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

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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 technique. Extracellular application of NFT induced a concentration-dependent decrease of macroscopic inward rectifier potassium current (i_{K1}) with ID_{50} of 198 μM, while tyramine (100 μM) was ineffective in producing an inhibitory effect on i_{K1}. NFT reduced the mean open time of i_{K1} 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, i_{K1}, 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[4]. We previously showed that the main extracts of *boi*, *N*-feruloyl tyramine (NFT) and tyramine, exert inhibitory action on sodium (Na) channels without affecting calcium (Ca) channels by suppressing the maximum conductance and also shifting the steady state inactivation curve in the hyperpolarizing direction[10]. Since no electrophysiological findings obtained so far explain the positive inotropic effect of *boi*[4], 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 (Seyama & Yamaoka, 1988). Briefly, a heart was mounted on a Langendorff apparatus and retrogradely perfused via the aorta with a Ca^{2+} 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 Ca^{2+} concentration (200 μM) for 30 min and then centrifuged for 1 min at 93 × *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 $MgSO_4$ without adding $CaCl_2$ (external-1). Single-channel currents were recorded with external-2 and internal-2 solutions.

Tyramine (4-hydroxyphenylethylamin) was purchased from Sigma Chemical Co. NFT was syn

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Table 1. Composition of the solutions (in mM)

	Whole-cell experiment		Single-channel current experiment	
	external-1	internal-1	external-2	internal-2*
NaCl	113.5	10.0	—	—
K_2ATP	—	5.0	—	—
MgATP	—	—	—	3.0
Na_2-creatine phosphate	—	—	—	3.0
KCl	5.4	30.0	110.0	113.5
K-aspartate	—	100.0	—	—
$CaCl_2$	—	—	1.0	0.62
$MgSO_4$	3.0	5.0	—	—
glucose	—	10.0	—	10.0
EGTA	5.0	5.0	—	5.0
HEPES	10.0	10.0	10.0	10.0
TTX	0.001	—	—	—
pH	7.2	7.0	7.2	7.0

*Free Ca^{2+} concentrations of internal-2 were calculated to be 1.0×10^{-8} M, using Schoenmakers' program[7].

thesized from tyramine and feruloic acid. Briefly, *O*-acetylferuloic acid was condensed with tyramine to give *O*-acetylferuloyl tyramine. NFT was obtained by alkaline hydrolysis of *O*-acetylferuloyl tyramine and purified by crystallization. The chemical structures of the reagents are shown in Fig. 1. Stock solutions of tyramine (100 mM) and NFT (100 mM) were prepared in dimehtylsulfoxide (DMSO). All the test solutions were freshly prepared before each experiment. All experiments were conducted at room temperature (23–26°C) unless otherwise stated.

Electrophysiological recording and analysis

The experiments were carried out in whole-cell and inside-out configurations using the conventional patch-clamp technique[3]. 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 through a low-pass filter of Axopatch 200A, having a –3 dB cut-off frequency of 5 kHz. Data were recorded by DAT sampled at 5 kHz and were filtered with 1 kHz for analysis. Data were expressed as mean ± standard error (S.E.). The statistical significance between groups was determined by the Mann-Whitney test, $p<0.05$ being considered signifcant.

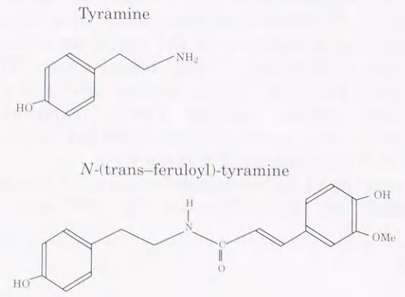

Fig. 1. Structure of *N*-feruloyl tyramine and tyramine.

RESULTS

Suppression of macroscopic inward-rectifier potassium current (i_{K1}) by external application of NFT

K channels in frog ventricular myocytes consist mainly of inwardly rectifying K channels and ATP-sensitive K (K_{ATP}) channels. Since we employed an ATP-rich internal solution for the whole-cell current recording, K current observed in this configuration should be mainly i_{K1}. 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[6,9] (Fig. 2). NFT (100 μM) in the external solution significantly suppressed i_{K1} at membrane potentials negative to 0 mV. However, 100 μM tyramine applied externally did not suppress i_{K1} in any of the three cells tested.

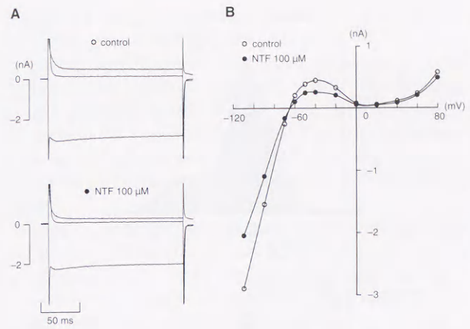

Fig. 2. A. Records of i_{K1} in the presence and absence of NFT (100 μM).
I_{K1}s were evoked by hyperpolarizing test pulses to –110, –40 and 0 mV from a holding potential of –65 mV. B. Current-voltage (I/V) relationship for i_{K1}. Amplitudes of the steady state i_{K1} current at the end of 175 ms test pulses are plotted against the membrane potential.

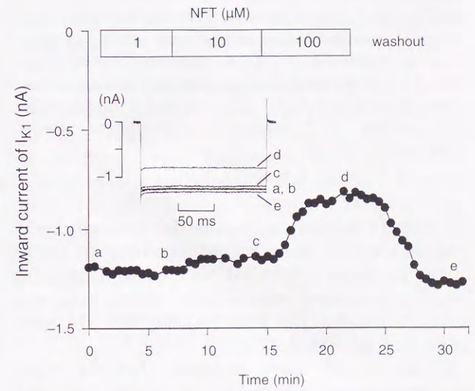

Fig. 3. Time course for the effect of NFT on steady state i_{K1}.
NFT (1, 10 and 100 μM) was consecutively applied externally. Test pulses to –110 mV from a holding potential of –65 mV were applied every 30 s. Amplitudes of i_{K1} at the end of 175 ms pulse (measured as a difference between the holding current and i_{K1}) were plotted against time.

Dose-response relationship for NFT action

The dose-response relationship was studied by measuring the macroscopic i_{K1} 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, i_{K1} 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. ID_{50} was estimated to be 198 μM with a Hill's coefficient of 0.26.

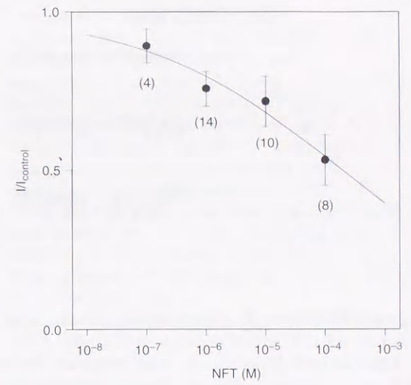

Fig. 4. Dose-response curve for NFT.
All data in NFT were referred to those in control and were plotted against the concentration of NFT. The line was fitted by the equation of $1/(1+([NFT]/ID_{50})^n)$, where ID_{50} indicates half inhibition dose of NFT for the channel and n, Hill's coefficient. Fitting the equation to the data gave ID_{50} of 1.98×10^{-4} M and n of 0.26. Vertical bars indicate mean ± standard error (S.E.). Number of cells examined is shown in parentheses.

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

To determine the mechanism underlying the suppression of i_{K1}, 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[1], K_{ATP} channels are thought to remain inert in a physiological condition. Therefore, the single-channel currents obtained in the following experiments were considered to be i_{K1}. The extrapolated I/V curves for i_{K1} with and without NFT were made from the collected data of several individual patches (Fig. 5). Since the lines adopted to data obtained with 110 mM $[K^+]_0$ and 113.5 mM $[K^+]_i$ cross the abscissa at 0 mV, regardless of the presence of NFT, the inwardly rectifying K channel is highly selective to K ions 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. 6). Amplitude histograms were compiled from the records

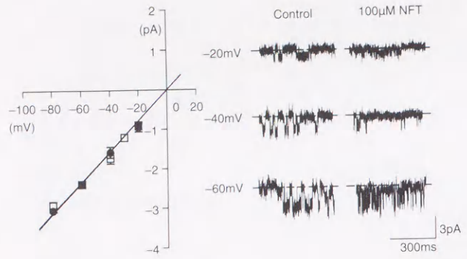

Fig. 5. The I/V relation for single-channel current of i_{K1}.
A. Data in the absence (□) and presence (●) of NFT gave almost identical linear conductances (38.9 pS and 38.5 pS with and without NFT, respectively) when fitted by straight lines. Data were obtained from inside-out patches of three cells. Vertical bars indicate mean ± S.E. B. These figures indicate representative records for each condition at different membrane potentials.

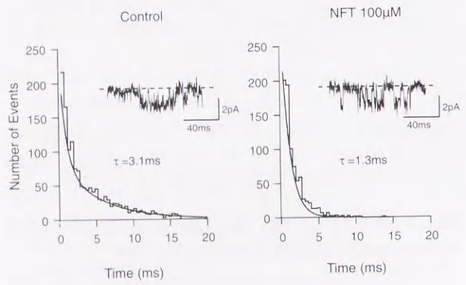

Fig. 6. Dwell time histograms and single-channel records for i_{K1} in the presence and absence of NFT. The patch membrane potential was –40 mV. A sample of the single-channel currents is shown in the inset. Open time histograms were fitted by a single exponential with time constants of 3.1 ms (control) and 1.3 ms (NFT), respectively.

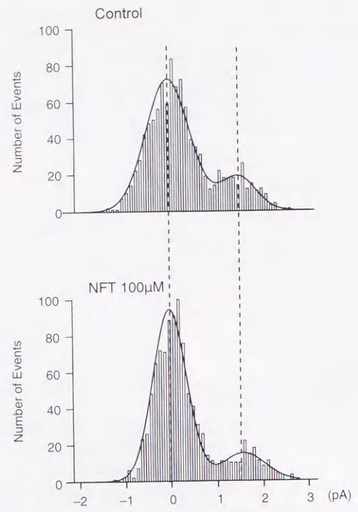

Fig. 7. Amplitude histograms for i_{K1} with and without NFT.
Amplitude histograms were constructed from the original records shown in the inset of Fig. 6 with the bin width set at 0.1 pA. Gaussian curves were fitted to these histograms and unitary conductances were determined to be 1.51 pA and 1.45 pA with and without NFT, respectively. Membrane potentials were –40 mV.

shown in Fig. 6 (Fig. 7). The single-channel amplitude was 1.84 ± 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 shortened the mean open time for inwardly rectifying K channels from 3.1 ms in control to 1.3 ms in the presence of 100 μM NFT without affecting the single-channel conductance. Third, ID_{50} was estimated to be 198 μM. Fourth, 100 μM tyramine had no effect on i_{K1}.

Hirose et al[4] have reported that the main pharmacological actions of *boi* containing NFT are a decrease in the maximum rate of rise of action potential, an increase in the duration of action potential, and positive inotropic action. Since the generation of action potential mainly depends on the excess inflow of Na ions to depolarize the membrane to the equilibrium potential for Na ion[2,5], partial suppression of the inflow of Na ion by *boi* does not affect the amplitude of action potential but reduces the maximum rate of rise of action potential. It is well known that the formation of the plateau phase in cardiac cells is due to the inflow of Ca ions through Ca channels and the limited outflow of K ions through inwardly rectifying K channels in the low value of the membrane conductance[2]. Since K current passing through inwardly rectifying K channels is responsible for the repolarization of action potential, the suppression of this K channel by NFT should prolong the plateau phase of action potential and, consequently, the influx of Ca ion may increase, resulting in the enhancement of contraction; the positive inotropic effect. The finding in our previous study[10] 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 seventeen (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[10]. When the results obtained in this study are reconciled with those on sodium channel, a hydrophobic 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 hydrophobic environment retain a strong negative cooperativity: several receptors around the binding site being subjected to a suppressive influence.

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