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SHORT COMMUNICATION

Activation of ATP-sensitive potassium channels by nicorandil is preserved in aged vascular smooth muscle cells in rats

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Abstract Nicorandil, an ATP-sensitive potassium (K_{ATP}) channel opener having the properties of a nitrate, causes vasodilation, particularly of coronary arteries, and has been reported to reduce the frequency of perioperative cardiac events. We previously demonstrated that isoflurane could activate vascular K_{ATP} channels through an intracellular signaling pathway, but that this isoflurane-induced channel opening is suppressed by aging. Here, we investigated whether advanced age modifies nicorandil-induced activation of vascular K_{ATP} channels. We used a cell-attached patch-clamp configuration to test the effects of nicorandil on K_{ATP} channel activity in vascular smooth muscle cells (VSMCs) obtained from 12- to 15-week-old (adult) and 24- to 25-month-old (aged) male Wistar rats. Bath application of nicorandil (0.1–100 μM) activated K_{ATP} channels to a level similar to that observed in VSMCs from the arteries of both adult and aged rats. Furthermore, concomitant bath application of nicorandil in the aged group dose-dependently ameliorated the age-related reduction in isoflurane-induced vascular K_{ATP} channel activation. Our findings indicate that nicorandil could be used effectively in elderly patients to directly activate vascular K_{ATP} channels during the perioperative period.

Keywords Nicorandil · Isoflurane · Potassium channels

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Adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels link membrane excitability to metabolism [1]. The intrinsic opening of K_{ATP} channels in vascular smooth muscle cells (VSMCs) during ischemia is critical for vasodilatation in target organs, enhancing hypoxic tolerance [2, 3]. Pharmacological activation of K_{ATP} channels by K^+ channel openers (KCOs) can mimic these beneficial actions, and thus KCOs have therapeutic potential for the treatment of ischemic conditions such as angina [3]. Indeed, it has been reported that the perioperative use of nicorandil, the first clinically available KCO with a nitrate-like action, reduces the frequency of cardiac events [4]. In addition to KCOs, volatile anesthetics produce vasodilatation by activating vascular K_{ATP} channels [5, 6]. However, we recently demonstrated that advanced age impaired isoflurane-induced K_{ATP} channel activation [7]. On the other hand, it was not known whether advanced age affects nicorandil-induced vascular K_{ATP} channel activation. The present study was undertaken to examine whether advanced age influences the effect of nicorandil on K_{ATP} channel activities in freshly isolated rat VSMCs.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. Twelve- to 15-week-old (adult group: weight 267 ± 16 g, $n = 6$) and 24- to 25-month-old (aged group: weight 401 ± 25 g, $n = 5$) male Wistar rats were used in the present study. VSMCs were isolated using a similar procedure to that reported previously [7]. Single-channel currents were recorded in the cell-attached patch-clamp configuration, which retains the physiological intracellular signaling milieu. Both bath and pipette solutions contained 140 mM KCl, 10 mM HEPES, 10 mM D-glucose, and 0.5 mM ethylene glycol tetraacetic acid (EGTA). Data acquisition and analyses were performed using pClamp 9.2 (Axon Instruments, Foster City, CA,

USA). Open-channel events were identified using a conventional 50 % current amplitude threshold level criterion. Open-channel probability (Po) was determined from the ratios of the areas under the peaks of amplitude histograms fitted to a Gaussian curve. Channel activity was calculated as NPo, where N is the number of observed channels in the patch from data samples of 60-s duration in the steady state. Results are presented as the mean ± SEM. Results are evaluated by one-way analysis of variance followed by Bonferroni post hoc tests, with $P < 0.05$ regarded as significant.

Single K_{ATP} channel openings in adult and aged VSMCs were recorded through cell-attached patches at a holding potential of −60 mV. Our previous studies have shown that, under these conditions, single K_{ATP} channel currents with a single-channel conductance of approximately 40 pS were observed, which were identical in the two age groups [7]. As shown in Fig. 1a, the bath application of nicorandil (0.1–100 μM) dose-dependently activated K_{ATP} channels in VSMCs from the arteries of both adult and aged animals. In both groups, the subsequent addition of 1 μM glibenclamide, a specific K_{ATP} channel inhibitor, reversed the effects of nicorandil. The nicorandil-induced NPo values were similar in VSMCs from adult ($n = 9$) and aged ($n = 9$) rats (Fig. 1b). Plasma levels of nicorandil during intravenous administration are reported to be approximately 1–4 μM [8, 9], and thus the concentrations of nicorandil that activated K_{ATP} channels in our experiments are clinically relevant.

In a separate experiment, coadministration of *N*-nitro-L-arginine methyl ester (L-NAME, 100 μM), a nonselective endogenous NO synthetase inhibitor, had no effect on nicorandil-induced K_{ATP} channel activation (data not shown; $n = 5$ in each group), indicating that the nitrate-like action of nicorandil is not involved in K_{ATP} channel activation.

We next examined the effects of concomitant administration of nicorandil on isoflurane-induced K_{ATP} channel activation in both age groups. In agreement with our previous data [7], the bath application of isoflurane (0.5 mM, 1.0 rat MAC) activated K_{ATP} channels in VSMCs from the arteries of adult rats, but this isoflurane-induced K_{ATP} channel activity was suppressed in VSMCs from the arteries of aged rats (Fig. 2a). Subsequent application of nicorandil (0.1–10 μM) in the aged group dose-dependently ameliorated this age-related suppression. The isoflurane-induced NPo in the presence or absence of nicorandil in the two age groups is summarized in Fig. 2b. In this experimental method, isoflurane-induced K_{ATP} channel activity in the aged group was too low to analyze the dose-dependent effects of isoflurane. Thus, we could not determine whether there is an additive or synergistic effect of isoflurane and nicorandil on K_{ATP} channels in VSMCs from the arteries of aged rats using isobologram analysis.

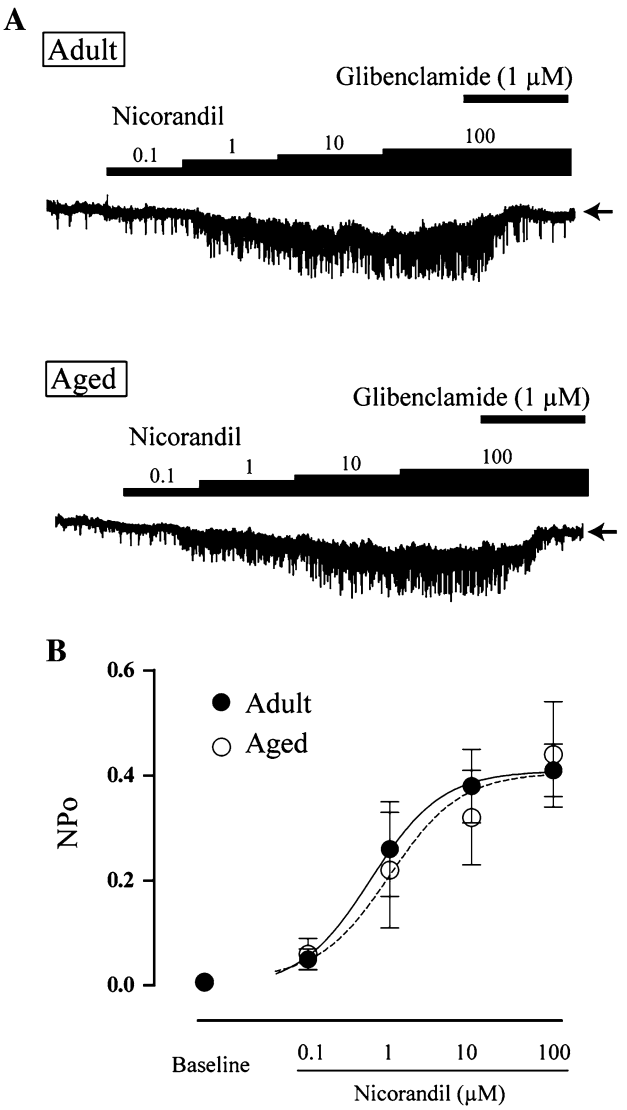

Fig. 1 Effects of nicorandil (0.1–100 μM) on ATP-sensitive potassium (K_{ATP}) channel activity in rat vascular smooth muscle cells from arteries of adult (12–15 weeks) and aged (24 months or older) rats. **a** Representative current traces of K_{ATP} channels in the adult and aged groups recorded in cell-attached configuration at a holding potential of −60 mV. Study drugs were superfused into the bath solution during the periods indicated by the *solid horizontal bars*. **b** Summation of changes in open channel probability (*NPo*) values. Values are shown as means ± SEM

Previous genetic studies have shown that vascular K_{ATP} channel-deficient mice exhibit phenotypes of impaired vascular function, coronary vasospasm, and a high rate of sudden death [10, 11]. Thus, activation of vascular K_{ATP} channels during the perioperative period may enhance the endogenous cardiovascular protective effects mediated by these channels. Although we previously found that isoflurane pharmacologically activates vascular K_{ATP} channels via the activation of protein kinase A [6], we also reported that K_{ATP} channel

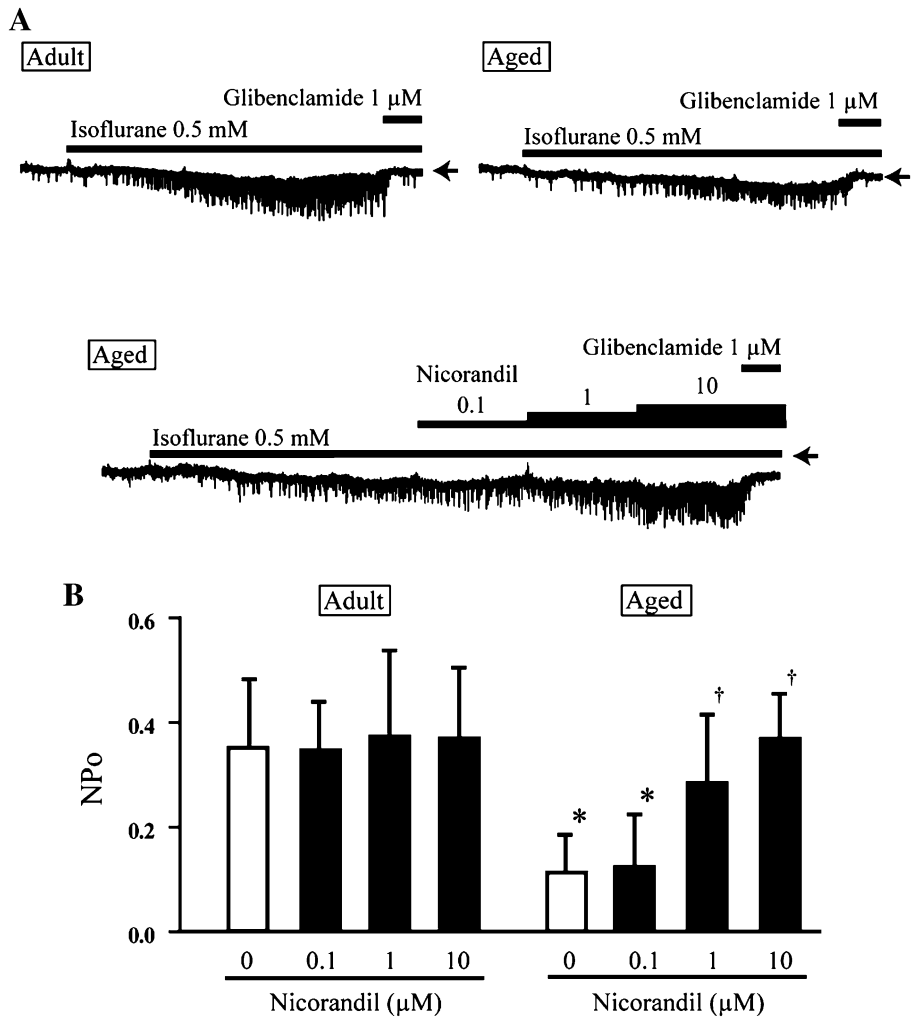

Fig. 2 Additive effect of nicorandil on isoflurane-induced ATP-sensitive potassium (K_{ATP}) channel activation in rat vascular smooth muscle cells. **a** Representative current traces of K_{ATP} channels in the adult (12–15 weeks) and aged (24 months or older) groups recorded in cell-attached configuration at a holding potential of −60 mV. Study drugs were superfused into the bath solution during the periods indicated by the *solid horizontal bars*. **b** Summation of changes in open channel probability (*NPo*) values. Values are shown as means ± SEM. Each *vertical bar* represents measurements from five patches. $^{*}p < 0.05$ versus adult group; $^{\dagger}p < 0.05$ versus baseline (isoflurane alone)

opening induced by isoflurane was significantly suppressed by aging [7]. In contrast, the present study demonstrates that nicorandil can activate vascular K_{ATP} channels with comparable efficiency in adult and aged groups (Fig. 1b) and ameliorate the age-related reduction in isoflurane-induced channel activation (Fig. 2b). Because nicorandil directly activates K_{ATP} channels by binding to channel subunits [2], the intrinsic single-channel properties per se may not be affected by aging. This finding indicates that K_{ATP} channels remain available for therapeutic targeting in aged tissue. In keeping with this hypothesis, it has been reported that nicorandil can reverse the impaired preconditioning of the aged heart [12].

In conclusion, nicorandil-induced activation of vascular K_{ATP} channels is preserved in aged rats. Our findings indicate that nicorandil may enhance the endogenous protective mechanisms mediated by vascular K_{ATP} channels, even in elderly patients.

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