r_i roughly reflects the uncoupling condition between the contiguous cardiac cells in the multicellular preparations.

Theoretical nonlinear fitting of the relationship between $\dot{V}_{max}$ and RMP under the $[K^+]_e$ alterations was performed according to the Boltzmann's equation as follows[12],

$$\dot{V}_{max} = \bar{\dot{V}}_{max} (1 + \exp [V - V_h] / s)^{-1} \quad [6]$$

where $\bar{\dot{V}}_{max}$ is $\dot{V}_{max}$ obtained theoretically under the condition apparently free from the steady-state inactivation of I_{Na} (i.e., $[K^+]_e$-free condition) but assumed as $\dot{V}_{max}$ under the minimum $[K^+]_e$ (i.e., 3.0 mM) in the present study. V and V_h are RMP providing respective $\dot{V}_{max}$ and half-inactivation of $\bar{\dot{V}}_{max}$, and s is a slope factor.

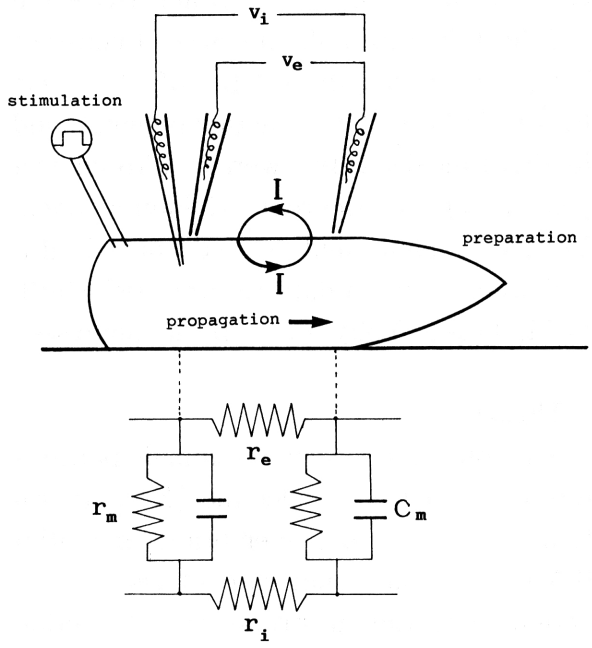

Fig. 1 Schematic illustration of the experiment (upper) and its equivalent circuit (lower) are presented. Constant stimulation is performed at the cut end of the preparation and excitation propagates to the rightward direction. c_m, membrane capacity per unit length; I, local circuit current; r_e, external longitudinal tissue resistance per unit length; r_i, internal longitudinal tissue resistance per unit length; r_m, membrane resistance times unit length; V_e, extracellular potential; V_i, intracellular potential.

Experimental protocols

After the preparation was subjected to half hour of equilibration, experimental protocol was commenced under the BCL of 1.0 sec. Aforementioned electrophysiologic parameters were evaluated under the different $[K^+]_e$ in the presence or absence of 100 μM nicorandil ($n = 5 \sim 8$). $[K^+]_e$ alteration was in the range of 3.0 to 12.0 mM, since further $[K^+]_e$ elevation induced the papillary muscle contracture and further $[K^+]_e$ reduction often evoked the abnormal automaticity of the preparation, both of which made the microelectrode impalement unstable. When data acquisition was disturbed by the poor microelectrode penetration, only the reliable data obtained under the stable impalement were analyzed. All experiments were conducted at the room temperature (22.3 ± 2.8 °C).

Data analysis and statistics

Data are presented as the mean ± SD. Comparison of various parameters among the different specific perfusion protocols was conducted by the paired t-test. Commercially available statistical software (Macintosh Expert Statview 4.0 system, Apple Japan, Tokyo, Japan) was used for practical analyses. Nonlinear regression analysis with least square method was conducted automatically by this computation. A p value less than 0.05 was considered statistically significant.

Results

As in Fig. 2, $\dot{V}_{max}$ was plotted as a function of RMP under the $[K^+]_e$ alterations ranging from 3.0 to 12.0 mM. In the control condition, $[K^+]_e$ elevation shifted RMP to the positive direction and reduced $\dot{V}_{max}$ dramatically, whereas $[K^+]_e$ reduction showed the opposite effects. This was the same when 100 μM nicorandil was introduced. $\dot{V}_{max}$ in the minimum $[K^+]_e$ was significantly greater in the absence than in the presence of nicorandil (198.6 ± 8.6 vs. 191.3 ± 9.4 V / sec, $p < 0.05$). In the nonlinear fitting, these two conditions produced individually a significant ($p < 0.05$) correlation between RMP and $\dot{V}_{max}$ ($r = 0.853$ and 0.903). The theoretical curve in the presence of nicorandil was superimposed completely by that in the control condition under the RMP less negative to −70 mV. V_h and s were not fundamentally different between the two conditions (s: 3.59 vs. 3.44 mV, V_h: −63.2 vs. −62.5 mV).

In the control condition, θ showed complicated changes during the course of progressive $[K^+]_e$ elevation, i.e., θ gradually increased as $[K^+]_e$ was elevated and was the maximum in the 9.0 mM $[K^+]_e$, which was recognized as a supernormal conduction[5)10)]. Thereafter, θ was reduced dramatically by the further $[K^+]_e$ elevation up to 12.0 mM. On the other hand, $[K^+]_e$ reduction (3.0 mM) induced conduction slowing. The alterations of θ as a function of $[K^+]_e$ were investigated in the presence of 100 μM nicorandil. Biphasic changes of θ under the $[K^+]_e$ alterations (i.e., 3.0 to 12.0 mM) were observed, while supernormal conduction was noted in the 9.0 mM $[K^+]_e$, as in the control condition (not shown). In the minimum $[K^+]_e$ of 3.0 mM, V_i / V_e reflecting r_i (equation [5]) was slightly increased from 13.1 to 14.9 by nicorandil. θ was slightly reduced (i.e., 58.8 to 56.5 cm / sec) and RMP shifted to the hyperpolarizing direction (i.e., −83.6 to −87.6 mV) by nicorandil (Fig. 3). By statistical analyses, conduction was slower (i.e., 57.0 ± 5.6 vs. 61.2 ± 6.1 cm / sec, $p < 0.05$) and RMP was more negative (−87.2 ± 2.0 vs.

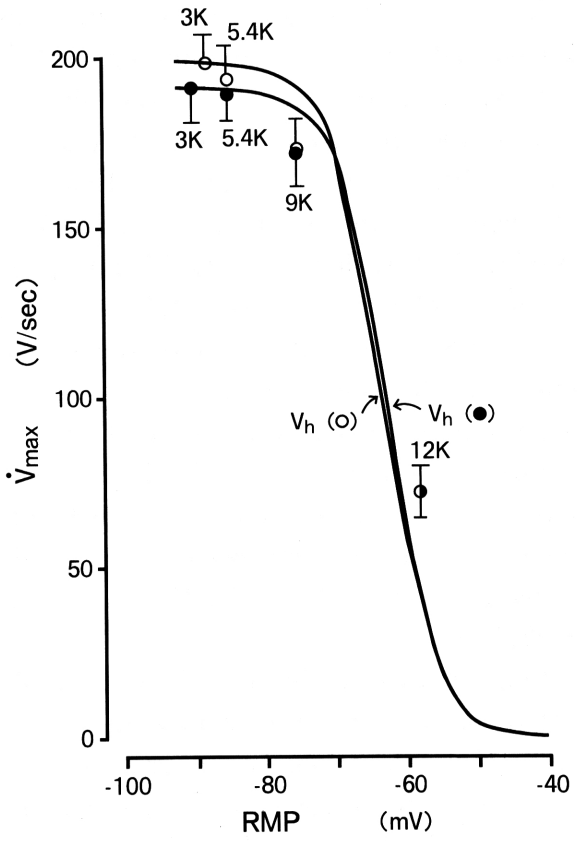

Fig. 2 The alteration of the maximum rate of rise of the action potential upstroke ($\dot{V}_{max}$) as a function of resting membrane potential (RMP) observed in the absence (open circles) or presence (closed circles) of 100 μM nicorandil. Nonlinear curve fitting was conducted by the Boltzmann's equation (equation [6] in the text). All symbols indicate mean ± SD. SD of RMP was too small to indicate outside of the symbols.

-84.4 ± 1.8 mV, $p < 0.05$) in the presence than in the absence of nicorandil.

The alterations of the three components of the longitudinal tissue resistance per unit length (i.e., r_{total}, r_i and r_e) under the course of $[K^+]_e$ alterations were presented in Table. These components were stable during the $[K^+]_e$ alterations (3.0 to 12.0 mM) in the control aerobic condition. When nicorandil was applied, r_{total} and r_e were constant under the $[K^+]_e$ alterations and statistically the same as those in the control. With respect to r_i, no significant changes were observed by introducing nicorandil in the lowest $[K^+]_e$ (22.7 ± 2.3 vs. 21.3 ± 2.0 KΩ / cm, $p = 0.07$) and by minimizing $[K^+]_e$ in the presence of nicorandil (22.7 ± 2.3 vs. 21.8 ± 2.1 KΩ / cm, $p = 0.08$).

Nicorandil and $[K^+]_e$ showed a great influence on the APD, in a complicated manner, irrespective of their dromotropic actions. APD showed a great $[K^+]_e$-dependence in the control condition, i.e., APD was lengthened as $[K^+]_e$ decreased and it was shortened as $[K^+]_e$ increased. Application of nicorandil generally shortened APD and this nicorandil-induced APD shortening was also $[K^+]_e$-dependent. This shortening was augmented as $[K^+]_e$ was lowered, i.e., it was significant ($p < 0.05$) in 3.0 and 5.4 mM $[K^+]_e$ but was not evident in 9.0 and 12.0 mM $[K^+]_e$ (Table).

Discussion

Nicorandil exerts hybrid properties as a conventional nitrate compound and a K_{ATP} channel opener[8)9)] and is recognized clinically as an antianginal and cardioprotective agent. Recently, this agent has been reported to be effective in specific kinds of arrhythmia which is not based on myocardial ischemia[13)]. Several basic investigations indicate that nicorandil exerts inhibitory actions on the afterdepolarization leading to the triggered arrhythmias in *in vivo*[14)] and *in vitro*[15)16)] models. This indicates that nicorandil-induced K_{ATP} channel opening accelerates the action potential repolarization, normalizes the repolarization abnormalities and inhibits the triggered activities. Relatively to such antiarrhythmic actions of nicorandil, the dromotropic effects of this agent remain unclear. Therefore, this study was designed to investigate the

dromotropic actions of nicorandil under the $[K^+]_e$ alterations using guinea pig myocardium, since $[K^+]_e$ *per se* also influences greatly the dromotropism and K^+ channel activities. The present study showed great dependence on $[K^+]_e$ (ranging from 3.0 to 12.0 mM) of various electrophysiologic parameters relating to the action potential propagation and these parameters were modulated by nicorandil in a $[K^+]_e$-dependent manner. Conduction speeding termed supernormal conduction[5)10)], in spite of a decrease in $\dot{V}_{max}$, was observed under the slightly elevated (i.e., 9.0 mM) $[K^+]_e$ and marked conduction slowing was obtained by further $[K^+]_e$ elevation in this study. Nicorandil slowed conduction only in the minimum $[K^+]_e$ of 3.0 mM (Fig. 3) without

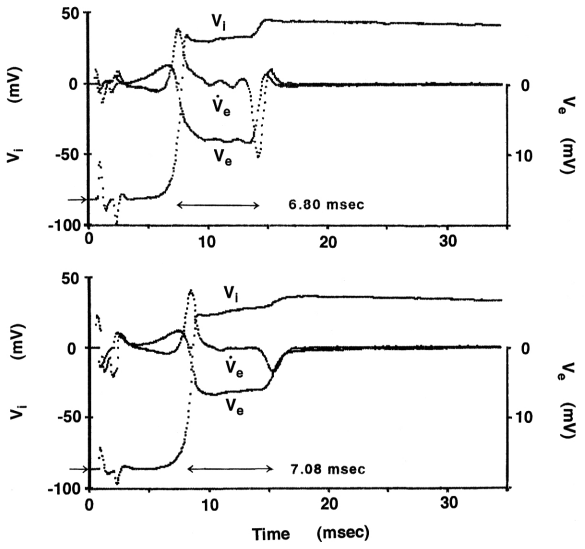

Fig. 3 Actual recording of the upstroke of V_i, V_e and the first time derivative of V_e ($\dot{V}_e$) in the 3.0 mM $[K^+]_e$ under the control condition (upper) or after the application of 100 μM nicorandil (lower). RMP was indicated by arrows on the ordinates (left). $\dot{V}_e$ clarified the interelectrode conduction time (CT) as indicated by the bidirectional arrows. CT was prolonged slightly by nicorandil. Interelectrode distance was 4.0 mm in this preparation. Note that the scale of ordinate for V_i (left) is different from that for V_e (right).

changing fundamental relation between θ and $[K^+]_e$ (Table). This is attributable to the nicorandil-induced small but significant fall in $\dot{V}_{max}$ (Table) observed only in the lowest $[K^+]_e$. Low $[K^+]_e$ reduces theoretically the K^+ conductance (g_k) of cardiac cell membrane, whereas nicorandil is expected to increase it as a K_{ATP} channel opener[17)18)], suggesting that electrophysiologic effects of nicorandil is manifest as $[K^+]_e$ decreases. $\dot{V}_{max}$, as a simple measure of I_{Na} responsible for conduction, is greatest in a condition free from both I_{Na} inactivation and outward K^+ current activation. In the present study, $\dot{V}_{max}$ observed at the lowest $[K^+]_e$ was suppressed by nicorandil (Table). This implies that nicorandil increased the background g_K which shifted RMP to the hyperpolarized direction toward the K^+ equilibrium potential (E_K), and augmented outward K^+ current which may have partly antagonized I_{Na} without influencing I_{Na} inactivation kinetics (Fig. 2). No suppression of 100 μM nicorandil on $\dot{V}_{max}$ in low (2.7 mM) $[K^+]_e$ was reported in the previous studies using canine Purkinje fibers[17)18)]. This is due in part to the difference in preparations, i.e., $\dot{V}_{max}$ in Purkinje fiber is far greater than that in myocardium.

Although nicorandil-induced cell-to-cell uncoupling was theoretically anticipated, no significant changes in r_i was observed by this agent (Table). Here, coupling coefficient (CC) is defined as a ratio of cellular gap junctional conductance (g_{gap}) divided by cellular membrane conductance (g_m) and is considered as one of the practical parameters responsible for cell-to-cell coupling, as follows[19)];

$$CC = g_{gap} / g_m \qquad [7]$$

Nicorandil is expected to increase g_m

(~ g_K at rest) as a K_{ATP} channel opener[17)18)] and is known to increase cGMP levels in cardiovascular tissue[20)] due to the nitrate moiety in its molecule[8)]. Although the effects of cGMP on myocardial gap junction are complicated[21)], Lucifer Yellow diffusion technique demonstrated no change in the diffusion coefficient in dog trabeculae[22)], whereas patch clamp technique using neonatal rat heart cell pairs revealed fall in g_{gap}[23)] by cGMP application. Based on equation [7], an increase in g_m alone or in combination with a possible decrease in g_{gap} leads theoretically to a decrease in CC, especially in the low $[K^+]_e$ condition. However, our study did not support this speculation probably due to an extent of the lowest $[K^+]_e$ in our experimental setting (i.e., 3.0 mM).

This study allows a few limitations. As a first one, the approximation of the steady state inactivation of I_{Na} by the $[K^+]_e$-dependent $\dot{V}_{max}$ alteration is problematic. V_h obtained by this study (i.e. −62 to −63 mV) shifted to the depolarized direction relative to V_h of I_{Na} (i.e. −72 to −94 mV) recorded in the single cardiomyocytes under the whole-cell patch clamp technique[24)25)]. Similarly, s in this study (about 3.5 mV) was exactly the same as in literature[26)] but quite smaller than that (ranging 5.1 to 6.9 mV) obtained by patch clamp study[24)25)]. These indicate that steady state inactivation of I_{Na} is not negligible at RMP of about −90 mV, which value was obtained by the lowest $[K^+]_e$ in this study. Secondly, intrinsic problem of

Table $[K^+]_e$-dependent, nicorandil-induced alterations of electrophysiologic parameters

$[K^+]_e$ (mM) (n)	3.0 (7)	5.4 (8)	9.0 (6)	12.0 (5)
θ (cm / sec)	61.2 ± 6.1#	68.0 ± 4.9	73.1 ± 5.9#	49.1 ± 5.3##
	57.0 ± 5.6#*	66.7 ± 5.4	71.9 ± 6.4#	46.3 ± 5.9##
$\dot{V}_{max}$ (V / sec)	198.6 ± 8.6#	193.7 ± 9.8	173.8 ± 8.7##	72.4 ± 7.1##
	191.3 ± 9.4*	189.1 ± 7.3	172.5 ± 9.2##	72.1 ± 7.5##
V_i (mV)	125.0 ± 5.1#	116.6 ± 4.2	102.7 ± 4.0#	83.6 ± 3.2##
	128.9 ± 5.6#	118.8 ± 4.3	103.0 ± 4.5#	83.9 ± 3.2##
V_e (mV)	9.4 ± 0.4#	8.7 ± 0.5	7.6 ± 0.3##	6.1 ± 0.4##
	8.6 ± 0.5#	8.8 ± 0.4	7.6 ± 0.4##	6.0 ± 0.3##
V_i / V_e	13.3 ± 1.2	13.4 ± 1.4	13.4 ± 1.3	13.6 ± 1.6
	14.9 ± 1.3*	13.7 ± 1.4	13.6 ± 1.5	13.8 ± 1.7
r_{total} (KΩ / cm)	1.74 ± 0.30	1.71 ± 0.26	1.70 ± 0.28	1.72 ± 0.24
	1.75 ± 0.29	1.72 ± 0.28	1.71 ± 0.27	1.71 ± 0.24
r_e (KΩ / cm)	1.87 ± 0.34	1.87 ± 0.33	1.85 ± 0.31	1.84 ± 0.32
	1.88 ± 0.34	1.88 ± 0.34	1.85 ± 0.30	1.83 ± 0.33
r_i (KΩ / cm)	21.3 ± 2.0	21.2 ± 1.9	20.9 ± 1.8	20.9 ± 1.7
	22.7 ± 2.3	21.8 ± 2.1	20.9 ± 2.0	21.0 ± 1.8
APD (msec)	232 ± 34##	184 ± 23	148 ± 19#	109 ± 18##
	207 ± 36##*	171 ± 24*	145 ± 21#	107 ± 20##

Data are mean ± SD. APD, action potential duration at 90 % repolarization; $[K^+]_e$, external K^+ concentration; r_e, r_i, r_{total}, external, internal and total longitudinal tissue resistance per unit length; θ, conduction velocity of excitation; V_e, V_i, extracellular and intracellular potential amplitudes, $\dot{V}_{max}$, the maximum rate of rise of the action potential upstroke. Upper and lower values in each parameter correspond to the absence and the presence of 100 μM nicorandil, respectively (n = 5 ~ 8). *$p < 0.05$ compared with respective control (i.e., drug-free) condition. #$p < 0.05$, and ##$p < 0.01$ compared with 5.4 mM $[K^+]_e$ condition. Basic cycle length (BCL) of the stimulation was 1.0 sec throughout.

superfusion technique using tissue preparation should be noted. An extent of 100 μM nicorandil-induced APD shortening in 3.0 mM $[K^+]_e$ in this study (~11 %) is quite less than that in the other studies using cardiomyocytes[9] or tissue preparations[17)18)] in the similar low (2.7 mM) $[K^+]_e$ condition (17 % to 57 %). This may raise the problems of drug permeation and K^+ or oxygen diffusion in the tissue preparation. Since APD is very sensitive to the local $[K^+]_e$ or partial oxygen pressure and is influenced by electrotonic interactions[10]. However, nicorandil-induced, $[K^+]_e$-dependent APD shortening confirmed in this study is in accordance with previous study[18]. Cellular ATP content may vary depending on the preparations, leading to the variations of the effects of nicorandil on APD.

In conclusion, 100 μM nicorandil demonstrated $[K^+]_e$-dependent, mild but significant negative dromotropism. This phenomenon was accounted for by the cable analysis including cell-to-cell coupling concept and may contribute to the antiarrhythmic actions of nicorandil in the low $[K^+]_e$ condition, which sometimes underlies the ischemia-related[27] or unrelated[14)15)16)] triggered arrhythmias.

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（和文抄録）

モルモット乳頭筋における外液 K^+ 濃度依存性のニコランジルの陰性変伝導作用

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ニコランジルの抗不整脈作用が近年注目されているが，その変伝導作用は明らかでない．今回これを検討するためにモルモット乳頭筋の表面潅流標本に微小電極法を用いて細胞内活動電位，細胞外電位を同時記録した．また細胞内抵抗，活動電位最大立ち上がり速度，伝導速度の関係を外液 K^+ 濃度を変化させて（3.0－12.0 mM），ニコランジル（100 μM）の有無で検討した．ニコランジルを投与すると低 K^+ 濃度（3.0 mM）下で有意な（$p < 0.05$）膜の過分極と活動電位最大立ち上がり速度の減少，さらに興奮伝導速度の減少を認めたが，これらの変化は正常（5.4 mM）ないし高 K^+ 濃度（9.0－12.0 mM）下ではみられなかった．またニコランジルは細胞間の電気的結合の指標である細胞内抵抗には影響しなかった．さらにニコランジルによる活動電位持続時間の短縮は K^+ 濃度が低下するにつれて顕在化した．今回明らかとなったニコランジルの低 K^+ 濃度下での陰性変伝導作用は，この様な状態で発生しやすい不整脈に対して抑制的に作用することが期待される．