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Antagonism by the suramin analogue NF279 on human $P2X_1$ and $P2X_7$ receptors

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Abstract

The effect of the suramin analogue 8,8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid) (NF279) was analyzed on human $P2X_1$ and $P2X_7$ receptor subtypes (human $P2X_1$ and human $P2X_7$) heterologously expressed in Xenopus oocytes using the two-microelectrode voltage-clamp technique. At activating ATP concentrations of 1 μM (human $P2X_1$) and 10 μM ATP (human $P2X_7$), IC_{50} values of 0.05 μM and 2.8 μM were found for human $P2X_1$ and human $P2X_7$ receptors, respectively. An increase in the activating [ATP] shifted the NF279 concentration–inhibition curve rightwards for both receptors. NF279 slowed the activation of both human $P2X_1$ and human $P2X_7$ as well as the desensitization of human $P2X_1$. The data support a model in which desensitization of $P2X_1$ is dependent on preceding activation of these P2X receptors. It is concluded that NF279 acts as a competitive antagonist with much higher potency at human $P2X_1$ than at $P2X_7$ receptors. NF279 may hence be suited to discriminate between both receptors in native tissues.

Keywords: ATP receptor; Oocyte; Antagonist; Voltage clamp; Whole cell; NF279 (8,8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid))

1. Introduction

Receptors for extracellular nucleotides have been classified into P2X and P2Y subtypes (Ralevic and Burnstock, 1998). According to the signal transduction mechanisms the P2X receptors constitute a new class of ligand-gated ion channels while the P2Y subtypes are G-protein coupled metabotropic receptors. To date, seven P2X receptor subunits ($P2X_{1-7}$) have been identified, functionally characterized and their transcript distribution has been described (North and Barnard, 1997; Soto et al., 1997). For $P2X_1$ as well as $P2X_3$ from rat, it was shown that the channels are composed of three homomeric subunits (Nicke et al., 1998). Furthermore, it has been demonstrated that many cell types express multiple P2X subunits and that heterooligomerization of different P2X subunits may form P2X receptors with distinctive biophysical and pharmacological profiles (Lewis et al., 1995; Torres et al., 1999). The functional biophysical and pharmacological characterization of P2X receptors has been performed by using mainly whole-cell patch clamp techniques in the heterologous systems and in native cells.

Selective antagonists or activators of certain P2X subunits are helpful in the identification of distinct P2X homo- or heteropolymers. Progress in the field of P2X receptors has been impeded in the past because of the lack of such selective ligands (Ralevic and Burnstock, 1998). Recently, it has been shown that the suramin analogue 8,8′-(carbonylbis(imino-3,1-phenylenecarbonylimino)bis-(1,3,5-naphthalenetrisulfonic acid) (NF023) is able to discriminate between $P2X_1$, $P2X_2$, $P2X_3$ and $P2X_4$ subtypes (Soto et al., 1999). A further suramin analogue, 8,8′-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid) (NF279), has been shown to be a selective P2X receptor antagonist in smooth muscle preparations with an IC_{50} of about 1 μM (Damer et al., 1998; Lambrecht et al.,

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1999). In these studies, the potency estimated for NF279 at the P2X receptors in rat vas deferens ($pIC_{50} = 5.72$) and guinea pig ileum ($pA_2 = 5.95$) were up to 50-fold higher compared to that found at P2Y receptors in guinea pig taenia coli ($pA_2 = 4.25$) and guinea pig ileum ($pA_2 = 4.97$). Whole cell voltage clamp measurements in Xenopus oocytes expressing homomultimeric P2X receptors have been performed to characterize the effect of NF279 on different P2X receptor subunits. They revealed a high potency of the drug towards the rat $P2X_1$ compared to rat $P2X_2$ and $P2X_3$ and human $P2X_4$ (Rettinger et al., 1999).

The aim of the present study was to characterize the effect of NF279 on human $P2X_1$ and $P2X_7$ receptors expressed in Xenopus oocytes to get further information about the P2X subunit selectivity of the drug.

2. Materials and methods

2.1. Materials

Chemicals were obtained from Sigma (Deisenhofen, Germany) if not otherwise stated. NF279 (as hexasodium salt) was synthesized using methods described for the synthesis of suramin analogues (Nickel et al., 1986), and purity was checked by high performance liquid chromatography (HPLC) (Kassack and Nickel, 1996).

2.2. RNA preparation

$P2X_1$ and $P2X_7$ were cloned from total RNA of human B lymphocytes by reverse transcriptase polymerase chain reaction (RT–PCR) using gene-specific primers. Respective primer sequences were designed from the published human sequences of $P2X_1$ (Valera et al., 1995) and $P2X_7$ (Rassendren et al., 1997). The PCR products were inserted into vector pNKS2 (Gloor et al., 1995) using appropriate restriction sites. Coding sequences were fully sequenced on both strands. The deduced amino acid sequence of $P2X_1$ coincided with data in literature (Valera et al., 1995), whereas $P2X_7$ deviated in two amino acid residues (G441 and A496) from the published sequence (Rassendren et al., 1997).

Capped cRNAs were synthesized from linearized templates with SP6 RNA polymerase (Pharmacia), purified by sepharose chromatography and phenol–chloroform extraction, and dissolved in 5 mM Tris/HCl, pH 7.2, at 0.5 μg/μl, using the optical density reading at 260 nm for quantification (OD 1.0 = 40 μg/μl).

2.3. Oocyte treatment

Xenopus laevis females were imported from African Xenopus Facility (Knysna, Republic of South Africa). In order to prepare the oocytes the animals were anaesthetized in an aqueous solution supplemented with tricaine (MS222, solution a, see Table 1). Parts of the ovary were removed through a small incision and treated with collagenase (solution b) for defolliculation overnight. Thereafter, the oocytes were washed several times in nominally Ca^{2+} free salt solution (solution c). The oocytes were kept in Petri dishes containing normal salt solution (solution d). One to four days before the experiments, stage V or VI oocytes were injected with 20–50 nl of cRNA and then incubated in normal salt solution supplemented with antibiotics (solution e) at 19°C until used.

2.4. Electrophysiology

All experiments were carried out at room temperature (≈ 22°C). The measurement of membrane currents was performed by the two-microelectrode voltage-clamp method. Microelectrodes were pulled from borosolicate glass and filled with 3 M KCl. Only electrodes with resistances of between 0.5 and 1 MΩ were used. Currents were recorded and filtered at 100 Hz using an oocyte clamp amplifier (OC-725C, Hamden, USA) and then sampled at 85 Hz. Data were stored and analyzed on a personal computer using self-programmed software.

Fast and reproducible solution exchange was achieved using a small tube-like chamber (0.1 ml) and a fast superfusion (≈ 75 μl/s). Switching between different bathing

Table 1
Extracellular solutions. Concentrations are in mM if not otherwise stated. pH was adjusted to 7.4 with NaOH (for solutions a to g and i) or with KOH (solution h), respectively

	a	b	c	d	e	f	g	h	i
NaCl		100	100	100	100	100	100	2.5	100
KCl		1	1	1	1	2.5	2.5	100	2.5
$MgCl_2$		1	1	1	1	1		1	2
$CaCl_2$		1		1	1	1		1	
HEPES	5	5	5	5	5	5	5	5	5
Flufenamic acid							0.1		
Penicillin (U/ml)					10,000				
Streptomycin (mg/ml)					10				
Collagenase (mg/ml)		1.5							
Tricaine (g/l)	2								

solutions was performed by a set of computer-controlled magnetic valves using a modified U-tube technique (Bretschneider and Markwardt, 1999). To test for the speed of solution exchange an Na^+-selective current was induced by clamping the oocyte for 1–3 min at +40 mV (Rettinger, 1999). The time course of solution exchange was measured as the change in holding current due to switching to a K^+-rich extracellular solution (solution h) and back to solution f (see Table 1). The mean 10–90% exchange times were 1210 ± 70 ms for the wash-in and 1770 ± 170 ms for the washout of solution h, respectively.

The impalement of the electrodes and measurement of the oocyte's membrane potential was carried out in normal oocyte Ringer (solution f). ATP-dependent currents were measured at a holding potential of −40 mV. To avoid activation of endogenous currents activated by Ca^{2+} ions permeating the P2X receptor channels, the following measurements of P2X receptor-dependent current and the blocking effect of NF279 were carried out in Ca^{2+} free bathing solutions (solution g). Magnesium ions were also omitted because the agonist of human $P2X_7$ receptors is ATP^{4-}. Leaving Mg^{2+} in the bathing solutions would have increased the total amount of ATP needed to obtain saturating concentrations of ATP^{4-} to values which would be insoluble and would lead to a large increase in osmolarity. The deprivation of divalent cations activated a large conductance which was blocked by 0.1 mM flufenamic acid (Weber et al., 1995). Flufenamic acid increased human $P2X_1$- and human $P2X_7$-dependent currents by factors of 1.58 ± 0.17 and 1.25 ± 0.14, respectively. To check possible effects of divalent cations and flufenamic acid on the action of NF279 additional measurements were carried out in bathing solution with 2 mM Mg^{2+} and without flufenamic acid (solution i).

For the detailed analysis of the dose-dependent effect of NF279 on the two subtypes of human P2X-receptors, different concentrations of the antagonist were applied at stochastic sequences. At the start of the experiment, ATP was administered three times for 6 s at an interval of 5 min and the third current response was taken as control current ($I_{P,\ cont}$) This procedure was chosen because pilot experiments showed that application of ATP at intervals of 5 min gave reproducible currents with amplitude variations < 10%. The P2X receptor-dependent current I_P was measured as the peak current (for human $P2X_1$) or the current after 5 s of agonist application (for human $P2X_7$), minus the holding current before agonist application. To keep the desensitization degree of human $P2X_1$ constant, each ATP application in NF279-containing bathing solution to human $P2X_1$-expressing oocytes was followed by an application of 100 or 1 μM ATP in NF279-free solution 24 s later. The next concentration of NF279 was then always applied 5 min later. To account for the various degree of human $P2X_1$ receptor expression the P2X receptor-dependent current at different [NF279] evoked by a certain concentration of ATP, I_P([ATP, NF279]) was normalized to the control P2X receptor-dependent current $I_{P,\ cont}$ evoked by ATP in NF279-free solution (see Eq. (1) in Section 3).To test the reversibility of the block by NF279 at the end of the experiment the drug was washed out for 5 min.

Nonlinear approximations and presentation of data were performed using the program Sigmaplot (Jandel, Corte Madeira, USA). Averaged data are given as means ± S.D. if not otherwise stated. Statistical data were analyzed by one-way repeated measures analysis of variance. Statistical significance of differences between means was tested using an all pairwise multiple comparison procedure (Student–Newman–Keuls Method) by the program Sigmastat (Jandel). Significance was taken at $P < 0.05$.

3. Results

3.1. Antagonism by NF279 on the human $P2X_1$ receptor

The effect of NF279 was tested by preincubation of Xenopus oocytes in divalent free extracellular solutions with different concentrations of the drug and activation of human $P2X_1$ by 1 and 100 μM ATP. As shown in Fig. 1A and B, the degree of block of human $P2X_1$-dependent currents is dependent on the ATP-concentration used to activate the receptor. To quantify this observation, the normalized peak current $I_{NF279,\ rel}$ (Eq. (1), see also Section 2) is drawn against the NF279 concentration as shown in Fig. 1C. The data were fitted according to Eq. (2):

$$I_{NF279,rel} = \frac{I_P([ATP, NF279])}{I_{P,cont}} \quad (1)$$

$$I_{NF279,rel} = \frac{1}{1 + \left(\frac{[NF279]}{10^{\log IC_{50}}}\right)^n} \quad (2)$$

where IC_{50} is the NF279 concentration required for half maximal current block and n is the Hill coefficient. The approximations for 1 and 100 μM ATP yielded $\log IC_{50}$ values (means ± S.E.M.) of -7.30 ± 0.03 and -6.80 ± 0.05 and Hill coefficients of 1.20 ± 0.06 and 0.81 ± 0.06, respectively. IC_{50} values as well as Hill coefficients are significantly different. In bathing solutions containing 2 mM Mg^{2+} but without flufenamic acid, NF279 blocked human $P2X_1$-dependent currents evoked by 1 μM ATP with a $\log IC_{50}$ of 7.24 ± 0.02 and a Hill coefficient of 1.47 ± 0.08. The Hill coefficient but not the $\log IC_{50}$ value is significantly different from the corresponding value for 1 μM ATP in divalent free bathing solution. NF279 could be completely washed out within 5 min (Fig. 1C).

As demonstrated in Fig. 1A and B, NF279 especially at concentrations of 0.1 μM and above also seems to slow down the time course for activation and desensitization of human $P2X_1$. Therefore, for a formal description of the

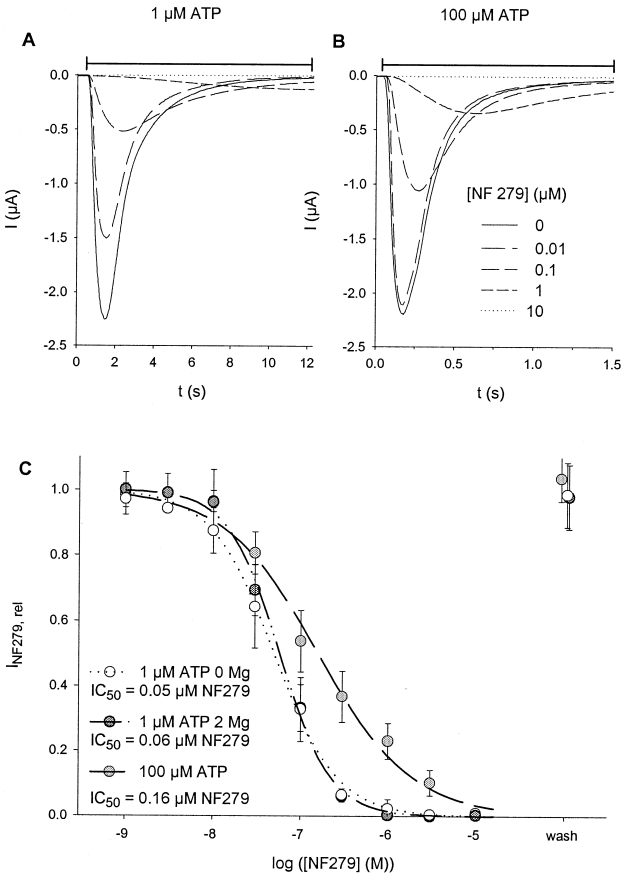

Fig. 1. Effect of NF279 on the human $P2X_1$ receptor. (A, B) Typical examples for the effect of NF279 on human $P2X_1$-dependent currents. Original current registrations from two different oocytes of currents elicited by 1 μM (A) or 100 μM ATP (B), after 30-s preincubation in bathing solution g supplemented with different concentrations of NF279 as indicated. Notice the different time scales. (C) Concentration–inhibition curves for the blocking effect of NF279 on peak human $P2X_1$-dependent currents. The currents were evoked by application of 1 or 100 μM ATP, respectively, in bathing solution g (''0 Mg'') or i (''2 Mg'') supplemented with the concentrations of NF279 as shown on the abscissa. The currents were normalized to the ATP-induced current without NF279 application (Eq. (1)). The blocking effect of 100 μM NF279 was washed out within 5 min (''wash''). Means from seven (1 μM ATP, 0 and 2 Mg) or eight (100 μM ATP) oocytes are shown. The data for ATP-induced currents were fitted according to Eq. (2).

kinetics of human $P2X_1$ a Hodgkin–Huxley-like model of independent activation and desensitization was used:

$$I_{P2X_1}(t) = I_{act}\left(1 - e^{-\frac{t}{\tau_{act}}}\right)e^{-\frac{t}{\tau_{des}}} + I_0 \quad (3)$$

where I_{act} is the activating current, I_0 is the steady-state current without ATP application, and τ_{act} and τ_{des} are the time constants for activation and desensitization, respectively. This model implies a complete desensitization of the human $P2X_1$-receptor by long lasting applications of ATP. Fig. 2B–D reveal a deceleration of both activation and desensitization of human $P2X_1$ by NF279 in divalent free bathing solution. Similarly, currents activated by 1 μM ATP in extracellular solutions containing 2 mM Mg^{2+} significantly increased their time constant of activation from 0.20 ± 0.06 to 0.42 ± 0.11 s as well as their time constant of desensitization from 0.45 ± 0.20 to 1.58 ± 0.58 s due to the application of 100 μM NF279.

3.2. Effect of NF279 on recovery from desensitization of human $P2X_1$ receptor currents

Supplementation of the bathing solution with NF279 not only changed the degree and kinetics of the first activation of human $P2X_1$ by ATP but also influenced the amplitude and time course of subsequent human $P2X_1$-dependent currents (''recovery currents'';) elicited by a second ATP application in NF279-free bathing solution after a recovery interval of 24 s (Fig. 3A, B). To evaluate the dependence of this effect on the concentration of NF279 the peak amplitude of the recovery current $I_{P,rec}$ was normalized to the human $P2X_1$-dependent currents $I_{P,cont}$

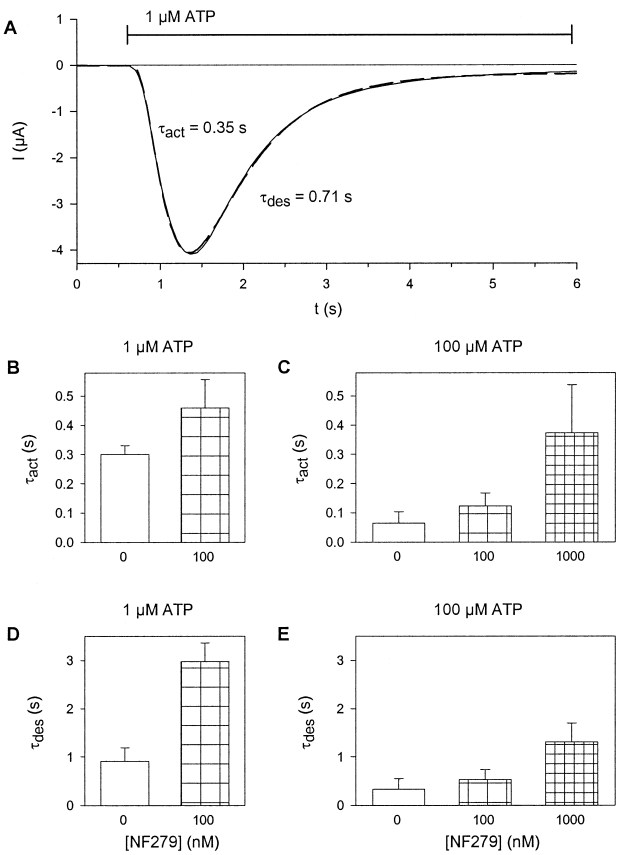

Fig. 2. Effect of NF279 on kinetics of human $P2X_1$-dependent currents. (A) Example of approximation of human $P2X_1$ receptor kinetics according to Eq. (3). The approximation is drawn as dashed line. The statistical analysis revealed a significant increase in the activation time constants τ_{act} for currents induced by the application of 1 μM (B) or 100 μM ATP (C). Desensitization was also significantly slowed by NF279 as shown by the effect of the drug on the desensitization time constants τ_{des} of currents evoked by 1 μM (D) or 100 μM ATP (E). All means are data derived from six different oocytes. Values for different concentrations of NF279 are statistically significant.

evoked at 5-min intervals in NF279-free bathing solution (Eq. 4) as described in Section 2. The normalized current $I_{rec,rel}$ was approximated by Eq. (5):

$$I_{rec,rel} = \frac{I_{P,rec}([ATP, NF279])}{I_{P,cont}} \quad (4)$$

$$I_{rec,rel} = \frac{1 - I_{rec,0}}{1 + \left(\frac{10^{logEC_{50}}}{[NF279]}\right)^{n}} + I_{rec,0} \quad (5)$$

where EC_{50} is the NF279 concentration at which the recovery current reaches 50% of the control current $I_{P,cont}$. $I_{rec,0}$ is the relative recovery current $I_{rec,rel}$ without preceding application of NF279 and n is again the Hill coefficient. The approximation for the measurement in divalent free bathing solution yielded significantly different values of $logEC_{50}$ for 1 μM (-6.24 ± 0.05, means ± S.E.M.) and 100 μM ATP (-5.78 ± 0.03). The approximated values of $I_{rec,0}$ (0.18 ± 0.02 and 0.16 ± 0.01) and n (1.01 ± 0.1 and 1.02 ± 0.08) were not statistically different at these two ATP concentrations (see Fig. 3C). In extracellular solution supplemented with 2 mM Mg^{2+} and without flufenamic acid for the activating ATP concentration of 1 μM, the values of $logEC_{50}$ (-6.16 ± 0.07), n(-6.16 ± 0.07), and $I_{rec,0}$ (0.14 ± 0.03) are not significantly different from those in Mg^{2+}-free solution containing flufenamic acid.

The model of Eq. (5) implied that at very high NF279 concentrations the recovery current would reach 100% of the control current. This model fits the data well up to NF279 concentrations of 30 μM, but for 100 μM NF279 the data deviate strongly from the approximation (see Fig. 3C). This may be caused by a direct effect of NF279 on the recovery current due to an incomplete washout of NF279 at this high concentration. This assumption is supported by the observation that for high concentrations of NF279 during the first ATP application the time to peak of the recovery current increases (see Fig. 3D). The slowing of activation and desensitization resulting in a prolonged time to peak current is a typical effect of NF279 as already shown in Fig. 1.

3.3. Antagonism by NF279 on human $P2X_7$ receptor currents

Fig. 4 shows the effect of NF279 on human $P2X_7$-dependent currents. Like for human $P2X_1$, the effect of NF279 on current amplitude seems to be dependent on the concentration of ATP used to activate the receptor. This was verified by the analysis of the concentration–response

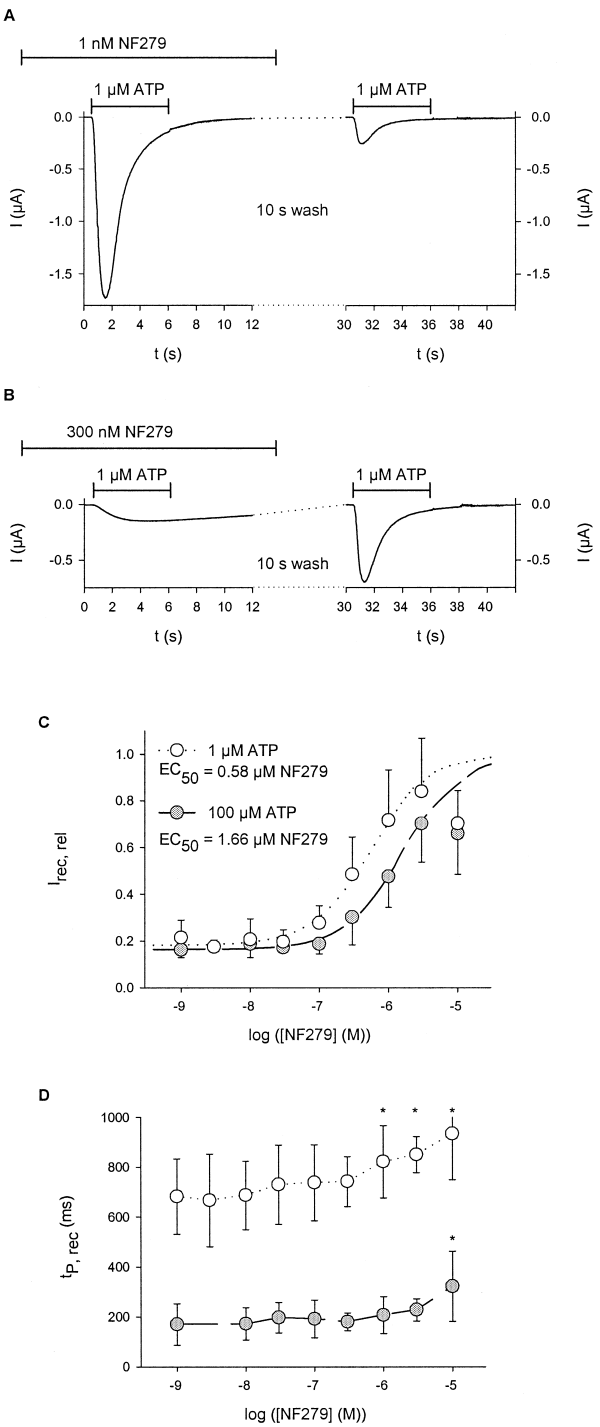

Fig. 3. Effect of NF279 on recovery from desensitization. Original current records show that compared to the effect of low concentrations of NF279 (A), high concentrations of NF279 (B) decrease the first ATP-dependent current and lead to an increased current evoked by the same ATP concentration after a recovery interval of 24 s. (C) Statistics of the concentration-dependent effect of NF279 on the amplitude of recovering human $P2X_1$-dependent current. The amplitude $I_{rec,rel}$ of the relative recovery current evoked after the foregoing ATP application in bathing solutions containing NF279 concentrations as indicated on the abscissa was calculated according to Eq. (4). The data were approximated according to Eq. (5). (D) shows the effect of NF279 added to the bath during a foregoing application of 1 or 100 μM ATP (as indicated) on the time to peak of the recovery current, $t_{P,rec}$. Asterisks mark $t_{P,rec}$ values significantly different from the mean calculated for an NF279 concentration of 1 nM.

curves shown in Fig. 4C. The approximation of the data by Eq. (2) yielded (means ± S.E.M.) $\log IC_{50}$ values of -5.55 ± 0.03, -5.14 ± 0.03 and -4.50 ± 0.04 as well as Hill coefficients of 1.22 ± 0.10, 1.16 ± 0.08 and 1.11 ± 0.11, for 10, 100, and 1000 μM ATP, respectively. The IC_{50} values but not the Hill coefficients are significantly different.

The effect of NF279 on kinetics of the human $P2X_7$ receptor is depicted in Fig. 5. The time course of activation of the human $P2X_7$-receptor current ($I_{P2X_7,act}(t)$) was fitted according to:

$$I_{P2X_7,act}(t) = I_{act}\left(I - e^{-\frac{t}{\tau_{act}}}\right) + ct + I_0 \quad (6)$$

where I_{act}, τ_{act} and I_0 have the same meaning as in Eq. (2) and c is the slope of the linearly rising current.

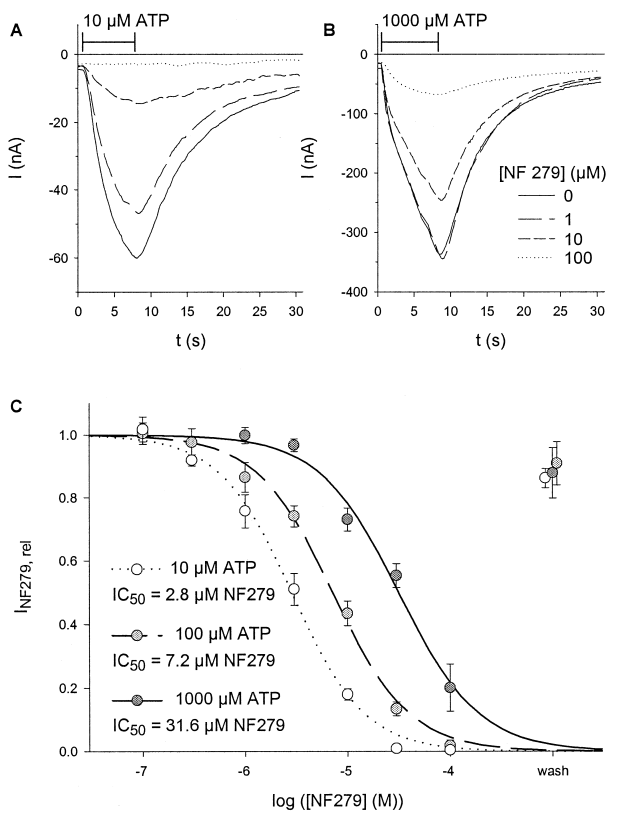

Fig. 4. Effect of NF279 on human $P2X_7$. (A, B) Typical examples for the effect of NF279 on human $P2X_7$-dependent currents. Original current registrations of currents elicited by application for 6 s of 10 μM (A) or 1000 μM ATP (B) after 30-s preincubation in bathing solutions with different concentrations of NF279 as indicated. (C) Concentration–inhibition curves for the effect of NF279 on human $P2X_7$-dependent currents. human $P2X_7$ receptor-dependent currents were evoked by the application of 10, 100 or 1000 μM ATP in bathing solution g supplemented with the concentrations of NF279 as shown on the abscissa. Relative currents ($I_{NF279,rel}$) were calculated according to Eq. (1). The blocking effect of 100 μM NF279 could be almost completely washed out within 5 min (''wash'';). Means from six (10 μM ATP), seven (100 μM ATP), or six (1000 μM ATP) oocytes are shown. The data for ATP-induced currents were approximated using Eq. (2).

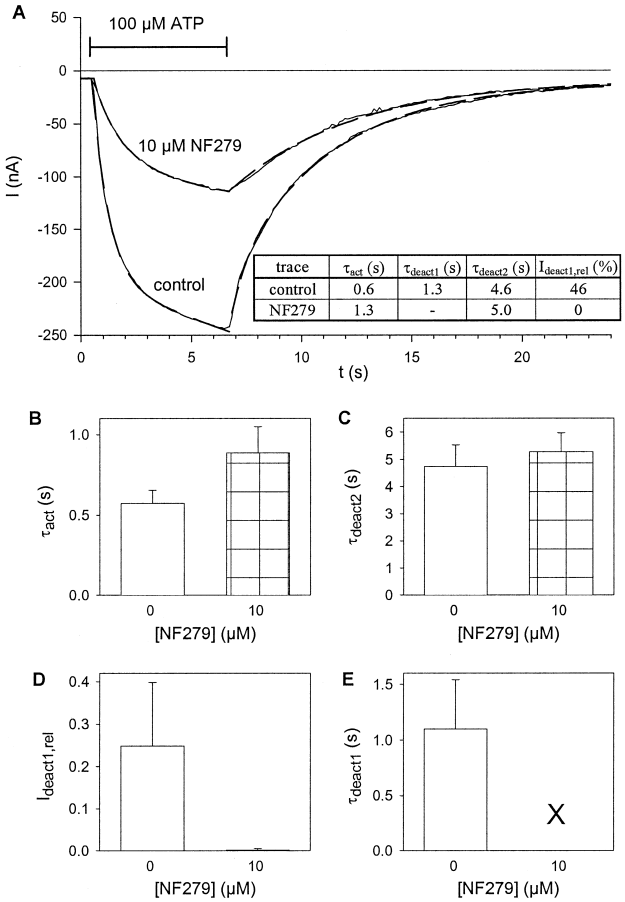

Fig. 5. Effect of NF279 on kinetics of human $P2X_7$-dependent currents. (A) Example of approximation of human $P2X_7$ receptor kinetics according to Eqs. (6) and (7). The approximations are drawn as dashed lines. Parameters of the approximations of the shown example are given in the inset. The statistical analysis (means from nine oocytes) of the effect of NF279 on kinetics of human $P2X_7$-dependent currents revealed a significant increase in the activation time constant for currents induced by the application of 100 μM NF279 (B). The time constant of the component of slow deactivation, τ_{deact2}, was slightly but significantly increased (C). The main effect of NF279 on deactivation was the total block of the component of fast deactivation, I_{deact1} (D). Therefore, the time constant of fast deactivation, τ_{deact1}, could only be determined for currents elicited in bathing solution without NF279 (E).

The deactivating current ($I_{P2X_7,deact}$) during washout of ATP was significantly better described bi-exponentially than by one exponential term only (Horn, 1987):

$$I_{P2X_7,deact}(t) = I_{deact1}e^{-\frac{t}{\tau_{deact1}}} + I_{deact2}e^{-\frac{t}{\tau_{deact2}}} + I_0 \quad (7)$$

I_0 has the same meaning as in Eq. (1) and I_{deact1} and I_{deact2} are the amplitudes and τ_{deact1} and τ_{deact2} are the time constants of the fast and slow deactivating component, respectively. The statistical analysis of the data reveals the main effect of NF279 on human $P2X_7$ kinetics to be

slowing of activation (Fig. 5B) and nearly complete block of the fast component of deactivation (Fig. 5D).

4. Discussion

The present study demonstrates a high potency of NF279 towards the human $P2X_1$ receptor ($IC_{50} = 50$ nM for [ATP] = 1 μM). This is similar to the strong effect of NF279 on the rat homologue where an even lower IC_{50} of 19 nM NF279 was found for activating ATP concentrations of 1 μM (Rettinger et al., 1999). The variance may be due to differences in the protein structure of the rat and human $P2X_1$ receptor. On the other hand, for another suramin analogue, NF023, similar IC_{50} values of 0.24 and 0.21 μM have been found for the rat and human $P2X_1$ homologue, respectively, heterologously expressed in Xenopus oocytes (Soto et al., 1999).

We had to consider that extracellular Mg^{2+} reduces the concentration of free ATP^{4-}, the agonist at $P2X_7$ and $P2X_7$-like native P2X receptors, for example in human B lymphocytes (Markwardt et al., 1997) (see also Section 2). Therefore, in most of the measurements we omitted extracellular Mg^{2+} for making the effects of different ATP concentrations on human $P2X_1$ and human $P2X_7$ comparable. Further experiments were performed in bathing solution without flufenamic acid and supplemented with 2 mM Mg^{2+}. The results make a significant influence of Mg^{2+} and flufenamic acid on the effect of NF279 on human $P2X_1$-dependent current amplitude and kinetics unlikely.

NF279 slows down the activation of human $P2X_1$. This together with the finding that higher ATP concentrations can shift the concentration–inhibition curve of NF279 rightwards can most easily be explained by a competition between NF279 and ATP at the ATP binding site. The same conclusion was drawn for the action of NF279 on smooth muscle preparations (Damer et al., 1998; Lambrecht et al., 1999) and the effect of NF023 on rat and human $P2X_1$ (Soto et al., 1999). The measured data for block of $P2X_1$ by NF279 and its competition with ATP are nevertheless biased by the fact that due to the very fast desensitization of the receptor a steady state equilibrium of the activating and blocking actions of drugs could not be reached during the short times of activation. Hence, the speed of solution exchange will also influence the measured block and competition. Particularly, a slow solution exchange will not leave enough time for the applied ATP to compete with NF279 at all binding sites occupied by the antagonist molecules. The true competition may be therefore even stronger than measured by our method.

NF279 also influences currents recovering from desensitization measured after a first application and subsequent washout of ATP and NF279. The higher the concentration of NF279 during the first receptor activation, the higher is the peak amplitude of the recovering current. This means that binding of NF279 not only blocks activation but also the desensitization of the human $P2X_1$ receptor. Again, this effect can be surmounted by increasing the activating ATP concentration. This can be explained by an action of NF279 on the ATP binding site only and the assumption that desensitization of $P2X_1$ is only possible after binding of ATP to the activating binding site(s) of the receptor. Then, like the inactivation process of voltage-gated Na^+ channels (Aldrich et al., 1983), the time course of macroscopic desensitization may primarily depend on the speed of activation. This model would be in accordance to our finding that NF279 decelerates macroscopic activation and desensitization as well. Further experiments are needed to clarify this issue.

NF279 blocks the fast component of deactivation of human $P2X_7$. The meaning of the two deactivating components is not yet clear but implies that the activated $P2X_7$ receptor may exist in two different states and that the one of them, which deactivates fast, is preferentially blocked by NF279. Also, this model requires further investigations.

For the blocking effect of NF279 on human $P2X_7$ receptors, a relatively high IC_{50} of about 3 μM was found for an activating ATP concentration of 10 μM. Therefore, NF279 is much less potent on human $P2X_7$ compared to human $P2X_1$ but much more effective than on the human $P2X_4$ receptor where the IC_{50} is greater than 300 μM (Rettinger et al., 1999). We have also investigated the effect of NF279 on the $P2X_7$/P2Z of the human lymphoblastoid cell line JY. Here, NF279 was applied in extracellular solutions supplemented with 0.5 mM $CaCl_2$. The IC_{50} of NF279 was measured at an activating ATP^{4-} concentration of 1 mM to be 68 μM (preliminary results). That is not far from the IC_{50} of 31.6 μM found for human $P2X_7$ in oocytes (this work) with the same activating ATP^{4-} concentration but in solutions free from Ca^{2+} and Mg^{2+}. So, it can be assumed, that, like for the human $P2X_1$ receptor, the inhibitory potency of NF279 on human $P2X_7$ is not much affected by divalent cations.

In the same study for the rat $P2X_3$ receptor, the IC_{50} at an activating ATP concentration of 1 μM amounted to 1.6 μM NF279. Assuming for $P2X_3$, that elevating the activating ATP concentration to 10 μM would also shift the concentration–inhibition curve rightwards, the potency of NF279 should be similar for human $P2X_7$ and rat $P2X_3$. Therefore, the potency profile of NF279 could be complemented after the present study to: rat $P2X_1$ > human $P2X_1$ ≫ rat $P2X_2$ > rat $P2X_3$ ≈ human $P2X_7$ ≫ human $P2X_4$. Measurements of the effect of NF279 on rat $P2X_7$ and on the human $P2X_3$ and $P2X_2$ have not yet been carried out. For NF023 the IC_{50} was more than three times higher for the human compared to the rat $P2X_3$ (Soto et al., 1999) similar to the effect of NF279 on $P2X_1$ (see above).

In conclusion, our results demonstrate that NF279 is a highly potent antagonist at the human $P2X_1$ receptor and moderately effective on human $P2X_7$. Furthermore, considering its very low potency at human $P2X_4$, it is a very useful tool to characterize P2X receptors in native tissues,

which express several P2X receptor subunits like human B lymphocytes where messages for $P2X_1$, $P2X_4$ and $P2X_7$ were found (Büttner et al., 1998).

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