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The antibacterial and NMDA receptor activating properties of aminoglycosides are dissociable

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Abstract

The use of aminoglycoside antibiotics is limited by side effects, the most critical of which are vestibular and cochlear toxicity. Recent evidence indicates that these effects result from an excitotoxic process mediated, at least in part, through a polyamine-like activation of NMDA receptors. This study investigated whether these positive modulatory effects of aminoglycosides at NMDA receptors are dissociable from their antibacterial properties. A group of structurally related apramycin derivatives was evaluated for the ability to enhance [^{3}H]dizocilpine binding to rat brain membranes, and for the ability to augment agonist responses on recombinant (NR1A/2B) NMDA receptors expressed in *Xenopus* oocytes. Based on the antibacterial potencies of these derivatives against *Staphylococcus aureus* and *Escherichia coli*, it is concluded that there is no correlation between the ability of an aminoglycoside to produce a positive modulation of NMDA receptors and minimum inhibitory antibacterial concentrations. These findings indicate that it may be possible to develop an aminoglycoside antibiotic with reduced potential for ototoxicity.

Keywords: Aminoglycoside; Ototoxicity; NMDA receptor; Dizocilpine

1. Introduction

Aminoglycosides remain an important component of antibiotic therapy (Begg and Barclay, 1995; Priuska and Schacht, 1997) despite the potential risk of serious side effects, including nephrotoxicity and ototoxicity. While nephrotoxicity is often reversible upon cessation of therapy, ototoxicity can result in permanent hearing loss. This limiting side effect has generally relegated aminoglycosides to second and third-line therapy in industrialized nations. However, several factors, including low cost, rapid onset of action, and synergy with β-lactam antibiotics sustain the use of aminoglycosides in developing countries. This continued use is exemplified by an estimate that two-thirds of the deaf-mutism in China is the result of aminoglycoside toxicity (Schacht, 1993).

Cochlear hair cells are particularly vulnerable to aminoglycosides, and the loss of these cells is likely to represent the cellular basis of hearing loss (Wersall, 1995). While the biochemical and molecular processes mediating aminoglycoside-induced ototoxicity are not fully understood, converging lines of evidence indicate that this is, at least in part, an excitotoxic process requiring NMDA receptor activation (Basile et al., 1996; Segal et al., 1999). Thus, in guinea pigs, both the hearing loss and damage to cochlear hair cells produced by aminoglycosides is substantially reduced by coadministration of NMDA receptor antagonists (Basile et al., 1996). Consistent with this hypothesis, electrophysiological and neurochemical studies have demonstrated that aminoglycosides produce a polyamine-like activation of NMDA receptors (Pullan et al., 1992; Lu et al., 1998; Harvey and Skolnick, 1999). These positive modulatory actions of aminoglycosides at NMDA receptors can be demonstrated at pharmacologically relevant concentrations — that is, at levels present in cochlear perilymph following ototoxic doses of aminoglycosides (Brummett et al., 1978).

The aminocyclitols and aminosugars that constitute these antibiotics cannot, either individually or combined in mixtures, recreate the potent, polyamine-like activation of

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NMDA receptors produced by the parent molecules (Segal and Skolnick, 1998). These findings indicate that the three-dimensional covalent structure of an aminoglycoside is necessary for activation of NMDA receptors, and raise the possibility that the potential mechanism responsible for aminoglycoside-induced ototoxicity may be dissociable from the antibacterial actions. To determine if a relationship exists between the NMDA receptor activation and antibacterial actions of aminoglycosides, these properties were evaluated in a series of structurally related apramycin derivatives. We now report that within this series of derivatives, there is no direct relationship between the potency of a molecule to activate NMDA receptors and its antibacterial strength, indicating these are dissociable properties of aminoglycoside antibiotics.

2. Materials and methods

2.1. [^{3}H]dizocilpine binding

2.1.1. Materials

[^{3}H]Dizocilpine (Sp. Act. 22.5 Ci/mmol) was obtained from DuPont-NEN (Boston, MA). Apramycin and derivatives were prepared within the Lilly Research Laboratories, Indianapolis, IN.

2.1.2. Membrane preparation

Adult male Sprague–Dawley rats (250–300 g; Harlan SD, Indianapolis, IN) were maintained in a vivarium under standard conditions (12-hour day/night cycle) with free access to food and water. Rats were decapitated and the forebrains rapidly removed. Tissue was disrupted using a Polytron (setting #7 for 30 s) in 10 volumes of ice-cold fresh 0.32 M sucrose-HTS (HTS: 5 mM HEPES/4.5 mM Tris buffer; pH 7.4), and the tissue suspension diluted to 50 volumes. The homogenate was centrifuged (4°C) at $1000 \times g$ for 10 min. The supernatant was decanted and centrifuged (4°C) at $20{,}000 \times g$ for 20 min. The resulting pellet was resuspended in 50 volumes of HTS and recentrifuged at $8000 \times g$ for 20 min. Approximately 2/3 of the supernatant was decanted and reserved, with the remainder used to collect the outer layer (buffy coat) pellet by swirling the pellet with gentle vortexing. The remaining pellet core was discarded and the combined buffy coat and supernatant centrifuged at $20{,}000 \times g$ for 20 min (4°C). The resulting pellet was resuspended in 50 volumes of 1 mM EDTA-HTS buffer. This centrifugation/resuspension procedure was repeated three more times, except the suspension medium was HTS for the final two times. The resulting pellets were resuspended in 5 volumes of HTS and stored in −70°C until assayed.

Tissues were thawed on ice and diluted to 50 volumes with 5 mM HEPES/4.5 mM Tris buffer (pH 7.4). The suspension was centrifuged (4°C) at $20{,}000 \times g$ for 20 min and resuspended in 50 volumes of buffer. Assays were performed in a total volume of 0.5 ml containing [^{3}H]dizocilpine (final concentration 3.5–4 nM), membrane suspension (0.25 ml containing ~60–70 μg of membrane protein), and test compounds. Non-specific binding was determined with 100 μM phencyclidine hydrochloride. All reagents and buffers were prepared in Milli-Q water and tissue was extensively ''washed'' to minimize glycine and glutamate. After a 2-h incubation (25°C), the assays were terminated by rapid filtration (MB-48R cell harvester; Brandel) over glass fiber filters (Brandel GF/B) presoaked in 0.03% polyethylenimine. The filters were washed with two 2–3 ml aliquots of ice-cold assay buffer. The radioactivity trapped by the filters was determined by liquid scintillation counting (Beckman model LS 6500). Protein concentrations were measured using the bicinchoninic acid protein assay reagent (Pierce, Rockford, IL).

2.2. Effects on recombinant NMDA receptors expressed in Xenopus oocytes

2.2.1. Materials

Xenopus laevis frogs were purchased from Xenopus-1 (Dexter, MI). Collagenase B was obtained from Boehringer Mannheim (Indianapolis, IN). All other compounds were obtained from Sigma Chemical (St. Louis, MO). The rat NMDA receptor NR1A clone was a gift from Dr. S. Nakanishi (Department of Immunology, Kyoto University, Japan). The rat NR2B clone was a gift from J. Sullivan (Salk Institute, La Jolla, CA). To optimize subunit expression levels in oocytes, most of the 5′ untranslated region was removed from the subunit clones using available restriction enzyme sites. The cDNAs encoding the NMDA subunits were subcloned into pcDNA3.

2.2.2. Injection of in vitro synthesized RNA into Xenopus oocytes

Capped cRNA was synthesized from linearized template cDNA encoding the subunits using mMESSAGE mMACHINE kits (Ambion, Austin, TX). Oocytes were injected with the NR1A and NR2B subunits in a ratio of 1:5 determined by gel electrophoresis. Mature *X. laevis* frogs were anesthetized by submersion in 0.1% 3-aminobenzoic acid ethyl ester, and oocytes were surgically removed. Follicle cells were removed by treatment with collagenase B for 2 h. Each oocyte was injected with 5–25 ng of cRNA in 50 nl of water and incubated at 19°C in modified Barth's saline (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 100 μg/ml gentamicin, and 15 mM HEPES, pH 7.6). Responses from oocytes were recorded after 1 to 7 days post-injection.

2.2.3. Electrophysiological recordings

Oocytes were perfused at room temperature in a Warner Instruments oocyte recording chamber #RC-5/18 (Hamden, CT) with perfusion solution (115 mM NaCl, 1.8 mM $CaCl_2$, 2.5 mM KCl, 10 mM HEPES, pH 7.2). Perfusion solution was gravity fed continuously at a rate of

15 ml/min. Compounds were diluted in perfusion solution, and applied until a steady state current was reached, typically 60 s. Current responses to agonist application were measured under two-electrode voltage clamp, at a holding potential of −35 mV. This potential is close to the reversal potential for a calcium activated chloride current endogenous to oocytes (Barish, 1983). Therefore, the contribution of this quickly desensitizing current to the plateau phase of the whole cell current is minimized.

Data was collected using a GeneClamp 500 amplifier and Axoscope software (Axon Instruments, Foster City, CA). Responses to concentrations of the co-agonists glutamate and glycine in the presence of aminoglycosides are reported as a percentage of the response to glutamate and glycine alone (''percent control response'', or ''% control''). Data were fitted to a four parameter logistic equation using GraphPad Prizm. Statistical significance was determined using a one-way ANOVA (analysis of variance) followed by a Bonferroni's multiple comparison post hoc test. The availability of 3-*N*-ethyl and 2′-*N*-phthalimido derivatives was insufficient to extend electrophysiological analysis beyond 1 mM. Since the concentration–response curves for apramycin reached a plateau at 1 mM, this concentration was fitted as the top of the curve for the derivatives as well.

2.3. Determination of antibacterial potency

Antibiotic susceptibility was determined by standard agar dilution methods according to the National Committee for Clinical Laboratory Standards, Approval Standard M7-A (Washington, 1985; Kirst et al., 1995).

3. Results

3.1. Activation of NMDA receptors

3.1.1. Neurochemistry

Apramycin (Fig. 1) produced a concentration dependent increase in [^{3}H]dizocilpine binding (Fig. 2). The potency

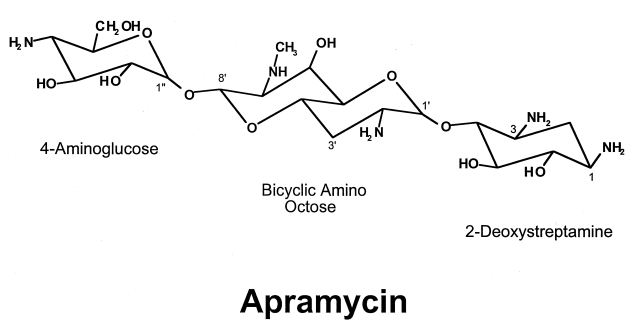

Fig. 1. The structure of apramycin. This compound contains 2-deoxystreptamine, a common aminocyclitol also present in neomycin, kanamycin, tobramycin and gentamicin. The aminocyclitol moiety is glycosidically linked at its 4-hydroxy position to a bicyclic aminooctose, which is linked to 4-aminoglucose via a 1,1′-bis-glycosyl bond. The positions that are substituted (Table 1) are indicated by numbers (Kirst, 1996).

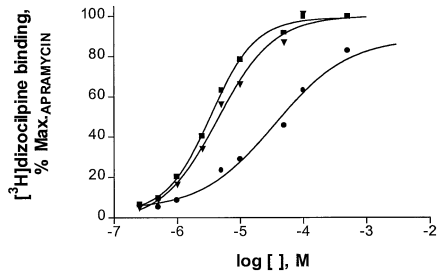

Fig. 2. Enhancement of [^{3}H]dizocilpine binding by apramycin (■), 3-*N*-ethylapramycin (▼), and 2′-*N*-phthalimidoapramycin (●). Rat forebrain membranes were extensively washed to reduce the concentrations of endogenous compounds (e.g., glycine, glutamate, and polyamines) known to affect [^{3}H]dizocilpine binding. Data are expressed as a percent of the maximal enhancement achieved with apramycin. In this representative experiment, EC_{50} values are 3.4, 4.4 and 186 μM for apramycin, 3-*N*-ethylapramycin, and 2′-*N*-phthalimidoapramycin, respectively; mean ± S.E.M. are presented in Table 1. In a typical assay, specific [^{3}H]dizocilpine binding was ~35 fmol/assay, and was increased to ~150 fmol/assay in the presence of maximally effective concentrations of aminoglycosides.

of apramycin in this assay (EC_{50} 3.9 ± 1.1 μM) is comparable to other clinically useful aminoglycosides such as streptomycin and neomycin (Basile et al., 1996). The series of apramycin derivatives demonstrated an ~70-fold range in potency to stimulate [^{3}H]dizocilpine binding (Table 1). Substitution of a relatively small functional groups on the 2-deoxystreptamine, bicyclic aminooctose or 4-aminoglucose rings did not remarkably affect the potency of apramycin derivatives to enhance [^{3}H]dizocilpine binding. For example, the 1-*N*-glycyl, 3-*N*-ethyl, 2′-*N*-[4-amino-2(*S*)-hydroxybutyryl] and 4″-*N*-acetyl derivatives enhanced [^{3}H]dizocilpine binding with potencies comparable to the parent compound apramycin. Moreover, the 3-*N*-ethyl derivative potentiated [^{3}H]dizocilpine binding with a similar efficacy as apramycin (Fig. 2).

In contrast, substitutions of larger functional groups and bridged derivatives on the bicyclic aminooctose ring resulted in a substantial reduction in potency to enhance [^{3}H]dizocilpine binding. For example, the potencies of the 6′-*O*-7′-*N*-cyclocarbonyl was ~5-fold lower than apramycin and the 2′-*N*-phthalimido derivative (Fig. 2) was ~40-fold lower than apramycin.

3.1.2. Electrophysiological studies

The ability of a compound to enhance [^{3}H]dizocilpine binding to brain membranes is a facile means of evaluating the potency of positive modulators at a mixed population of native NMDA receptors. However, this measure cannot discriminate the multiple actions produced by aminoglycosides at specific NMDA receptor subtypes (Lu et al., 1998; Harvey and Skolnick, 1999). Therefore, the activity of selected apramycin derivatives on recombinant NMDA receptors composed of NR1A/NR2B subunits was examined. This receptor subunit combination exhibits the full range of positive modulatory responses to polyamines

Table 1
Polyamine-like action of apramycin derivatives at NMDA receptors: comparison with antibacterial properties
[^{3}H]dizocilpine binding to NMDA receptors was assayed in thoroughly washed membranes prepared from rat forebrain. This preparation is nominally free of glycine and glutamate. Values represent the mean ± S.E.M. of ≥ 3 assays. Minimum inhibitory concentration values are expressed relative to apramycin (N/A = not active in disc diffusion assay at 1 mg/ml). A Spearman rank correlation (nonparametric) analysis of the EC_{50} values to enhance [^{3}H]dizocilpine binding and minimum inhibitory concentrations for which definitive values are established demonstrated no significant interdependence (vs. *E. coli* $r^2 = 0.01$; vs. *S. aureus* $r^2 = 0.33$).

Compound	EC_{50} (μM), [^{3}H]dizocilpine binding	Minimum inhibitory concentration (μg/ml)	
		S. aureus X1	*E. coli* EC14
1-*N*-glycylapramycin	2.2 ± 0.6	16	32
3-*N*-ethylapramycin	3.2 ± 0.5	32	> 128
2′-*N*-[4-amino-2(*S*)-hydroxybutyryl] apramycin	3.3 ± 0.8	16	32
Apramycin	3.9 ± 1.1	4	16
4″-*N*-acetylapramycin	7.8 ± 1.3	16	16
4″,7′-di-*N*-acetylapramycin	12.1 ± 1.6	> 128	> 128
6′-*O*-7′-*N*-cyclocarbonylapramycin	19.0 ± 2.6	8	64
6′-*O*-7′-*N*,4″-*N*-6″-*O*-biscyclocarbonylapramycin	37.7 ± 5.5	> 128	> 128
2′-*N*-phthalimidoapramycin	145.0 ± 11.6	N/A	N/A

(Williams, 1997), and aminoglycosides (Lu et al., 1998; Harvey and Skolnick, 1999) described in native receptors (Benveniste and Mayer, 1993). Similar to other therapeutically useful aminoglycosides (Harvey and Skolnick, 1999), apramycin augmented agonist responses under both glycine-independent (i.e., saturating glycine and glutamate) and glycine-dependent (i.e., subsaturating glycine with saturating glutamate) conditions (Figs. 3 and 4). Under glycine-independent conditions, the 3-*N*-ethyl derivative and apramycin potentiated agonist responses with similar potencies (EC_{50} values of 268 and 226 μM, respectively, Fig. 4A). In contrast, the 2′-*N*-phthalimido derivative (EC_{50} = 577 μM) was significantly ($P < 0.001$) less potent than the parent aminoglycoside, apramycin. The maximum responses produced by both 1 mM 3-*N*-ethyapramycin (343 ± 28% control) and 2′-*N*-phthalimidoapramycin (174 ± 13% control) were significantly lower than apramycin (425 ± 40% control; $P < 0.05$ and $P < 0.001$, respectively).

Under glycine-dependent conditions, apramycin and its 3-*N*-ethyl analog potentiated agonist responses with similar potencies (278 and 303 μM, respectively, Fig. 4B). In contrast, the 2′-*N*-phthalimido derivative (EC_{50} = 429 μM) was significantly less potent ($P < 0.001$) than the parent aminoglycoside. At 1 mM, the 3-*N*-ethyl derivative (975 ± 83% control, $P < 0.001$) produced a greater potentiation than apramycin (618 ± 24% control), while the 2′-*N*-phthalimido derivative was significantly less efficacious (336 ± 29% control, $P < 0.01$) than the parent compound.

3.2. Antibacterial potencies

Structural modification of apramycin had a significant effect on the minimum inhibitory concentrations against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterium (Table 1). Substitution on the 2-deoxystreptamine ring at the 1-*N* or 3-*N* position resulted in loss of activity against both organisms. For example, 3-*N*-ethyl substitution reduced potency against *S. aureus* by 8-fold and abolished activity against *E. coli*. Substitution on the bicyclic aminooctose ring at the 2′-*N* position by 4-amino-2(*S*)-hydroxybutyryl amide resulted in a small loss of antibacterial activity (4- and 2-fold

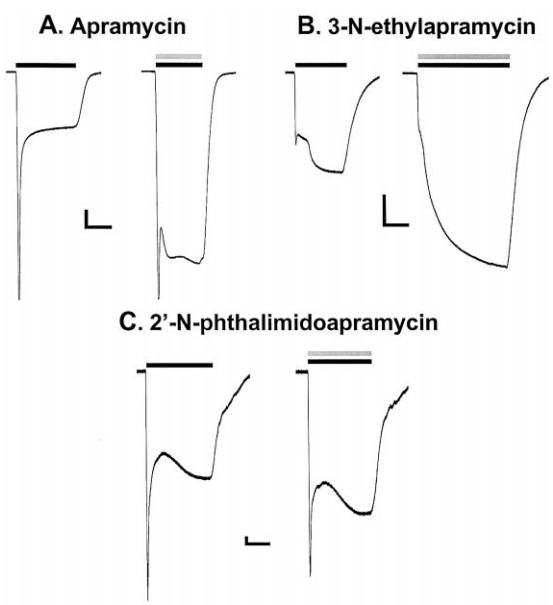

Fig. 3. Enhancement of NMDA (NR1A/2B) receptor responses by apramycin (A), 3-*N*-ethylapramycin (B), and 2′-*N*-phthalimidoapramycin (C). Representative voltage clamp traces of *Xenopus* oocytes during bath perfusion of 30 μM glutamate and 10 μM glycine for time period indicated by black bar (*left traces*), followed by perfusion of co-agonists together with 300 μM of indicated aminoglycoside for time period indicated by gray bar (*right traces*). The initial inward spike is derived from a calcium activated chloride current endogenously expressed in oocytes, whereas the NMDA receptor response is represented by the later plateau phase (Leonard and Kelso, 1990). Scale bars: 100 nA, 25 s.

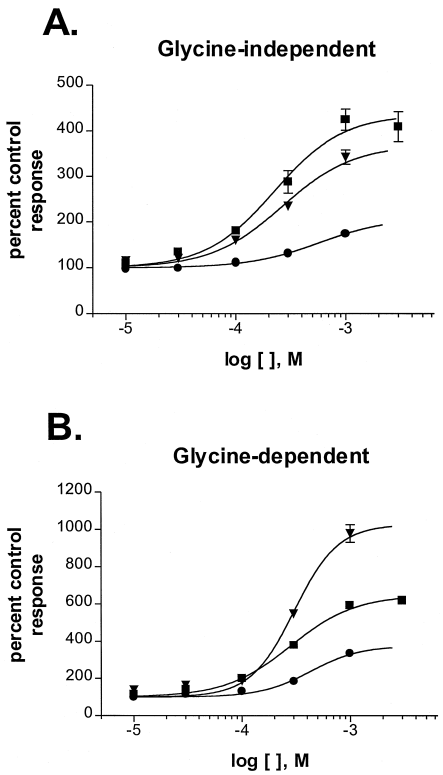

Fig. 4. Concentration–response curves for enhancement of NMDA (NR1A/2B) receptor responses by apramycin (■), 3-*N*-ethylapramycin (▼), and 2′-N-phthalimidoapramycin (●). (A) Potentiation of agonist responses by compounds on voltage clamped *Xenopus* oocytes under glycine-independent conditions (saturating glycine = 10 μM; saturating glutamate = 30 μM). The fitted $-\log EC_{50} \pm$S.E. values from three pooled experiments are 3.645 ± 0.08694, 3.572 ± 0.03478, and 3.233 ± 0.03597 for apramycin, and the 3-*N*-ethyl and 2′-*N*-phthalimido derivatives, respectively. (B) Potentiation of agonist responses by compounds on voltage clamped *Xenopus* oocytes under glycine-dependent conditions (subsaturating glycine = 100 nM; saturating glutamate = 30 μM). The fitted $-\log EC_{50} \pm$S.E. values from three pooled experiments are 3.554 ± 0.0362, 3.518 ± 0.02356 and 3.367 ± 0.0308 for apramycin, 3-*N*-ethyl and 2′-*N*-phthalimido derivatives, respectively. Symbols are mean ± S.D. of three separate oocytes and fitted to a four parameter logistic equation.

loss against *S. aureus* and *E. coli*, respectively), while an *N*-phthalimido group at this position eliminated activity against both bacterial strains.

Substitution on the 4-aminoglucose ring at the 4″ position by N-acetyl moiety did not affect activity against *E. coli*, and produced a 4-fold loss in activity against *S. aureus.* An additional N-acetyl substitution on the 7′-*N* position of the bicyclic aminooctose ring resulted in a complete loss of antibacterial activity against these two strains.

4. Discussion

Aminoglycosides are used to treat a wide range of bacterial infections ranging from pneumonia to drug-resistant tuberculosis (Begg and Barclay, 1995). However, the use of these drugs is tempered by the risk of significant side effects including nephrotoxicity and ototoxicity. While kidney function can be monitored during therapy, aminoglycoside induced ototoxicity is not easily monitored. Aminoglycosides can cause damage to both the cochlea and vestibular apparatus (Begg and Barclay, 1995). While compensatory mechanisms greatly dampen the impact of impaired vestibular function (Hodges, 1984), cochlear damage can result in permanent hearing loss. Despite attempts to limit dosing and careful monitoring of blood levels, measurable signs of hearing loss are detectable in 10–15% of patients receiving aminoglycosides (Priuska and Schacht, 1997). Thus, the development of an aminoglycoside antibiotic lacking this ototoxic liability would be regarded as a significant therapeutic advance.

A high correlation has been reported between the relative cochleotoxicities of a series of aminoglycosides in humans and the potencies of these compounds to produce a polyamine-like enhancement of [^{3}H]dizocilpine binding to NMDA receptors (Basile et al., 1996). Furthermore, the ability of specific NMDA receptor antagonists to attenuate both the cochlear (Basile et al., 1996) and vestibular (Basile et al., 1999) effects of aminoglycosides has led to the hypothesis that this damage is an excitotoxic process mediated, at least in part, by a polyamine-like activation of NMDA receptors. The principal objective of the present study was to determine if the antibacterial and NMDA activating properties of aminoglycosides are dissociable. If these properties are dissociable, then it may be possible to synthesize an aminoglycoside antibiotic with reduced ototoxicity.

Based on an examination of individual aminocyclitols and aminosugars common to many aminoglycosides, it appears necessary to maintain three-dimensional covalent structure (Segal and Skolnick, 1998) in order to produce a potent, polyamine-like activation of NMDA receptors. While most clinically used aminoglycosides maintain a broad structural similarity — an aminocyclitol ring joined through a glycosidic linkage to one or more aminosugars — both the wide variety of substituents on these ring systems and the structural diversity of these substituents complicates direct inferences about the relationship between antibiotic potency and NMDA receptor activating properties among aminoglycosides. Therefore, the availability of a series of structurally related apramycin derivatives afforded the opportunity to directly compare antibiotic potency and NMDA receptor activating properties. Within this series of apramycin derivatives, no relationship was evinced between the positive modulatory actions of these aminoglycosides at NMDA receptors and the concentrations required to inhibit the growth of either *S. aureus* or *E. coli*. Thus, small substituents on the aminicyclitol and bicyclic aminooctose rings had little effect on potency to enhance of [^{3}H]dizocilpine binding, yet had dramatic effects on antibacterial activity. For example, the 1-*N*-glycyl, 3-*N*-ethyl and 2′-*N*-[4-amino-2(*S*)-hydroxybutyryl]

derivatives were at least as potent as apramycin in potentiating [^{3}H]dizocilpine binding, yet demonstrated 4- to 8-fold less activity against *S. aureus*. In the case of 3-*N*-ethyl-apramycin, this substitution abolished activity against *E. coli*. The 6′-*O*-7′-*N*-cyclocarbonyl derivative was ~5-fold less potent than apramycin in enhancing [^{3}H]dizocilpine binding, yet exhibited only 2-fold less activity against *S. aureus*; moreover, the 4″,7′-di-*N*-acetyl derivative was an ~3-fold less potent enhancer of [^{3}H]dizocilpine binding and was ineffective against either bacteria. A Spearman rank correlation analysis of [^{3}H]dizocilpine binding enhancement potencies and minimum inhibitory concentrations for which definitive values were established indicated no significant interdependence between these values. In principle, these findings establish that these two properties of aminoglycoside antibiotics are dissociable. Nonetheless, no compounds emerged from this series with the ideal profile: high antibacterial potency together with a low potency to activate NMDA receptors.

Under similar assay conditions, clinically useful aminoglycosides enhance [^{3}H]dizocilpine binding to brain membranes with EC_{50} values of ~10 μM (Pullan et al., 1992; Basile et al., 1996; Segal and Skolnick, 1998) while peak concentrations in the cochlear perilymph after ototoxic doses of aminoglycosides such as gentamicin and amikacin are ~20 μM (Brummett et al., 1978) These neurochemical assays are performed using extensively washed cortical membranes, and the enhancement of [^{3}H]dizocilpine binding is likely to reflect both the glycine-dependent and -independent actions of aminoglycosides at a heterogeneous population of NMDA receptors. Because the relative contribution of the glycine-dependent and -independent actions of aminoglycosides to the ototoxic potential of aminoglycosides is unclear (Harvey and Skolnick, 1999), each action was examined in a subset of apramycin derivatives using recombinant NMDA NR1A/2B receptors (Fig. 4). This subunit combination was chosen because binary receptors composed of NMDA NR1A and either NR2A, NR2C, or NR2D do not manifest the entire spectrum of positive modulatory (i.e., glycine-dependent and -independent) responses produced by aminoglycosides (Harvey and Skolnick, 1999). Similar to what is observed with both endogenous polyamines and many clinically useful aminoglycosides, apramycin and its analogs were more potent in activating NMDA receptors under glycine-dependent compared to a glycine-independent conditions (Fig 4). The absolute difference in potency between neurochemical and electrophysiological measures obtained for this series of aminoglycosides is consistent with previous findings (Pullan et al., 1992; Basile et al., 1996; Lu et al., 1998) and reflects, at least in part, the ability to fully optimize assay conditions in radioligand binding assays compared to the *Xenopus* oocyte expression system. Nonetheless, the rank order potency under both glycine-dependent and -independent conditions corresponds to that obtained in radioligand binding studies using native NMDA receptors. These observations are consistent with the concept that the ability of aminoglycoside antibiotics to produce a positive modulatory action at NMDA receptors is not directly related to antibiotic potency. If these properties are indeed dissociable, then it may be possible to develop aminoglycoside antibiotics with a reduced risk for ototoxicity.

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