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Study of the reversal effect of NF449 on neuromuscular blockade induced by d-tubocurarine

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ABSTRACT

Aims: The aim of this study was to investigate the mechanism for the reversal effect of NF449 (a suramin analogue) on the neuromuscular block induced by D-tubocurarine (D-TC).
Main methods: Nerve-stimulated muscle contractions and end-plate potentials were performed in mouse phrenic nerve-diaphragm preparations. Acetylcholine (ACh)-induced muscle contractions were performed in the chick biventer cervicis preparations. Presynaptic nerve terminal waveform recordings were performed in mouse triangularis sterni preparations.
Key findings: Amongst the suramin analogues in this study, only the NF449 and suramin were able to reverse the blockade effect produced by D-TC on nerve-stimulated muscle contractions. Each of these suramin analogues (NF007, NF023, NF279 and NF449) alone has no significant effect on the amplitude of nerve-stimulated muscle contractions. NF449 and suramin also showed the antagonising effects on the inhibition of end-plate potentials induced by D-TC. Furthermore, pre-treatment with NF449 can antagonise the inhibition of D-TC in ACh-induced contractions of chick biventer cervicis muscle. NF449 produced a greater rightward shift of the dose–response inhibition curve for D-TC than did suramin. Because other purinergic 2X (P2X) receptor antagonists, NF023 and NF279, do not have the reverse effects on the neuromuscular blockade of D-TC, the effect of NF449 seems irrelevant to inhibition of P2X receptors.
Significance: These data suggest that NF449 was able to compete with the binding of D-TC on the nicotinic ACh receptors, and the effect of NF449 was more potent than suramin in reducing the inhibition of D-TC. The structure of NF449 may provide useful information for designing potent antidotes against neuromuscular toxins.

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Introduction

The neuromuscular junction consists of the presynaptic motor nerve terminal and the motor end-plate of skeletal muscle. Acetylcholine (ACh) is synthesized within the presynaptic terminal and stored in vesicles (Huh and Fuhrer, 2002; Fagerlund and Eriksson, 2009). The arrival of an action potential at the presynaptic terminal leads to the release of ACh that then diffuses across the junctional cleft and binds to nicotinic ACh (nACh) receptors at the postsynaptic membrane. Some chemical compounds can interrupt the neuromuscular transmission and cause muscle relaxation. The studies of the structure–activity relationship of agonists and antagonists have revealed how they bind to orthostatic binding sites (Albuquerque et al., 2009; Zouridakis et al., 2009; Azam and McIntosh, 2009). Previous studies showed that suramin not only reversed the effects of neuromuscular blockers (Henning et al., 1993) but also inhibited the prejunctional Ca^{2+} channels (Henning et al., 1996). However, the mechanism of effect of suramin on the neuromuscular junction is still unclear. Suramin is a polysulphonated naphthylurea derivative that is used, as a therapeutic agent in the treatment of human African trypanosomiasis (sleeping sickness) and onchocerciasis (Schneider, 1960; Schulz-Key et al., 1985). Recent studies have shown that suramin possesses a variety of biological activities. The compound can inhibit reverse transcriptase and prevent HIV entry into the cell (Jentsch et al., 1987), and it has been used experimentally in the

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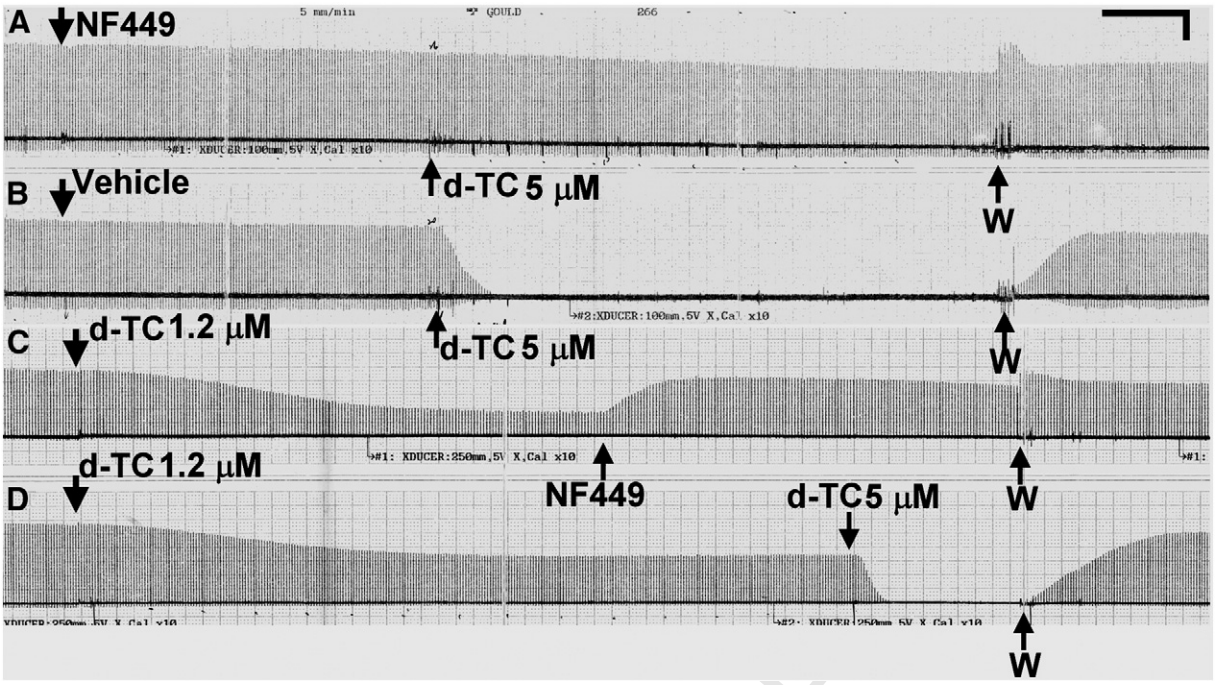

Q1 **Fig. 1.** The reverse effect of NF449 on the neuromuscular blockade induced by D-tubocurarine (D-TC) in the mouse phrenic nerve-diaphragm. Pretreatment with NF449 (100 μM) can prevent the block of nerve-stimulated muscle contractions induced by D-TC (5 μM) (A). Pretreatment with vehicle served as a control for the comparison (B). The partial inhibition of nerve-evoked muscle contractions induced by a lower concentration of D-TC (1.2 μM) can also be reversed by the after-treatment with NF449 (C). The blocking effects of D-TC (5 μM) were reversible with washout (D, B). Calibrations: 5 min; 0.5 g.

treatment of cancer (Dhar et al., 2000; Ord et al., 2005; Villalona-Calero et al., 2008). Suramin is also an antagonist of P2 purinoceptors (Dunn and Blakeley, 1988; Inoue et al., 1991). Previously, we have demonstrated that suramin can reverse the inhibitory action of a tripeptide, carbobenzoxy-Gly-Gly-Arg-β-naphthylamide, which acts both at the postsynaptic nACh receptors and presynaptic autoreceptors (Lin-Shiau and Lin, 1998). Recently, a series of novel suramin analogues have been developed (Kassack et al., 2004; Ullmann et al., 2005) that are specifically designed for antitumor activity (Krejci et al., 2010). Most of these analogues have a symmetrical structure

t1.1 **Table 1**
The blocking effect of D-tubocurarine (D-TC) on the nerve-evoked muscle contractions of mouse phrenic nerve–diaphragm pretreated with suramin and suramin analogues (NF007, NF023, NF279 and NF449). Suramin and suramin analogues were pretreated for 15–25 min prior to the application of D-TC (5 μM).

Treatment	*n*	Time to complete blockade (min)	The percentage of inhibition of amplitude (before/20 min after the application of D-TC)
Group 1			
Pretreatment with NF007 (100 μM) + D-TC	5	3.2 ± 0.8	Complete blockade[†]
D-TC alone	5	2.8 ± 0.5	Complete blockade
Group 2			
Pretreatment with NF023 (100 μM) + D-TC	4	7.7 ± 0.3	Complete blockade
D-TC alone	4	3.4 ± 0.8	Complete blockade
Group 3			
Pretreatment with NF279 (100 μM) + D-TC	4	4.3 ± 0.6	Complete blockade
D-TC alone	4	3.6 ± 0.6	Complete blockade
Group 4			
Pretreatment with Suramin (100 μM) + D-TC	11	18.5 ± 3.1[*]	Complete blockade
D-TC alone	11	4.1 ± 0.5	Complete blockade
Group 5			
Pretreatment with NF449 (30 μM) + D-TC	4	Without complete blockade[#,*]	75.1 ± 5.9%[*]
D-TC alone	4	4.4 ± 0.6	Complete blockade
Group 6			
Pretreatment with NF449 (100 μM) + D-TC	9	Without complete blockade[#,*]	10.7 ± 6.8%[*]
D-TC alone	9	5.0 ± 0.8	Complete blockade

Data are presented as mean ± S.E.M.
The experimental animals were divided into six groups. The *diaphragm of each* individual animal *was divided* into *two* equal parts, one is pretreatment with the suramin analogue and the other is a treatment for D-TC only (pair study).
[*] $P<0.05$ as compared with D-TC alone within each group.
[#] Without complete blockade: the nerve-evoked muscle contractions cannot be blocked completely.
[†] Complete blockade = 100% inhibition of nerve-evoked muscle contractions.

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with a central urea bridge except for NF007. Amongst these analogues, the structure of NF279 is very similar to suramin. These analogues are polysulphonates with six negative charges for suramin and NF279 and eight negative charges for NF449. Therefore, we investigated the effects of suramin analogues NF007, NF023, NF279 and NF449 on the neuromuscular junctions. It turned out that NF449, but not the other analogues, was an effective protecting agent against the inhibition of D-tubocurarine (D-TC) on the nACh receptor, and its effect was more potent than that of suramin.

Materials and methods

Mouse phrenic nerve-diaphragm preparations

Mice of the ICR strain (17–22 g) were anaesthetised with carbon dioxide followed by cervical dislocation. Phrenic nerve-diaphragm preparations were isolated and suspended in 10 ml of modified Krebs solution with the compositions (in mM): NaCl 131, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 12.5 and glucose 11. The modified Krebs solution was saturated with carbogen (95% O_2/5% CO_2) and kept at a constant pH (7.3–7.4). Muscle contractions were elicited by electrical stimulation of the phrenic nerve with supramaximal rectangular pulses of 0.05 ms in duration at a frequency of 0.1 Hz and recorded by the isometric transducer on a Gould TA240 polygraph. The muscle resting tension was adjusted to 1 g and the preparation was allowed to equilibrate for 20–30 min before starting the experimental protocol.

Chick biventer cervicis muscle preparations

The preparations were isolated from baby Leghorns, 4–7 days old, according to previously described methods (Ginsborg and Warriner, 1960). The organ bath contained 10 ml modified Krebs solution. The contraction of the muscle was recorded isometrically with 0.5 g initial tension. Each muscle contraction was elicited by the addition of 30 μM ACh to the organ bath for about 1 min followed by the washout of ACh. The interval between applications of ACh was 20 min to prevent desensitisation of the nACh receptors.

Miniature end-plate potential and end-plate potential recordings

Miniature end-plate potential (mepp) and end-plate potential (epp) were measured by an intracellular glass microelectrode with a high impedance amplifier (Axoclamp 2B) in bridge mode. The microelectrodes were filled with 3 M KCl resistance of 5–12 MΩ. Epps were evoked by stimulation of the phrenic nerve using a bipolar suction electrode at a frequency of 0.1 Hz with 0.02 ms supramaximal rectangular pulses (A–M systems model 2100 stimulator), and the diaphragm was immobilised by the cut muscle method (Barstad and Lilleheil, 1968). The signals of epps, mepps and presynaptic currents were digitalised (Digidata 1440A and pClamp 10, Axon Instruments) and stored for later analysis (pClamp-Clampfit 10, Axon Instruments).

Mouse triangularis sternus preparation and nerve terminal waveform recordings

The thin layer (2–3 muscle layers) of mouse left triangularis sterni intercostal nerve–muscle preparation was isolated according to the method described previously (McArdle et al., 1981; Mallart, 1985). The isolated preparations were pinned out in the Sylgard-coated glass chamber 1–2 ml and visualised at 400× magnification by a Zeiss microscope (Axioskop FS plus). Preparations were continuously perfused at a rate of 2–4 ml/min with an oxygenated (95% O_2 plus 5% CO_2) modified Krebs solution containing (mM): NaCl 131, KCl 4.8, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 12.5 and glucose 11, pH 7.2–7.4. The intercostal nerves were stimulated by a suction electrode using supramaximal voltage and square-wave pulses of 0.02 ms in duration at 0.2 Hz (A–M systems model 2100 stimulator). Presynaptic waveforms were obtained from extracellular recording close to the nerve terminal endings of intercostal nerves with glass microelectrodes filled with 2 M NaCl (7–13 MΩ) placed inside the perineural sheath (Mallart, 1985). Postsynaptic activity was blocked by adding 20–30 μM D-TC to the bathing medium. The signals of the presynaptic waveforms were displayed and analysed on pClamp 10 software. All animal care was performed in accordance with the guidelines of the Committee of the CSMU as previously described (Su et al., 2009).

Drugs and chemicals

Suramin (8, 8′-[Carbonyl*bis* [imino-3,1-phenylenecarbonylimino (4-methyl-3,1-phenylene) carbonylimino]] *bis*-1,3,5-naphthalenetrisulphonic acid hexasodium salt), NF023 (8, 8 (carbonylbis (imino-3,1-phenylenecarbonylimino)) bis-(1,3,5-naphthalenetrisulfonicacid)),

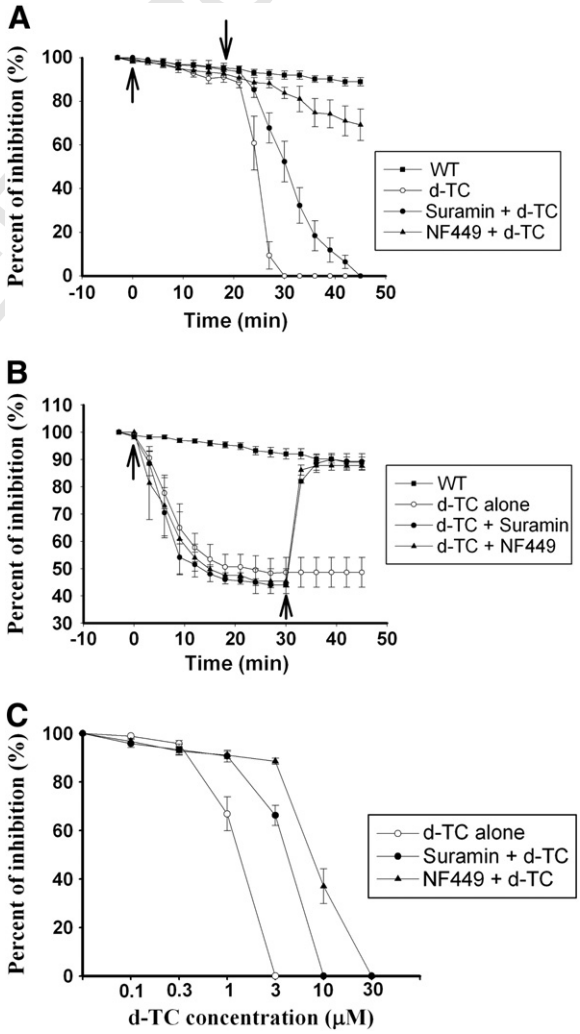

Fig. 2. The inhibition curves of nerve-evoked muscle contractions induced by D-tubocurarine in the mouse phrenic nerve-diaphragm pretreated with or without NF449 (or suramin). The pretreatment with NF449 100 μM (or suramin 100 μM) significantly prevented the decrease in nerve-evoked muscle contractions induced by D-TC (5 μM) treatment (A). The partial inhibition of muscle contractions induced by 1.2 μM D-TC were reversed with the after-treatment of NF449 100 μM (or suramin 100 μM) (B). The arrow at time 0 min indicates the application of suramin analogues (A) and D-TC (B), respectively. The arrow at time 20 min and 30 min indicates the addition of D-TC (A) and suramin analogues (B), respectively. NF449 caused a greater rightward shift of the concentration–inhibition curve of D-TC than did suramin (C).

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NF279 (8, 8′,-(carbonylbis (imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino)) bis (1,3,5-naphthalenetrisul fonic acid)) and NF449 (4, 4′, 4″, 4‴-(carbonylbis (imino-5,1,3-benzenetriylbis-(carbonylimino))) tetrakis-benzene-1,3-disulphonic acid octasodium salt) were purchased from Tocris biosciende Inc. (UK). NF007 (8-(3-nitrobenzamido)-1,3,5-naphthalenetrisulphonic acid) was purchased from Calbiochem Inc. (USA). D-TC was purchased from Fluka Inc. (USA).

Statistics

The data are given as mean ± S.E.M. The statistical significance of differences was evaluated using a paired or unpaired Student's *t*-test. When more than one group was compared with one control, significance was evaluated using one-way analysis of variance (ANOVA). Probability values (*P*) less than 0.05 were considered significant.

Results

Effects of NF449 on nerve-stimulated muscle contractions

As compared to the use of vehicle alone (Fig. 1B), pretreatment with NF449 (100 μM) can prevent the block of muscle contraction by D-TC (Fig. 1A). Another experiment showed that the partial inhibition of muscle contraction caused by 1.2 μM D-TC (Fig. 1C and D) can be reversed by subsequent treatment with NF449 (Fig. 1C). All effects of the blockade were reversible by washout. Pretreatment with other suramin analogues, NF007, NF023 and NF279, were unable to prevent the block of nerve-evoked muscle contractions induced by D-TC

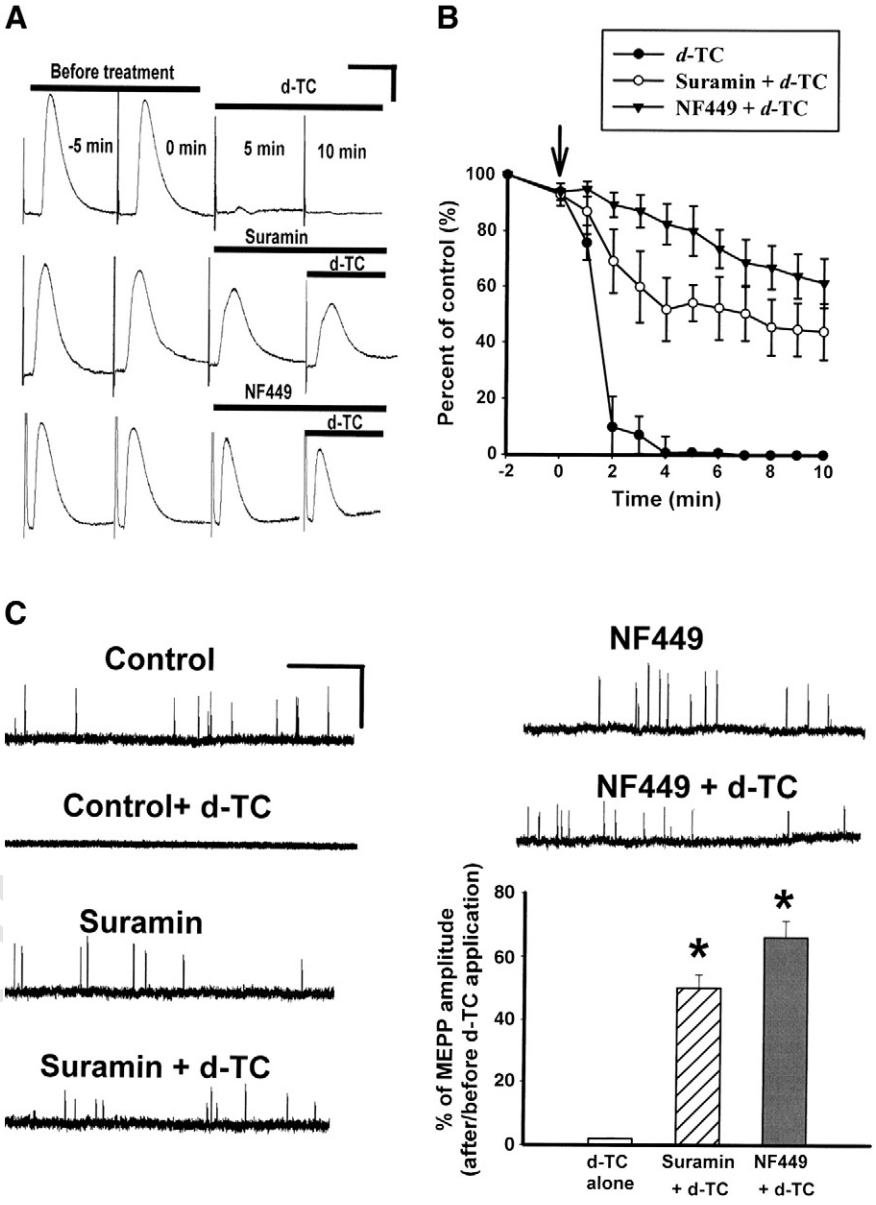

Fig. 3. Effect of pretreatment with NF449 on the inhibition of end-plate potentials (epp) or miniature end-plate potentials (mepp) induced by D-TC. (A) Representative epp traces recorded from mouse phrenic nerve diaphragm preparation. (B) The summarised plots of the D-TC-inhibition curves are shown for D-TC alone, NF449 and suramin pretreatment. The arrow indicates the application of NF449 or suramin or vehicle. Calibrations: 4 ms; 2 mV. (C) The recording traces showed the mepp amplitudes by addition of D-TC alone, and pretreatment with suramin or NF449 followed by addition of D-TC. The statistical bar chart showed the reversal effect of suramin and NF449 on the inhibition of mepp amplitudes induced by D-TC. Calibrations: 10 s; 1 mV.

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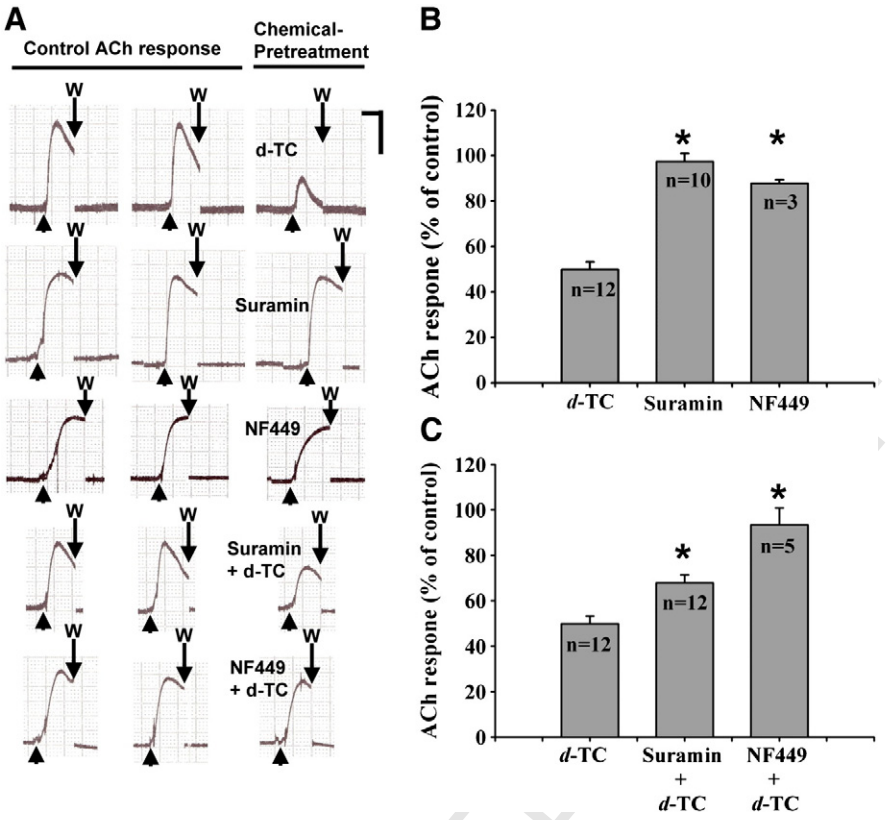

Fig. 4. Effects of the pretreatment with NF449 on the muscle contractions of chick biventer cervicis induced by ACh (30 μM). The representative traces of muscle contractions induced by ACh are shown in (A). ACh-induced muscle contractions caused by the application of ACh (30 μM) and then washout after the muscle contractions reached the maximal response. The upright arrow and down arrow indicate the application of ACh and washout (W), respectively. The effect of NF449 or suramin alone on the ACh-induced muscle contractions is summarised in (B). Pretreatment with NF449 or suramin on ACh-induced contractions in the presence D-TC is summarised in (C).* $P<0.05$ as compared with D-TC alone. Calibrations: 10 s; 1 g.

(Table 1). The time course of inhibition curves of D-TC when pre-treated or after-treated with NF449 are shown in Fig. 2A and B, respectively. Fig. 2C shows the concentration–inhibition curve of D-TC. The IC_{50} (inhibition concentration 50%) of D-TC alone was about 1.2 μM. Suramin and NF449 significantly shifted the curves to the right (IC_{50} from ~1.2 to ~4 μM for suramin and from ~1.2 to ~8 μM for NF449).

Effects of NF449 on the end-plate potential (epp) and miniature end-plate potential (mepp)

Application of D-TC (5 μM) to the extracellular solution produced a complete blockade of epps within 5 min (Fig. 3A). The time course of inhibition curves showed that pretreatment with either NF449 or suramin significantly reduced the percent inhibition of the epps induced by D-TC (Fig. 3B). The percent inhibitions of epps for D-TC alone, pretreatment with suramin and NF449 were 0%, 43.9 ± 10.1% and 61.4 ± 8.9%, respectively at 10 min after the application of D-TC. Similarly, NF449 and suramin significantly reduced the inhibition of mepps induced by D-TC (~~data not shown~~). D-TC alone produced a complete inhibition of mepps after 5 min of incubation. The percent inhibitions of mepps (amplitude of before/after the D-TC) were 50.2 ± 4.1% for suramin and 66.1 ± 5.2% for NF449. NF449 and suramin did not change the frequency of mepps (NF449: 0.95 ± 0.08 Hz; suramin: 0.88 ± 0.07 Hz; and control: 0.97 ± 0.08 Hz).

Effect of NF449 on the ACh-induced contraction in the chick biventer cervicis muscle

The contractions of chick biventer cervicis muscle (4–7 days old) can be induced by the addition of ACh (Fig. 4A). The time interval between each application was 20 min to prevent the nicotinic receptors from desensitising. Only the muscle preparations with less than 5% amplitude variance between two applications of ACh

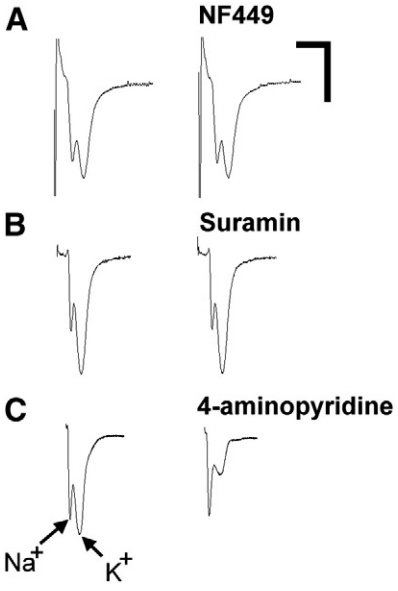

Fig. 5. Effects of NF449 and suramin on the presynaptic terminal waveforms. Treatment with NF449 (A, 100 μM) and suramin (B, 100 μM) did not affect the presynaptic Na^+ and K^+ current waveforms. The K^+ channel blocker, 4-aminopyridine (100 μM), showed inhibition of the K^+ current waveform as a comparison (C). Calibrations: 2 ms; 2 mV.

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(30 μM) were used in the further experiments. The ACh-induced contraction was significantly inhibited by the addition of D-TC (5 μM; D-TC alone, 49.8 ± 3.3% of control). Treatment with either NF449 or suramin alone produces only slight changes in the amplitudes of ACh-induced contraction (Fig. 4B). The pretreatment with either NF449 or suramin significantly reduced the inhibition of the ACh-induced contractions by D-TC (Fig. 4C; suramin: 67.8 ± 3.4% of control; NF 449: 93.3 ± 7.6% of control; $P<0.05$ as compared with D-TC alone).

Effects of NF449 on the presynaptic terminal waveforms

To determine whether NF449 and suramin can affect the presynaptic currents, we examined the presynaptic terminal waveforms with the application of NF449 or suramin. The results showed that NF449 (Fig. 5A) and suramin (Fig. 5B) did not affect the presynaptic Na^+ and K^+ waveforms. The effect of 4-aminopyridne (50 μM), a K^+ channel blocker, acts as the positive control for this comparison (Fig. 5C).

Discussion

In the present study, we demonstrated that NF449 prevents the neuromuscular blockade induced by D-TC via nicotinic ACh (nACh) receptors. NF449 can significantly reduce the inhibition of D-TC on the amplitudes of nerve-stimulated muscle contractions, end-plate potentials (epps) and ACh-induced contractions. Furthermore, NF449 did not affect the presynaptic Na^+ and K^+ waveforms. All the evidence indicated that the effect of NF449 was that it could compete with the binding of D-TC to the nACh receptors.

It has been reported that NF449 is a selective P2X purinergic receptor antagonist (Braun et al., 2001; Kassack et al., 2004), fibroblast growth factor receptor 3 (FGFR3) inhibitor (Gunosewoyo and Kassiou, 2010; Krejci et al., 2010) and Gs protein inhibitor (Hohenegger et al., 1998). Furthermore, suramin and suramin analogue NF023 act as direct antagonists of heterotrimeric G proteins by blocking of the rate-limiting step of G protein activation (Beindl et al., 1996). Because other P2X receptor antagonists (NF007, NF023 and NF279) (Van Rhee et al., 1994; Damer et al., 1998; Soto et al., 1999), FGFR inhibitors (NF279) (Krejci et al., 2010) and G protein uncouplers (NF023) (Beindl et al., 1996) cannot reverse the inhibitory effect induced by D-TC (Table 1), the effect of NF449 on the neuromuscular junction is not likely related to its effects on P2X, FGFR3 receptors or G protein.

In this study, NF449 protects the cholinergic receptor from the action of D-TC and this suggests that the pharmacological competition between these agents reflects a common binding site on the receptor protein (Miledi and Potter, 1971). The structure of suramin analogues and D-TC are shown in Fig. 6. NF449, NF023 and NF279 exhibit close structural similarity with suramin that belongs to a group of

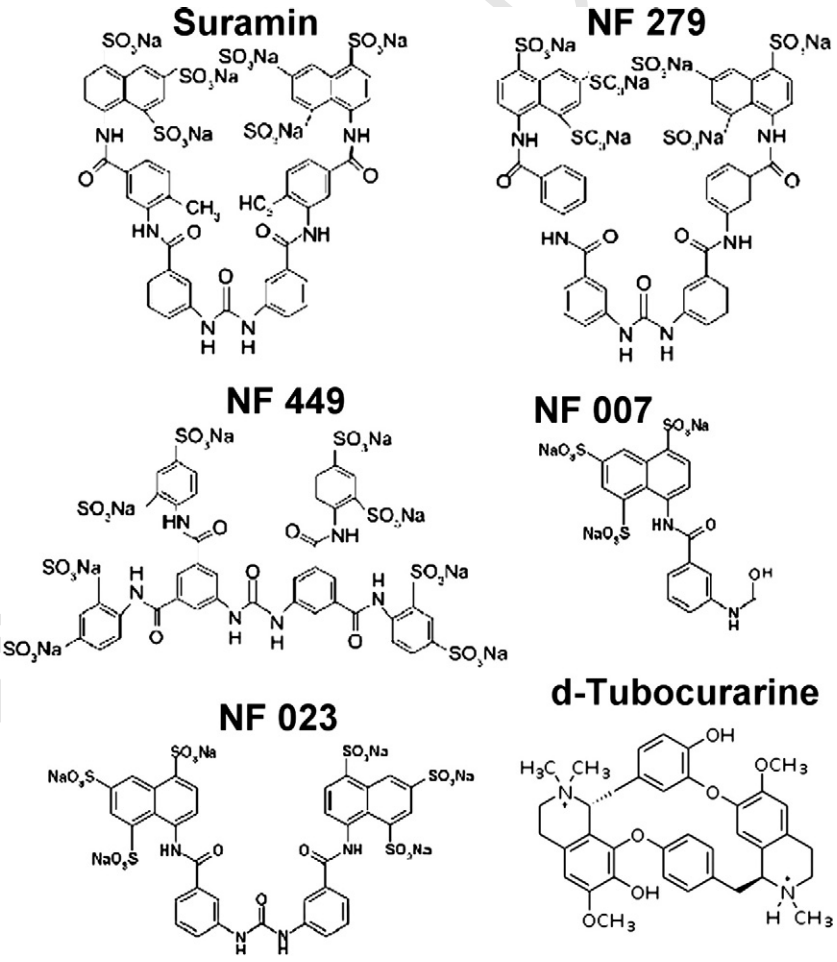

Fig. 6. The structures of NF449, NF007, NF023, NF279, suramin and ntubocurarine. NF007: 8-(3-nitrobenzamido)-1,3,5-naphthalenetrisulphonic acid; NF023: 8,8 -[carbonylbis (imino-3,1-phenylenecarbonylimino)]bis-1,3,5-naphthalenetrisulphonic acid; NF279: 8,8′-[carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino)]bis-1,3,5-naphthalenetrisulphonic acid; NF449: 4,4′,4″,4‴-(carbonylbis (imino-5,1,3-benzenetriylbis-(carbonylimino)))tetrakis-benzene-1,3-disulphonic acid octasodium salt; Suramin: 8,8′-[Carbonylbis [imino-3,1-phenylenecarbonylimino(4-methyl-3,1-phenylene)carbonylimino]]bis-1,3,5-naphthalenetrisulphonic acid hexasodium salt.

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polysulphonated napthylurea derivatives (Kassack et al., 2004). How does NF449 act on the nicotinic ACh receptor but NF007, NF023 and NF279 do not? Further investigation is needed to find the information concerning the structural determinants of ligand–receptor interactions (Horti et al., 2010). The structure of NF279 is the most similar to suramin, both are symmetrical polysulphonated naphthylamine derivatives of urea. The only slight difference between suramin and NF279 is that NF279 lacks two methyl groups located on the benzene moiety as compared with suramin. It has been reported that NF279 is a novel and potent antagonist selective for $P2X_1$ (Rettinger et al., 2000). However, suramin, but not NF279, prolonged the blocking time caused by D-TC. Another suramin analogue, NF449, is also a potent antagonist selective for $P2X_1$ (Hülsmann et al., 2003). In contrast to NF279, the treatment with NF449 can significantly prevent the blockade of nerve-evoked muscle contractions induced by D-TC. NF449 caused approximately a 5-fold (~1.2 μM to 8 μM) rightward shift in the concentration–inhibition curve of D-TC (Fig. 2), and its effect on the neuromuscular junction is more potent than that of suramin. Based on the structure–activity relationship of NF449 at P2 receptors, any deletion or change of position of the sulphonic acid groups, or replacing the central urea bond by the terephthalic acid bisamides reduced the potency at $P2X_1$ by at least 90% (Inoue et al., 1991). We don't know whether the groups of the central urea and sulphonic acid are important for the effect of NF449 on the nACh receptor. NF449 and suramin have a symmetrical structure with a central urea group and numbers of negatively charged sulphonate groups on the benzene or naphthyl rings. Based on the observation that the asymmetrical structure of NF007 does not have an effect on the neuromuscular junction, the symmetrical polysulphonated naphthylamine is likely important for the protecting effect on the nACh receptor by preventing neuromuscular block induced by D-TC.

A previous report showed that a stimulatory effect of suramin on the growth factor receptor indicated suramin is both a partial agonist and competitive inhibitor for the high affinity NGF receptor (Gill et al., 1996). Because NF449 and suramin have a slight inhibition of ACh-induced muscle contractions in chick biventer cervicis muscle, NF449 and suramin could possibly act as partial antagonists for the nACh receptor.

In summary, NF449 can prevent the blockade of neuromuscular transmission induced by D-TC. The effect of NF449 is to compete with the binding of D-TC on the nACh receptor site, and its effect is more potent than that of suramin. Even though this study provides evidence from animal experiments, the findings seem to have a potential clinical relevance. Nondepolarising neuromuscular blockers (muscle relaxants) are widely used as an adjunct for general anaesthesia or an anticonvulsant in clinical practice. It is suggested that NF449 may be useful in the treatment of muscle relaxants (nACh receptor blockers) overdose. The chemical structure of NF449 may provide important information in developing more effective antidotes against neuromuscular toxins.

Conflict of interest statement

None.

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References

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW. Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 2009;89(1):73-120.
Azam L, McIntosh JM. Alpha-conotoxins as pharmacological probes of nicotinic acetylcholine receptors. Acta Pharmacol Sin 2009;30(6):771–83.
Barstad JA, Lilleheil G. Transversely cut diaphragm preparation from rat, an adjuvant tool in the study of the physiology and pbarmacology of the myoneural junction. Arch Int Pharmacodyn Ther 1968;175(2):373–90.
Beindl W, Mitteraur T, Hohenegger M, Ijzerman AP, Nanoff C, Freissmuth M. Inhibition of receptor/G protein coupling by suramin analogues. Mol Pharmacol 1996;50(2):415–23.
Braun K, Rettinger J, Ganso M, Kassack M, Hildebrandt C, Ullmann H, et al. NF449: a subnanomolar potency antagonist at recombinant rat P2X1 receptors. Naunyn Schmiedebergs Arch Pharmacol 2001;364(3):285–90.
Damer S, Niebel B, Czeche S, Nickel P, Ardanuy U, Schmalzing G, et al. NF279: a novel potent and selective antagonist of P2X receptor-mediated responses. Eur J Pharmacol 1998;350(1):R5–6.
Dhar S, Gullbo J, Csoka K, Eriksson E, Nilsson K, Nickel P, et al. Antitumour activity of suramin analogues in human tumour cell lines and primary cultures of tumour cells from patients. Eur J Cancer 2000;36(6):803–9.
Dunn PM, Blakeley AG. Suramin: a reversible P2-purinoceptor antagonist in the mouse vas deferens. Br J Pharmacol 1988;93(2):243–5.
Fagerlund MJ, Eriksson LI. Current concepts in neuromuscular transmission. Br J Anaesth 2009;103(1):108–14.
Gill JS, Connolly DC, McManus MJ, Maihle NJ, Windebank AJ. Suramin induces phosphorylation of the high-affinity nerve growth factor receptor in PC12 cells and dorsal root ganglion neurons. J Neurochem 1996;66(3):963–72.
Ginsborg BL, Warriner J. The isolated chick biventer cervicis nerve–muscle preparation. Br J Pharmacol Chemother 1960;15:410–1.
Gunosewoyo H, Kassiou M. P2X purinergic receptor ligands: recently patented compounds. Expert Opin Ther Pat 2010;20(5):625–46.
Henning RH, Nelemans A, Houwertjes M, Agoston S. Reversal by suramin of neuromuscular block produced by pancuronium in the anaesthetized rat. Br J Pharmacol 1993;108(3):717–20.
Henning RH, Rowan EG, Braga MF, Nelemans A, Harvey AL. The prejunctional inhibitory effect of suramin on neuromuscular transmission in vitro. Eur J Pharmacol 1996;301(1–3):91–7.
Hohenegger M, Waldhoer M, Beindl W, Boing B, Kreimeyer A, Nickel P, et al. Gsalpha-selective G protein antagonists. Proc Natl Acad Sci USA 1998;95(1):346–51.
Horti AG, Gao Y, Kuwabara H, Dannals RF. Development of radioligands with optimized imaging properties for quantification of nicotinic acetylcholine receptors by positron emission tomography. Life Sci 2010;86(15–16):575–84.
Huh KH, Fuhrer C. Clustering of nicotinic acetylcholine receptors: from the neuromuscular junction to interneuronal synapses. Mol Neurobiol 2002;25(1):79-112.
Hülsmann M, Nickel P, Kassack M, Schmalzing G, Lambrecht G, Markwardt F. NF449, a novel picomolar potency antagonist at human P2X1 receptors. Eur J Pharmacol 2003;470(1–2):1–7.
Inoue K, Nakazawa K, Ohara-Imaizumi M, Obama T, Fujimori K, Takanaka A. Selective and competitive antagonism by suramin of ATP-stimulated catecholamine-secretion from PC12 phaeochromocytoma cells. Br J Pharmacol 1991;102(3):581–4.
Jentsch KD, Hunsmann G, Hartmann H, Nickel P. Inhibition of human immunodeficiency virus type I reverse transcriptase by suramin-related compounds. J Gen Virol 1987;68(Pt 8):2183–92.
Kassack MU, Braun K, Ganso M, Ullmann H, Nickel P, Böing B, et al. Structure-activity relationships of analogues of NF449 confirm NF449 as the most potent and selective known P2X1 receptor antagonist. Eur J Med Chem 2004;39(4):345–57.
Krejci P, Murakami S, Prochazkova J, Trantirek L, Chlebova K, Ouyang Z, et al. NF449 is a novel inhibitor of fibroblast growth factor receptor 3 (FGFR3) signaling active in chondrocytes and multiple myeloma cells. J Biol Chem 2010;285(27):20644–53.
Lin-Shiau SY, Lin MJ. Studies on curare-like action of the tripeptide carbobenzoxy-Gly-Gly-Arg-beta-naphthylamide in mouse diaphragm. Eur J Pharmacol 1998;343(1):51–6.
Mallart A. Electric current flow inside perineurial sheaths of mouse motor nerves. J Physiol 1985;368:565–75.
McArdle JJ, Angaut-Petit D, Mallart A, Bournaud R, Faille L, Brigant JL. Advantages of the triangularis sterni muscle of the mouse for investigations of synaptic phenomena. J Neurosci Methods 1981;4(2):109–15.
Miledi R, Potter LT. Acetylcholine receptors in muscle fibres. Nature 1971;233(5322):599–603.
Ord JJ, Streeter E, Jones A, Le Monnier K, Cranston D, Crew J, et al. Phase I trial of intravesical Suramin in recurrent superficial transitional cell bladder carcinoma. Br J Cancer 2005;92(12):2140–7.
Rettinger J, Schmalzing G, Damer S, Müller G, Nickel P, Lambrecht G. The suramin analogue NF279 is a novel and potent antagonist selective for the P2X1 receptor. Neuropharmacology 2000;39(11):2044–53.
Schneider J. Treatment of African trypanosomiasis (sleeping sickness). Presse Med 1960;68:881–3.
Schulz-Key H, Karam M, Prost A. Suramin in the treatment of onchocerciasis: the efficacy of low doses on the parasite in an area with vector control. Trop Med Parasitol 1985;36(4):244–8.
Soto F, Lambrecht G, Nickel P, Stühmer W, Busch AE. Antagonistic properties of the suramin analogue NF023 at heterologously expressed P2X receptors. Neuropharmacology 1999;38(1):141–9.
Su TR, Chen CH, Huang SJ, Lee CY, Su MC, Chen GH, et al. Functional study of the effect of phosphatase inhibitors on KCNQ4 channels expressed in Xenopus oocytes. Acta Pharmacol Sin 2009;30(9):1220–6.

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Ullmann H, Meis S, Hongwiset D, Marzian C, Wiese M, Nickel P, et al. Synthesis and structure–activity relationships of suramin-derived P2Y11 receptor antagonists with nanomolar potency. J Med Chem 2005;48(22):7040–8.
Van Rhee AM, van der Heijden MPA, Beukers MW, Ijzerman AP, Soudijn W, Nickel P. Novel competitive antagonists for P2 purinoceptors. European J Pharmacology Molecular Pharmacology 1994;268(1):1–7.
Villalona-Calero MA, Otterson GA, Wientjes MG, Weber F, Bekaii-Saab T, Young D, et al. Noncytotoxic suramin as a chemosensitizer in patients with advanced non-small-cell lung cancer: a phase II study. Ann Oncol 2008;19(11):1903–9.
Zouridakis M, Zisimopoulou P, Poulas K, Tzartos SJ. Recent advances in understanding the structure of nicotinic acetylcholine receptors. IUBMB Life 2009;61(4):407–23.

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