Full Paper

Comparative Pharmacological Studies on the A₂ Adenosine Receptor Agonist 5'-*n*-Ethyl-carboxamidoadenosine and Its F¹⁹ Isotope Labelled Derivative

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Abstract. Adenosine receptors are expressed in various mammalian tissues where they mediate the effects of adenosine on cellular functions through a number of signalling mechanisms. 18F-NECA is the positron-emitting derivative of the A₂-receptor agonist NECA (5'-*n*-ethyl-carboxamidoadenosine) and is a radioligand for PET imaging of adenosine receptors. Contractility and relaxation studies were performed on guinea pig atrial myocardium, pulmonary artery, and thoracic aorta to compare the pharmacological effects of NECA and F-NECA (a non-emitting derivative) on tissues. Furthermore, the effect of NECA and F-NECA on the potassium conductance was investigated in DDT1 MF-2 smooth muscle cells with the patch-clamp technique. Both NECA and F-NECA reduced the contractile force in atrial myocardium and evoked phasic contraction in pulmonary artery (A₁ adenosine-receptor-mediated actions) in a dose dependent manner; however, the apparent affinity was lower for F-NECA. No difference was found in relaxation induced by these compounds in 1 μM noradrenaline-precontracted aorta and pulmonary artery (in the presence of DPCPX, an A₁ adenosine receptor antagonist, tissue containing A_{2B} adenosine receptors). NECA (5 μM) and F-NECA (5 μM) also decreased the peak current and accelerated activation and inactivation properties of the potassium channels, but F-NECA was less effective. These results suggest that while NECA and F-NECA are equivalent agonists of vascular A_{2B} receptors, they mediate different changes of some parameters. When evaluating the data obtained by the use of radiolabelled ligands, one has to take into consideration the possible physiological effects of the ligands besides its binding properties to tissues.

Keywords: adenosine receptor, PET, myocardium, vascular tissue, potassium channel

Introduction

Adenosine receptors are cell surface receptors having adenosine as their natural ligand. These receptors are distributed in many human tissues and can be grouped into three subclasses: A₁, A₂, and A₃ on the basis of their effect on cyclic AMP activity. A₁ and A₃ receptors inhibit, while A₂ stimulates cyclic AMP formation (1). The latter can further be subdivided into A_{2A} and A_{2B}

subfamilies. The signal transduction mediated by A₁ or A₂ receptors is G-protein coupled and can lead to an increase in free cytosolic calcium concentration and an elevated level of inositol trisphosphate (IP₃) (2, 3). The interaction of adenosine with its receptor can evoke a number of physiological effects such as alteration of myocardial contractility (4, 5), hyperpolarization of vascular smooth muscle (6), and vasorelaxation (7). In DDT1 MF-2 smooth muscle cells derived from hamster vas deferens, voltage dependent potassium channels as well as a close relationship between the activity of A₁ and A₂ adenosine receptors have been described (8, 9).

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It was shown in follicular *Xenopus* oocytes that adenosine receptor activation can modify potassium currents (10), and activation of A₁ or A₂ adenosine receptors inversely modulates potassium currents and membrane potential in smooth muscle cells (11). A possible technique for the visualization of adenosine receptor distribution in the central nervous system and myocardium is positron emission tomography (PET). Several protocols have been developed in the last decade with different ligands for these investigations (12–15). The use of this technique requires radiolabelled ligands with high affinity and specificity for their receptors. 18F-NECA is the radiolabelled derivative of the A₂ adenosine receptor agonist 5'-N-ethyl-carboxamido-adenosine (NECA), where a positron-emitting fluor atom is bound to the original compound. 18F-NECA has already proved to be a suitable radioligand for PET imaging of adenosine receptors (16, 17), but its binding properties to receptors and the changes in physiological functions evoked by this radioligand needs further investigation. In our study we aimed to examine the A₁ and A₂ adenosine receptor-induced contraction and relaxation responses of some tissues due to F-NECA, the non-emitting counterpart of 18F-NECA compared to those caused by NECA, as well as the effects of this compound on the potassium conductance in single cells.

Materials and Methods

Reagents

5'-N-ethyl-carboxamidoadenosine (NECA) was purchased from Sigma Chemical (St. Louis, MO, USA), 1,3-dipropyl-8-cyclopentylxanthine was obtained from RBI (Natick, MA, USA), noradrenaline bitartrate was from Gedeon Richter (Budapest, Hungary), and 8-phenyltheophylline was obtained from Calbiochem-Behring (La Jolla, CA, USA). 18F-NECA and its stable analogue were prepared in our laboratory according to the procedure described before (16). All other chemicals were obtained from Sigma Chemical. Stock solutions of NECA and F-NECA were prepared in DMSO and kept at –20°C in the dark. On the day of the experiments, fresh dilutions of the stocks were made keeping the final concentration of DMSO below 0.5% v/v. Phosphate-buffered saline (PBS) contained 140 mM NaCl, 5 mM KCl, 8 mM Na₂HPO₄, and 3 mM NaH₂PO₄, pH 7.3.

Cell culture

The hamster vas deferens smooth cell line (DDT1 MF-2) was obtained from the European Collection of Animal Cell Cultures (Salisbury, UK). DDT1 MF-2 cells were cultured at 37°C in a humidified atmosphere

of 5% CO₂ and 95% O₂. The growth medium was Dulbecco's modified Eagle's medium supplemented with 2 mM L-glutamine and 10% (v/v) fetal calf serum. Cells were collected by vigorous shaking of the flask, washed with PBS, and resuspended in a Ringer-like solution used as extracellular solution in the electrophysiological measurements.

Animals

Male, Hartley guinea pigs, weighing 420–560 g, were used. The studies were carried out in accordance with guidelines outlined by the Institutional Animal Care and Use Committee of University of Debrecen.

Isolated atrial myocardium

Guinea pigs were sacrificed by a blow on the head under ether anesthesia. Then the chest was opened and the heart was quickly excised and washed in oxygenated Krebs solution at 30°C. Left atria were isolated and set up in 10-ml vertical organ baths (TSZ-04; Experimetria, Budapest, Hungary) containing Krebs solution (37°C), of the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.0 mM NaH₂PO₄, 1.2 mM MgCl₂, 24.9 mM NaHCO₃, and 11.5 mM glucose, pH 7.4, when gassed with 95% O₂ and 5% CO₂. The atria were electrically paced (3 Hz, 1 ms, twice the threshold voltage) by a programmable stimulator (PST-02, Experimetria). Isometric contractions were measured by means of a transducer (SG-01D, Experimetria), recorded by a polygraph (WR 3101; HSE, Hugstetten, Germany). Preparations were allowed to equilibrate for 50 min under an initial resting tension of 10 mN. After stabilization of the contractile parameters, cumulative NECA or F-NECA concentration-response curves (E/[A]) were generated.

Blood vessel preparations

Circular segments (rings cut open, 2 mm in length, endothelium not removed) were prepared from the middle part of the main pulmonary arteries and the thoracic aorta of guinea pigs according to the method described by Miller and Vanhoutte (18). The tissues were fixed vertically with a silk thread in a 10 ml, temperature-controlled (37°C) tissue chamber containing Krebs solution. The composition of the Krebs solution was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.0 mM NaH₂PO₄, 1.2 mM MgCl₂, 24.9 mM NaHCO₃, 0.004 mM disodium EDTA, 0.11 mM ascorbic acid, and 11.5 mM glucose at pH 7.4, when gassed with 95% O₂ and 5% CO₂. Tension of pulmonary and aortic vascular strips was measured isometrically by transducers (SG-01D, Experimetria) and the output fed to potentiometric recorders (SP-K2V,

Riken Denshi). The specimens were given an initial tension of 10 mN and allowed to stabilize at this level of tension for at least 3 h. Following the equilibration period, preparations were exposed to 1 μ M noradrenaline until a steady level of tension was reached. It is known that adenosine and adenosine analogues are only able to evoke contractile responses in precontracted (conventionally by noradrenaline) pulmonary artery preparations, but not in resting preparations. The tissue bath was then washed with fresh nutrient solution 5 times at 3-min intervals and then left undisturbed for 30 min. When the contraction was stable, 10 μ M NECA was applied to the organ bath and was allowed to produce a maximal response. The tissues were then washed several times with fresh, noradrenaline-containing (1 μ M) Krebs solution and allowed to recover. Experiments were started after producing at least two successive stable and reproducible contractile responses of the vessels to NECA. In pulmonary arteries, non-cumulative method of application of NECA and F-NECA was used, whereas in thoracic aorta, cumulative administration of adenosine analogues was applied. Substance modifying the purine actions (8-PT) was applied under the resting state for 30–40 min, and tissues then recontracted to the control level of tone with noradrenaline. At the completion of the experiments, where A_1 adenosine receptor mediated contractions of NECA and F-NECA were evaluated, tissues were lightly blotted, measured, and weighed. The cross-sectional area of each preparation was calculated using the following formula: cross-sectional area (mm^2) = weight (mg) / (length (mm) $\times$ density (mg/mm^3)). The density of the vessels was assumed to be 1.05 mg/mm^3 . Responses to purine analogues were then calculated as the increase in tension (mN) in response to each concentration of agonist/cross-sectional area of tissue (mm^2).

Electrophysiology

Patch-clamp measurements were performed in whole-cell configuration with an Axopatch-200-A patch-clamp amplifier in conjunction with a Digidata 1200 computer interface from Axon Instruments (Foster City, CA, USA). Low-pass filtering was applied at half of the sampling frequency. Patch-electrodes of 3–4 M Ω resistance were fabricated from GC150 F-15 borosilicate glass capillaries (Clark Electromedical Instruments, Reading, UK). The pipettes were filled with KF intracellular solution (140 mM KF, 2 mM MgCl_2 , 1 mM CaCl_2 , 11 mM EGTA, and 10 mM HEPES, pH 7.22). The cells were bathed in a Ringer-like solution (145 mM NaCl, 5 mM KCl, 1 mM MgCl_2 , 2.5 mM CaCl_2 , 10 mM HEPES, and 5.5 mM glucose, pH 7.35). The measured

osmolality of the solutions was 320 mOsm. Gigaseals in the order of 20–100 G Ω were formed by applying 50–70 H $_2$ O cm negative pressure to the interior of the patch-pipette. Subsequently, whole-cell measuring configurations were established by the application of negative pressure pulses. In most of the experiments the leak was negligible compared to the K^+ currents. The access resistance was 3–10 Mohm; we used for compensation 80% correction and 40% prediction. All patch-clamp measurements were carried out at 20°C. Current determination protocols were started 12 min after entering the whole-cell configuration or later. K^+ channel properties of the examined cells remained stable for up to several hours as they were kept on ice until the patch-clamp experiments. During the measurements, 80% correction and 40% prediction compensation was used for the series resistance. NECA and F-NECA were dissolved in the Ringer-like extracellular solution and taken to the cells by a continuous perfusion system.

Data analyses

For patch-clamp data analyses, the pClamp8 program package (Axon Instruments, Inc.) was used. The activation and inactivation time constants were determined by a fitting routine in Clampfit 8.0, using the Levenberg-Marquard search method with a minimization method of sum of squared errors. All experiments were repeated at least three times. Averaged data are indicated as the mean $\pm$ S.D. and were calculated using n experiments, where n indicates the number of independent experiments. The purine-induced decrease in mechanical activity was expressed as the percentage reduction of contractile force (electrically paced atrial myocardium, pulmonary artery after blockade of A_1 adenosine receptors). Contraction was calculated as an enhancement of the tone above the predrug value and expressed in mN/mm^2 . Individual $E/[A]$ curve data were fitted by means of a least-square iterative computer program to a logistic function of the form: $E = E_{\text{max}}[A]^{n_H} / [A]^{n_H} + [EC_{50}]^{n_H}$, where E denotes the effect, E_{max} is the asymptote, $[A]$ is the concentration of the agonist, EC_{50} is the concentration producing a half-maximal response, and n_H is the midpoint slope parameter. In some cases, where appropriate, linear regression analysis was carried out to calculate EC_{25} values (EC_{25} : the concentration of agonist to induce a 25% relaxation). For the contractility experiments, data are expressed as the mean $\pm$ S.E.M. The EC_{50} values are expressed as their negative base 10 logarithms (pD_2 values) throughout the text. Statistical analyses of differences between groups were carried out with Student's t -test and a probability of 0.05 was taken as the level of statistical significance.

Results

Contractility studies on atrial myocardium and pulmonary artery

Both NECA and F-NECA evoked a concentration-dependent decrease of contractile force in electrically stimulated atrial myocardium, a tissue containing A₁ adenosine receptors (18, 19). In tissue investigations, we examined the range of concentration for NECA or F-NECA to take a proper dose-response curve. As shown in Table 1, E_{max} and midpoint slope parameters for E/[A] of NECA and F-NECA did not differ significantly, whereas the apparent affinity of F-NECA was lower than that of NECA (7.50 ± 0.18 and 8.23 ± 0.12 , respectively; $P < 0.05$). In isolated strips of guinea pig pulmonary arteries precontracted with noradrenaline (1 μ M), NECA exerted a concentration-dependent phasic contraction (Fig. 1B and Table 1). The noradrenaline-induced sustained tension was 12.4 ± 2.1 mN. In the original recordings (Fig. 3), one can see that NECA and F-NECA exerted not only phasic contraction in pulmonary arteries of guinea pig, but also tonic contraction and slow relaxation. E_{max} and slope parameters for E/[A] of F-NECA, similarly to the results found in atrial myocardium, did not differ from those obtained for NECA. The pD₂ values for NECA and F-NECA dose-response curves, however, were significantly different (at $P < 0.05$, Table 1).

Studies on A_{2B} adenosine receptor-induced relaxation in guinea pig aorta and pulmonary artery

In guinea pig thoracic aorta preparations, which is a

tissue containing A_{2B} adenosine receptors (20), precontracted with 1 μ M noradrenaline, both NECA and F-NECA induced dose-related relaxations. As shown in Fig. 2A and Table 1, no significant differences were found for both maximum responses, slope factors, and apparent affinities in E/[A] curves of these compounds. In another series of experiments, guinea pig pulmonary arteries (a tissue containing A₁ and A_{2B} adenosine receptors, (21)) were used, where the vascular A₁ adenosine receptors were inhibited by DPCPX, a specific A₁ adenosine receptor antagonist. This way, the A_{2B} adenosine receptor-induced fast relaxation could be reliably examined. The application of DPCPX did not affect the contractility of the tissues. In pulmonary arteries (in the presence of 0.3 μ M DPCPX), non-cumulative concentration-response curves were taken for NECA or F-NECA. No differences were observed in the maximum responses, the slope factors and pD₂ values for both NECA and F-NECA (Fig. 2: B and C, Table 1). To evaluate the methylxanthine-sensitivity of the purine analogues, E/[A] curves were also constructed in the presence of 1 μ M 8-PT (a non-selective antagonist on adenosine receptors). In the presence of 8-PT, the dose-response curves for both NECA and F-NECA were shifted to the right and the maximum relaxant responses were also developed, indicating the competitive nature of the antagonism. EC₂₅ values for NECA and F-NECA E/[A] curves were determined in the absence and in the presence of 8-PT, and then concentration ratios were calculated. Concentration ratios for NECA and F-NECA were as follows: 3.83 ± 0.69 and 4.94 ± 0.54 , respectively ($P < 0.05$).

Table 1. Effects of A₁ and A_{2B} adenosine receptor-mediated actions of NECA and F-NECA in atrial myocardium, aorta, and pulmonary artery of guinea pigs

Compound	pD ₂	n _H	E _{max}	n
Atrial myocardium (cardiodepressive action: A ₁ adenosine receptor-mediated effect)				
NECA	8.23 ± 0.12	1.04 ± 0.06	83 ± 3	5
19F-NECA	$7.50 \pm 0.18^*$	0.86 ± 0.15	87 ± 2	4
Pulmonary artery (phasic contraction: A ₁ adenosine receptor-mediated action)				
NECA	6.58 ± 0.05	1.20 ± 0.14	5.00 ± 0.14	4
19F-NECA	$6.20 \pm 0.01^*$	1.59 ± 0.09	4.88 ± 0.15	4
Aorta (relaxation: A _{2B} adenosine receptor-mediated effect)				
NECA	6.58 ± 0.16	0.88 ± 0.13	87 ± 8	5
19F-NECA	6.43 ± 0.04	1.06 ± 0.03	85 ± 2	4
Pulmonary artery (fast relaxation in the presence of DPCPX: A _{2B} adenosine receptor-mediated effect)				
NECA	5.47 ± 0.16	0.64 ± 0.07	61 ± 5	6
19F-NECA	5.49 ± 0.12	0.75 ± 0.04	53 ± 3	4

pD₂: the negative common logarithms of EC₅₀ values, n_H: midpoint slope parameter, E_{max}: the maximum % relaxation of noradrenaline-induced precontraction (with the exception of phasic contraction induced in pulmonary artery, where E_{max} values are expressed in absolute values of contraction: mN/mm²). *: Significantly different at $P < 0.05$.

Fig. 1. Adenosine A_1 receptor-mediated effects of various concentrations of NECA and F-NECA on two different tissues. Panel A: Cardiodepressive action of NECA ($n=4$) and F-NECA ($n=4$) on electrically stimulated guinea pig atrial myocardium. Panel B: Contraction of the guinea pig pulmonary artery by NECA ($n=4$) and F-NECA ($n=4$). Preparations were precontracted with $1 \mu\text{M}$ noradrenaline. The agonist-induced increases in tension are normalized for vessel cross-sectional area, and absolute values of the increase in tension are shown. Data represent the mean $\pm$ S.E.M. of n independent experiments (n is indicated in Table 1).

Electrophysiological measurements on smooth muscle cells

The whole cell potassium current recorded on hamster vas deferens DDT1-MF2 cells had two components: an inward, voltage dependent sodium current and an outward, voltage dependent potassium current. From the holding potential of -120 mV , when cells were given test pulses to -80 mV or higher voltages up to $+70 \text{ mV}$, the sodium current opened at -30 mV and closed at $+70 \text{ mV}$. The potassium current opened at -20 mV and remained open at higher voltages. The sodium current activated in 1 ms and inactivated in 2 ms , while the potassium current activated in 80 ms and inactivated in 2 s . The earlier activation and inactivation of the sodium current enabled the separation of the potassium current

Fig. 2. Effect of NECA and F-NECA on tissues with A_{2B} adenosine receptor dominance in signal transduction. Panel A: Concentration-dependent relaxation of guinea pig thoracic aortas by NECA ($n=4$) and F-NECA ($n=4$). Relaxant responses are expressed as a percent of the noradrenaline-induced contraction. Panel B: Effects of NECA on guinea pig pulmonary artery in the absence and in the presence of 8-PT under complete blockade of A_1 adenosine receptors by $0.3 \mu\text{M}$ DPCPX. Panel C: Effects of F-NECA on guinea pig pulmonary artery in the absence and in the presence of 8-PT under complete blockade of A_1 adenosine receptors by $0.3 \mu\text{M}$ DPCPX. Data represent the mean $\pm$ S.E.M. of n independent experiments (n is indicated in Table 1).

Fig. 3. Effect of NECA and F-NECA on the potassium conductance of DDT-1 MF-2 smooth muscle cells. Normalized potassium current values are plotted as a function of the test potential for cells externally treated with NECA (panel A, dashed line) or F-NECA (panel B, dashed line), with control values (normal line). In the whole-cell configuration, voltage steps were applied every 30 s for 100 ms in 20-mV increments from -80 to 60 mV using a holding potential of -120 mV. The drugs were transported to the cells with a continuous, valve controlled perfusion system. Representative current traces are displayed for a cell before (upper trace in panel C) NECA and after (lower trace in panel C) NECA treatment. Panel D shows the effect of F-NECA. Displayed data are the mean $\pm$ S.D. values of three experiments.

from the sodium current.

The basal current amplitude of the cells was between 800 and 3000 pA. We also performed a pharmacological analysis of the voltage dependent, outward potassium conductance, using specific potassium channel blockers. The non-peptide potassium channel blocker tetraethylammonium (TEA) reduced the current and the same could be seen for the two peptide blockers charibdotoxin (ChTx) and margatoxin (MgTx). The EC₅₀ values of the blockers were 2.1 mM for TEA, 43 pM for MgTx,

and 910 pM for ChTx.

Hamster vas deferens DDT1 MF-2 cells responded with a significant ($P < 0.05$) decrease in whole cell potassium current to both NECA and F-NECA treatment. The basis of the selection of the concentration for NECA or F-NECA in electrophysiological measurements was that NECA is an effective agonist for both A₁ and A_{2B} receptors at a concentration of 5 μ M [1]. The peak current evoked by depolarization from -120 to $+40$ mV fell to $61.2 \pm 8.3\%$ ($n = 3$) of the control

value upon the administration of 5 μ M NECA, while it was diminished only to $80.6 \pm 4.7\%$ when F-NECA was applied. The shift in voltage-current relationship (Fig. 3: A and B) of the potassium conductance of DDT1 MF-2 cells also shows a voltage-independent decrease due to the application of the adenosine analogues. The difference between the two agents can also be seen on these curves. The treatment with both NECA or F-NECA significantly ($P < 0.05$) accelerated the inactivation of the membrane current as can be seen in Fig. 3, C and D. Accordingly, the inactivation time constant was reduced to $55.1 \pm 18.0\%$ of the original value when cells were perfused by 5 μ M NECA, while 5 μ M F-NECA had less effect, reducing the value only to $80.1 \pm 15.3\%$. Also, a significant difference was found between the effects of the two derivatives in the modification of the activation time constant of the current: while 5 μ M NECA resulted in a decrease to $73.9 \pm 10.0\%$, the application of 5 μ M F-NECA did not produce any significant change, showing an increase to $104.6 \pm 1.8\%$.

Discussion

On the basis of the present experiments on isolated tissues (containing A_1 or A_{2B} adenosine receptors), it was observed that in electrically driven atrial myocardium and pulmonary artery (tissues containing A_1 adenosine receptors), the pD_2 value of F-NECA is significantly lower than that of NECA. This indicates a less prominent affinity to both myocardial and vascular A_1 adenosine receptors. The similar maximum effects and midpoint slope parameters could be explained by supposing that NECA and F-NECA operate by similar signaling mechanisms. The actions of NECA and F-NECA were also studied in two independent tissues containing A_{2B} adenosine receptors. It has been proven that (a) aorta contains A_{2B} receptors (19) and (b) after inhibition of A_1 adenosine receptors in pulmonary artery, the A_{2B} adenosine receptor-induced relaxation is predominant (21). The basic parameters for the concentration-response curves of NECA (E_{max} , n_H , pD_2) did not differ from those of F-NECA; therefore, it is suggested that NECA and F-NECA are equivalent agonists on vascular A_{2B} adenosine receptors. This suggestion was also confirmed by the use of 8-PT, an adenosine receptor antagonist, which induced virtually the same rightward shifts of both the NECA and F-NECA $E/[A]$ curves. Although 8-PT is a nonselective antagonist of the adenosine receptors, but the samples were pre-incubated in 0.3 μ M DPCPX, thus the modulation evoked by 8-PT is selectively produced via A_{2B} receptors, as A_1 receptors are fully antagonized by DPCPX.

In the electrophysiological measurements, we exam-

ined the changes of the whole-cell potassium current of DDT1 MF-2 cells evoked by NECA and F-NECA. According to the pharmacological analysis of this potassium current we could conclude that the properties of the measured conductances are close to those of the Kv1.3 Shaker family voltage-dependent potassium channels.

The two adenosine analogues, NECA and its fluorine-labelled derivative F-NECA, have different effects on the potassium conductance of DDT1 MF-2 smooth muscle cells. This can be a consequence of the difference in their chemical structure which may lead to changes in A_1 and A_{2B} adenosine receptor affinity of these compounds. As both A_1 and A_{2B} receptors are found to be present on DDT1 MF-2 cells (9), and NECA is an agonist for both receptors (22), the elicited function upon the ligation of NECA or F-NECA is the summation of the effects mediated via A_1 and A_{2B} subtypes. The regulation of potassium current by adenosine analogues depends on the generation of cAMP (23). Our results suggest that the stimulation of cAMP dependent signaling pathways via receptor binding is different for NECA and F-NECA. Similarly to our investigations on atrial myocardium, aorta, and pulmonary artery, NECA and F-NECA also show a difference in the regulation of some functions in DDT1 MF-2 cells. The significantly smaller blocking effect on the peak potassium current by 5 μ M F-NECA compared to that of the administration of 5 μ M NECA can be attributed to the alterations in the affinity of these analogues. This can also be an explanation for the differences in the evoked changes in the potassium current kinetics, especially for the activation, where no effect was found for F-NECA. Although the alteration in electrophysiological properties caused by F-NECA is less prominent, both compounds reduce potassium current. Therefore F-NECA can be used as a moderately less effective analogue from the electrophysiological point of view.

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