Effect of N-Feruloyl Tyramine (an Analogue of Tyramine) on Inwardly Rectifying Potassium Channel in Frog Ventricular Myocytes

Makoto MUNEMORI, Kaoru YAMAOKA, Irawan YUSUF, Kotaro SUMI, Hidenki OTSUKA, and Kazuo YAMASAKI

1) Department of Physiology, Hiroshima University School of Medicine
2) Institute of Pharmaceutical Science, Hiroshima University School of Medicine, Hiroshima, Japan

ABSTRACT

Electrophysiological effects of N-feruloyl tyramine (NFT), an analogue of tyramine, on potassium currents in frog ventricular myocytes were examined using single-channel recording and whole-cell voltage clamp techniques. Extracellular application of NFT induced a concentration-dependent decrease of macroscopic inward rectifier potassium current (iK1) with ID50 of 19.11 M, while tyramine (100 pM) was ineffective in producing an inhibitory effect on iK1. NFT reduced the mean open time of iK1 to 1.3 ms from 3.1 ms in control without affecting the amplitude of single-channel conductance. It is indicated that boi containing NFT produces a prolongation of the plateau phase caused by the suppression of inwardly rectifying K channel. Thus, this prolongation may induce an increase in the inflow of Ca ions, which in turn leads to a positive inotropic effect.

Key words: NFT, iK1, Suppression

It has been reported that a Chinese folklore medicine, boi, containing extracts from Sinomenium acutum, suppresses the maximum rate of rise of action potential and prolongs its duration in the frog heart, and that consequently the contraction force is increased by 50% of the control. We previously showed that the main extracts of boi, N-feruloyl tyramine (NFT) and tyramine, exert inhibitory action on sodium (Na) channels with suppressing the maximum conductance and also shifting the steady state inactivation curve in the hyperpolarizing direction. Since no electrophysiological findings obtained so far explain the positive inotropic effect of boi, we have examined the effect of NFT and tyramine on the potassium (K) channels in single ventricular frog myocytes.

MATERIALS AND METHODS

Cell preparation

The method for the isolation of single ventricular cells from the frog (Rana catesbeiana) was essentially the same as that in a previous experiment (Neyama & Yamaska, 1998). Briefly, a heart was mounted on a Langendorff apparatus and retrogradely perfused via the aorta with a Ca2+ free solution containing collagenase (0.1 mg/ml; Yakult, Tokyo, Japan) and trypsin (0.06 mg/ml; type I, Sigma Chemical, St. Louis, MO, USA) for 20 min at 32°C. Then the isolated ventricle was cut into pieces and dispersed by pipette agitation. The single cells were kept in a solution containing a low Ca2+ concentration (200 nM) for 30 min and then centrifuged for 1 min at 93 x g and stored in Leibovitz's L-15 medium (GIBCO, Grand Island, NY, USA) for experimental use.

Solutions and chemicals

The compositions of the solutions are given in Table 1. Whole-cell currents other than K currents were eliminated by the use of an external solution containing 1 μM tetrodotoxin (TTX; Sankyo Co. Ltd, Tokyo, Japan) and 3.0 mM MgSO4 without adding CaCl2 (external-1). Single-channel currents were recorded with external-2 and internal-2 solutions. Tyramine (4-hydroxyphenylethylamine) was purchased from Sigma Chemical Co. NFT was syn
Electrophysiological recording and analysis

The experiments were carried out in whole-cell and inside-out configurations using the conventional patch-clamp technique[5]. The records obtained by a patch-clamp amplifier (Axopatch 200A, Axon Instruments, Inc., Foster City, CA, USA) were stored on tapes using a DAT tape-recorder (DTC-1000 ES, SONY, Tokyo, Japan). Data were filtered with 1 kHz for analysis. All data in NFT were referred to those in control and were plotted against the concentration of NFT. The line was fitted by the equation of 1/(1+ IC50/D)[4], where IC50 indicates half inhibition dose of NFT for the channel and n: Hill's coefficient. Fitting the equation to the data gave IC50 of 198 x 10^-6 M and n of 0.26. Vertical bars indicate mean ± standard error (S.E.). Number of cells examined is shown in parentheses.

RESULTS

Suppression of macroscopic inward-rectifier potassium current (iK+) by external application of NFT

K channels in frog ventricular myocytes consist mainly of inwardly rectifying K (KIR) channels and ATP-sensitive K (KATP) channels. Since we employed an ATP-rich internal solution for the whole-cell current recording, K current observed in this configuration should be mainly iK+. On applying 175 ms pulses of various amplitudes from a holding potential of -65 mV, the resultant current-voltage (I/V) curve showed a characteristic N-shaped configuration indicating that the currents recorded in this condition were mainly carried by K ion through inwardly rectifying K channels[8]. Fig. 2: NFT (100 μM) in the external solution significantly suppressed iK+ at membrane potentials negative to 0 mV. However, 100 μM tyramine applied externally did not suppress iK+ in any of the three cells tested.

Dose-response relationship for NFT action

The dose-response relationship was studied by measuring the macroscopic iK+ during a 175 ms test pulse to −110 mV from a holding potential of −65 mV. The test pulses were applied every 30 s. As shown in Fig. 3, iK+ was slightly decreased at 10 μM NFT and the suppression became more pronounced at 100 μM NFT. The dose-response curve is shown in Fig. 4. IC50 was estimated to be 198 μM with a Hill's coefficient of 0.26.

Effect of NFT on the single-channel activities of inwardly rectifying K channels

To determine the mechanism underlying the suppression of iK+ we recorded single-channel currents for inwardly rectifying K channels in frog ventricular myocytes. Since frog cardiac myocytes have been reported to contain 2–3 mM ATP[5], KATP channels are thought to remain inert regardless of the presence of NFT, the inwardly rectifying iK+ channel is highly selective to K ion and its ionic selectivity is not affected by NFT. The conductance was estimated to be 38.9 pS and 38.5 pS with and without NFT, respectively. Combined open time histograms were made from three patches. The bin width was set to 0.4 ms. The mean open time were shortened from 3.1 ms to 1.3 ms by application of NFT (Fig. 5). Amplitude histograms were compiled from the records obtained at 100 mM NFT.
**Fig. 5.** The I/V relation for single-channel current of K:\nA. Data in the absence control of NFT gave almost identical linear conductances (33.9 pS and 33.5 pS with and without NFT, respectively) when fitted by straight lines. Data were obtained from inside-out patches of three cells. Vertical bars indicate mean ± S.E. B. These figures indicate representative currents for each condition at different membrane potentials.

**Fig. 7.** Amplitude histograms for K:\nThe single-channel amplitudes were constructed from the original recorded in the inset of Fig. 6 with the bin width set at 0.1 pA. Gaussian curves were fitted to these histograms and unitary conductances were determined to be 1.51 pA and 1.45 pA with and without NFT, respectively. Membrane potential were -40 mV.

shown in Fig. 6 (Fig. 7). The single-channel amplitudes were 1.94 ± 0.24 pA (n = 3) in the control solution and 1.72 ± 0.28 pA (n = 3) in the presence of NFT, i.e., the conductance was not affected by NFT.

**DISCUSSION**

The present study has unveiled several characteristics of the action of NFT on inwardly rectifying K channels in frog ventricular myocytes. First, NFT suppressed inwardly rectifying K channels at membrane potentials negative to 0 mV when applied extracellularly. Second, NFT should prolong the plateau phase of action potential and, consequently, the influx of Ca ions may increase, resulting in the enhancement of contraction; the positive inotropic effect. The finding in our previous study\(^2\) that main pharmacological action of tyramine and NFT is the suppression of Na channels without affecting calcium channels accords well with the notion discussed above.

Since tyramine contains two hydrophilic groups per eight carbon atoms and NFT three per seven-\(^2\)teen (see Fig. 1), it is reasonable to assume that tyramine is more hydrophilic than NFT. In comparing their inhibitory action on Na channel, NFT has been reported to be more potent than tyramine\(^1\). When the results obtained in this study are reconciled with those on sodium channel, a hydrophilic character seems to be critical to pharmacological action on channels. Thus, one can assume that the binding site in K channels accessible from the external surface may be in a more hydrophobic environment. Another noticeable finding is that Hill's coefficient = 0.26. It is suggested that receptors for NFT in a hydrophilic environment retain a strong negative cooperativity; several receptors around the binding site being subjected to a suppressive influence.

**ACKNOWLEDGMENTS**

We would like to express our sincere thanks to Prof. I. Seiyama for his guidance and for reviewing the manuscript. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science and Culture (Grant number 02257101, 03253101 and 04248101, and the Uehara Memorial Foundation.


