Effects of 4-Aminopyridine on the Fade Response to Vagal Nerve Stimulation in the Isolated Dog Atrium

Yasuyuki FURUKAWA, Kimiaki SAEGUSA and Shigetoshi CHIBA*

Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan

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Abstract—Intramural parasympathetic nerve stimulation (5 Hz, 2 min) after propranolol treatment evoked negative chronotropic and inotropic responses in the isolated dog atrium. The cardiac responses were not maintained during stimulation; they reached maximum values and then faded back toward their control values ("fade" response). 4-Aminopyridine increased the maximum values of both responses, and it increased the fade of the chronotropic response but not that of the inotropic response. These results suggest that the fade response to parasympathetic nerve stimulation is modulated at the parasympathetic nerve terminals of the isolated dog atrium.

Although vagal nerve stimulation induced frequency-dependent decreases in heart rate and contractile force, continuous high frequency stimulation could not maintain the maximum changes in atrial rate and contractile force and they usually faded (1, 2). Kilbinger and Löffelholz (3) demonstrated that the release of acetylcholine (ACh) to vagal nerve stimulation at a low frequency of 3 Hz was constant and that ACh overflow gradually faded at a high frequency of 20 Hz in the isolated, perfused chicken heart. We suggested previously that parasympathetic nerve stimulation at a frequency of 5 Hz induced relatively constant release of ACh in the isolated dog atrium (2). It was reported that 4-aminopyridine (4-AP) prolonged the action potential duration due to inhibition of K+ outward current in the giant axon of the cockroach (4) and that it increases the Ca++ inward current in sartorius muscles of the frog (5). In the isolated and perfused dog atrium, we observed that 4-AP potentiated dose-dependently the maximal cardiac responses to ACh release by intramural parasympathetic nerve stimulation (6). In the present study, therefore, it was investigated whether 4-AP potentiated steadily the negative cardiac responses during continuous vagal nerve stimulation at a low frequency of 5 Hz in the isolated, blood-perfused dog atrium.

The isolated right atrial muscle was obtained from a mongrel dog, which weighed from 9 to 18 kg, after the dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and sodium heparin was injected (200 USP units/kg, i.v.). The isolated atrial preparation was perfused through the sinus node artery with heparinized arterial blood conducted from another anesthetized dog, which weighed 10–22 kg. The details of the preparation have been described in a previous paper (7). The isometric tension development and atrial rate were continuously recorded during the experiment. After 1 mg/kg of propranolol hydrochloride (Sumitomo Chemicals) was injected intravenously to the support dog, intramural parasympathetic nerve fibers of the isolated atrium were stimulated by means of an electrical stimulator (Nihon Kohden SEN-7103) at a frequency of 5 Hz for 2 min. The pulse duration of the stimuli was 0.3 msec, and the voltage (3–6 V) was adjusted just below the threshold for activation of the pacemaker cells and myocardial fibers but above the threshold for activation of intramural parasympathetic nerve fibers of the isolated atrium.
nerve fibers (2). In order to investigate effects of 4-AP on the vagal-induced cardiac effects, after 4-aminopyridine (4-AP, base, Wako Pure Chemicals), dissolved in physiological saline, treatment at a dose of 3, 30 or 300 μg into the sinus node artery, parasympathetic nerve fibers were stimulated at a frequency of 5 Hz for 2 min. Figure 1 shows the changes in atrial rate and contractile force during parasympathetic nerve stimulation at a frequency of 5 Hz in a representative experiment (panel A) and how to calculate the maximum value (Max) and fade of the responses (panel B). Data were analyzed by means of a three-way mixed model analysis of variance and Student’s t-test.

When 4-AP was injected at a dose of 3 to 300 μg after treatment with propranolol, a slight positive chronotropic and positive inotropic effects were observed as shown by Furukawa et al. (6).

Intramural parasympathetic nerve stimulation at a frequency of 5 Hz for 2 min decreased rapidly the atrial rate and contractile force (Fig. 1A). After reaching maximum negative cardiac responses, the negative inotropic response faded 44±5.7 (mean±S.E.) % back toward the prestimulation level, and the negative chronotropic response faded a little (12±4.9%). Treatment with 4-AP dose-dependently potentiated the maximum negative chronotropic and inotropic responses to vagal nerve stimulation (Fig. 2, left panel; P<0.05). The fade of the chronotropic response to parasympathetic nerve stimulation was dose-dependently (P<0.05) augmented by 4-AP, whereas the fade of the inotropic response was not changed (Fig. 2, right panel).

When vagal nerve fibers were stimulated successively at a low frequency (less than 5 Hz), the maximum negative chronotropic effect was maintained at almost the constant level in the isolated kitten or dog heart tissue (1, 2). In the isolated perfused chicken heart, ACh overflow was maintained even when the vagal nerves were stimulated at a frequency of 3 Hz for more than 5 min (3). In the present study, the negative chronotropic response to parasympathetic nerve stimulation at a frequency of 5 Hz rapidly reached to the maximum level, and then it faded a little back toward the prestimulation level. It would be expected that the release of ACh to parasympathetic nerve stimulation at a frequency of 5 Hz was almost constant for 2 min. Previously, we (6) investigated effects of 4-AP on the isolated, blood-perfused dog atrium. It was shown that 4-AP potentiated the maximal negative cardiac responses to vagal

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**Fig. 1.** A: The changes in atrial rate and contractile force during intramural parasympathetic nerve stimulation at a frequency of 5 Hz in a representative experiment of an isolated, blood-perfused dog atrium. The pulse duration of the stimuli was 0.3 msec and the voltage was 4 V. B: Schematic response of the isolated atrium to parasympathetic nerve stimulation to illustrate how the maximum value (Max) and fade of the responses were calculated. a, control value before the start of the stimuli; b, maximum value of the response; c, magnitude of the response just before cessation of the stimulation.
nerve stimulation but not similar responses to exogenous ACh. Finally, we suggested that the potentiating effect of 4-AP on the negative cardiac responses to vagal nerve stimulation is due to an increase in ACh release. Thus, it would be expected that 4-AP potentiated steadily the negative cardiac responses to continuous parasympathetic nerve stimulation. In the present study, however, the fade of the chronotropic response was increased after treatment with 4-AP in a dose-dependent manner. Infusion of ACh did not induce a fade of the chronotropic response (2). Therefore, it is suggested that 4-AP modulates the fade of the chronotropic response to parasympathetic nerve stimulation at a prejunctional site, i.e., 4-AP increases the release of ACh from the nerve terminals but the increase gradually fades during stimulation. The temporal decrease of the ACh release may be induced by a limitation of the depolarization due to prolongation of the action potential duration (4, 5), muscarinic suppression by released ACh at the presynaptic nerve terminals (8, 9), or other mechanisms.

Whereas the maximum negative inotropic response to vagal nerve stimulation was potentiated by 4-AP, the fade was not augmented. This discrepancy from the fade of the chronotropic response might be related to other additional mechanisms, i.e., frequency-dependent changes in contractile force or a different sensitivity to ACh between pacemaker activity and contractility.

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