Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 461 (2003) 105–111
www.elsevier.com/locate/ejphar

Therapeutically relevant concentrations of neomycin selectively inhibit P-type Ca^{2+} channels in rat striatum

Dobromir Dobrev*, Ursula Ravens

Department of Pharmacology and Toxicology, Carl Gustav Carus Medical School, Dresden University of Technology, Fetscher Str. 74, D-01307 Dresden, Germany

Received 26 August 2002; received in revised form 24 December 2002; accepted 7 January 2003

Abstract

The effects of neomycin on voltage-activated Ca^{2+} channels (VACCs) were studied by Ca^{2+}-dependent K^+- and veratridine-evoked [^{3}H]dopamine release from rat striatal slices. Neomycin (0.01–1 mM) concentration dependently reduced K^+-evoked [^{3}H]dopamine release (IC_{50} ~25 μM), producing ~98% inhibition at 1 mM. Contribution of N-, P- and Q-type Ca^{2+} channels to this neomycin-sensitive [^{3}H]dopamine release was tested by the combined application of 100 μM neomycin and selective Ca^{2+} channel blockers. The effects of neomycin combined with 1 μM of ω-conotoxin GVIA (N-type Ca^{2+} channels) or with 100 nM of ω-conotoxin MVIIC (Q-type Ca^{2+} channels) were additive, excluding involvement of N- and Q-type Ca^{2+} channels. However, the combined effects of neomycin with 30 nM of ω-agatoxin-IVA (P-type Ca^{2+} channels) were not additive, suggesting involvement of P-type Ca^{2+} channels in neomycin-induced inhibition of [^{3}H]dopamine release. On the other hand, veratridine-evoked [^{3}H]dopamine release was shown to be mediated by Q-type Ca^{2+} channels only. In addition, neither the inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase thapsigargin (500 nM) nor the blocker of sarcoplasmic reticulum ryanodine Ca^{2+} channels ryanodine (30 μM) modulate veratridine-evoked [^{3}H]dopamine release, suggesting no contribution of intracellular Ca^{2+} stores. Neomycin (up to 100 μM) did not affect veratridine-evoked [^{3}H]dopamine release, suggesting that intracellular Ca^{2+} stores are not a prerequisite for the action of neomycin. Lack of inhibitory effect of neomycin is taken as additional indirect evidence for the involvement of P-type Ca^{2+} channels. In conclusion, therapeutically relevant concentrations of neomycin preferentially block P-type Ca^{2+} channels which regulate dopamine release in rat striatum. This block could be responsible for aminoglycoside-induced toxicity.

Keywords: Striatum; Dopamine release; Neomycin; Veratridine; ω-Conotoxin; ω-Agatoxin-IVA

1. Introduction

Aminoglycoside antibiotics like neomycin inhibit a variety of ion channels, and these actions are thought to contribute to some of the clinical side-effects such as acute nephrotoxicity and ototoxicity, respiratory arrest and neuromuscular blockade (Fischel-Ghodsian, 1999; Chambers, 2001).

Neomycin has been shown to inhibit neurotransmitter release from brain slices and synaptosomal preparations (Diamant et al., 1987; Keith et al., 1992; Carvalho et al., 1995; Elverfors et al., 1997; Bergquist et al., 1998), to block K^+-evoked $^{45}Ca^{2+}$ influx into synaptosomes (Atchison et al., 1988; Keith et al., 1992) and to reduce intracellular-free Ca^{2+} in synaptosomal preparations (Duarte et al., 1996). Because neomycin inhibits the specific binding of selective N-type Ca^{2+} channel blocker [^{125}I]ω-conotoxin GVIA (Knaus et al., 1987; Stumpo et al., 1991; Pichler et al., 1996), it was suggested that the inhibition of release by aminoglycosides is mediated by the blockade of N-type Ca^{2+} channels (Knaus et al., 1987). However, neomycin also affects non-L/non-N-type Ca^{2+} channels (Carvalho et al., 1995; Pichler et al., 1996; Elverfors et al., 1997; Toner and Stamford, 1997; Bergquist et al., 1998) and does not appear to be specific for voltage-activated Ca^{2+} channels (VACCs) because modulation of inositol 1,4,5-trisphosphate- and ryanodine-induced intracellular Ca^{2+} release channels may also contribute to its inhibitory effects on neurotransmitter release (Sayers and Michelangeli, 1993; Mack et al., 1992).

* Corresponding author. Tel.: +49-351-458-6279; fax: +49-351-458-6315.
E-mail address: dobrev@rcs1.urz.tu-dresden.de (D. Dobrev).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01319-0

Therapeutically relevant concentrations of aminoglycosides inhibit the specific binding of the P/Q-type VACC blocker [^{125}I]ω-conotoxin MVIIC to guinea-pig cerebellum membranes and block P/Q-type Ca^{2+} currents in *Xenopus* oocytes (Pichler et al., 1996). The authors conclude that inhibition of these channels may be responsible for some of the harmful toxic reactions of aminoglycoside antibiotics. Interestingly, P/Q-type Ca^{2+} channels are widely distributed and were also recently detected in the smooth muscle of renal resistance vessels and aorta (Hansen et al., 2000). However, despite clinical interest in identifying the type of VACCs affected preferentially by therapeutic concentrations of aminoglycosides, there are no data about specificity of aminoglycoside block of L-, N-, P- and Q-type Ca^{2+} channels in native expression systems.

The small size of nerve terminals does not allow direct measurement of Ca^{2+} currents. Here, we investigated the effects of neomycin on K^+- and veratridine-evoked [^{3}H]dopamine release from rat striatal slices because these neurons express L-, N-, P- and Q-type VACCs (Olivera et al., 1994; Mermelstein et al., 1999). In addition, K^+-evoked [^{3}H]dopamine release is mediated by P- and Q-type Ca^{2+} channels with minor contribution of N-type Ca^{2+} channels (Carvalho et al., 1995; Soliakov and Wonnacott, 1996; Dobrev and Andreas, 1997; Dobrev et al., 1999), whereas veratridine-evoked [^{3}H]dopamine release involves Q-type Ca^{2+} channels only (Dobrev et al., 1998). The peak plasma concentration of neomycin was estimated to be 1–4 μg/ml, yielding a free concentration of ~ 10 μM (Chambers, 2001). Moreover, because aminoglycosides do not bind to plasma proteins, the tissue concentration is expected to correspond to the peak plasma concentration. Here we found that in the therapeutically relevant concentration of 10 μM, neomycin selectively blocks P-type Ca^{2+} channels. However, at higher neomycin concentrations (1 mM), contribution of N- and Q-type Ca^{2+} channels to the inhibition of [^{3}H]dopamine release cannot be excluded.

2. Methods

All studies complied with the German Home Office regulations governing the care and use of laboratory animals.

2.1. Drugs

[^{3}H]Dopamine (specific activity of 21.5 Ci/mmol) was from American Radiolabeled Chemicals, St. Louis, MO, USA. Nitrendipine, nomifensine maleate and pargyline hydrochloride were from RBI (Natick, MA, USA). L-Ascorbic acid, ω-agatoxin-IVA, ω-conotoxin-GVIA, ω-conotoxin-MVIIC, neomycin, thapsigargin and veratridine were from Sigma (Deisenhofen, Germany). Ryanodine was from Calbiochem-Novabiochem (Bad Soden, Germany).

2.2. [^{3}H]Dopamine release experiments

Striatal slices were obtained from 2- to 3-month aged male Wistar rats (weight ~ 150–250 g; Charles River, Sulzfeld, Germany). The release experiments were performed as previously described (Dobrev and Andreas, 1997; Dobrev et al., 1998, 1999). In brief, 200-μm-thick striatal slices were prepared and pre-incubated for 20 min in Krebs-phosphate buffer (KPB, in millimolars: NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4 \cdot 7H_2O$ 1.2, $Na_2HPO_4 \cdot 2H_2O$ 15.9, ascorbic acid 0.6 and glucose 5.6; pH = 7.4) at room temperature (22–24 °C). Then, the slices were incubated with 0.1 μM of [^{3}H]dopamine for an additional 30 min at 37 °C. After loading, the slices were placed into a superfusion chamber (37 °C) and were superfused with KPB at a flow rate of 0.6 ml/min. The superfusion KPB contained 10 μM of nomifensine to inhibit [^{3}H]dopamine re-uptake, 10 μM of pargyline to block its metabolism and 1.3 mM of Na_2–EDTA to prevent autooxidation of [^{3}H]dopamine.

After 20 min of washout superfusion, 30 or 40 successive 1-min fractions were collected. The [^{3}H]dopamine release was evoked either once with 25 μM of veratridine (S_1) or twice with 50 mM of KCl at fractions 7 (S_1) and 27 (S_2) (i.e. 27 and 47 min after the onset of superfusion, Fig. 1). At the conclusion of each experiment, the slices were sonicated. The radioactivity (^{3}H) present in the collected fractions and the tissue slices was measured by liquid scintillation spectroscopy.

2.3. Data presentation and statistics

The release of [^{3}H]dopamine was calculated as previously described (Dobrev and Andreas, 1997). In brief, [^{3}H]dopamine release was expressed as a fraction of the total amount of [^{3}H]dopamine present in the slices at the

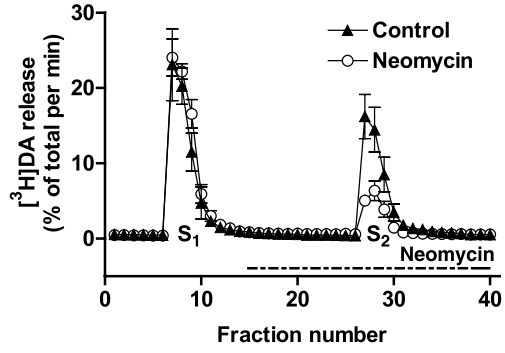

Fig. 1. Effect of neomycin (350 μM) on K^+-evoked [^{3}H]dopamine release. After 20 min of washout period, 1-min fractions of superfuse were collected. [^{3}H]Dopamine release was evoked twice (S_1 and S_2) for 2 min with 50 mM of KCl, neomycin was added to the buffer 12 min before S_2. The S_2/S_1 ratios were 0.79 ± 0.03 (n = 15) for control and 0.20 ± 0.02 (n = 4) for neomycin, respectively (P < 0.05). Each point on the graph represents the average radioactivity collected in each 1-min fraction expressed as a percentage of total radioactivity present in the slices just before the respective collection period with error bars larger than symbols.

onset of the corresponding collection period (Fig. 1). The stimulation-evoked [^{3}H]dopamine release was calculated from the total release during the 2-min stimulation period and the subsequent 9 (in the case of KCl) or 15 min (in the case of veratridine) corrected for basal release during this time. The stimulation-evoked [^{3}H]dopamine release during the first (S_1) and second (S_2) stimulation was then expressed as a percentage of the total radioactivity present in the slices just before the respective stimulation (S_1% and S_2%). To quantify drug effects on [^{3}H]dopamine release evoked by K^+ stimulation, the drugs were added 12 min before S_2 and the obtained S_2/S_1 ratio was expressed as a percentage of the control ratio. In the case of veratridine, S_1 values obtained in the presence of Ca^{2+} channel blockers were expressed as a percentage of those in the control group.

Data are given as means ± S.E.M. for the indicated number of experiments. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple comparisons test by using the computer program SPSS for Windows (version 10.0, München, Germany). $P < 0.05$ was considered statistically significant.

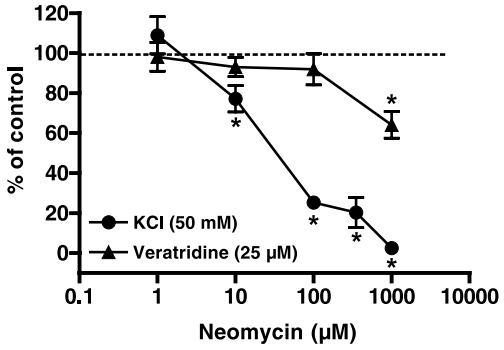

Fig. 2. Concentration–response curves for the inhibition produced by neomycin on K^+- and veratridine-evoked [^{3}H]dopamine release. Neomycin was added 12 min before S_2. Each point represents either mean S_2/S_1 ± S.E.M. for release evoked by 50 mM of K^+ or S_1 values when release was stimulated with 25 μM of veratridine. Data were from four to eight independent experiments for each drug concentration, with error bars larger than symbols. The control S_2/S_1 ratio for K^+-evoked [^{3}H]dopamine release was 0.79 ± 0.03 ($n = 15$) and the control S_1 value for veratridine-evoked [^{3}H]dopamine release was 55.2 ± 0.9% ($n = 9$), respectively. These values are defined as 100%. Statistical analysis was performed on untransformed data (S_2/S_1 or S_1) by one-way ANOVA and Student–Newman–Keuls multiple comparison test. *$P < 0.05$ compared with the respective controls.

3. Results

3.1. Effect of neomycin on K^+-evoked [^{3}H]dopamine release

We have previously demonstrated that K^+-evoked [^{3}H]dopamine release from striatal slices is mediated by N-, P- and Q-type VACCs (Dobrev and Andreas, 1997; Dobrev et al., 1999). Neomycin inhibited this release in a concentration-dependent manner (IC_{50} value of ~ 25 μM), the maximum inhibition being ~ 98% at 1 mM (Figs. 1 and 2).

3.2. Involvement of N- and P-type VACCs in neomycin-induced inhibition of [^{3}H]dopamine release

To examine whether neomycin interacts with N-, P- or Q-type channels to decrease dopamine release, we performed exclusion experiments. First, we investigated the effects of neomycin (10–100 μM) in the presence of P-type channel block. In the presence of 30 nM of ω-agatoxin-IVA, which produces maximum inhibition of P-type channels (Mintz et al., 1992; Wheeler et al., 1994), 10 μM of neomycin did not induce any additional block of evoked release and the inhibition was not statistically different when compared to ω-agatoxin-IVA alone (Fig. 3A). Conversely, in the presence of 100 μM of neomycin, 30 nM of ω-agatoxin-IVA was not able to further reduce K^+-evoked [^{3}H]dopamine release, for example, their effects were not additive (Fig. 3B). To validate the preferential block of P-type VACCs by neomycin, its effects were studied in the presence of N-type Ca^{2+} channel blocker ω-conotoxin-GVIA (Olivera et al., 1994). The effects of the co-application of neomycin (10–100 μM) and 1 μM of ω-conotoxin-GVIA were fully additive (Fig. 3A and B), excluding significant interaction between neomycin and N-type VACCs. Thus, therapeutically relevant concentrations of neomycin appear to inhibit preferentially P-type Ca^{2+} channels.

3.3. Involvement of Q-type VACCs in neomycin-induced inhibition of [^{3}H]dopamine release

Two strategies were used to test for the possible involvement of Q-type Ca^{2+} channels in neomycin-induced inhibition of release, that is, addition of 100 nM of ω-conotoxin-MVIIC to block Q-type Ca^{2+} channels (Wheeler et al., 1994; Olivera et al., 1994) and use of veratridine-evoked [^{3}H]dopamine release which recruits Q-type Ca^{2+} channels only (see above). The effects of the co-application of 100 μM of neomycin and 100 nM of ω-conotoxin-MVIIC on K^+-evoked [^{3}H]dopamine release were additive (Fig. 3B), excluding significant contribution of Q-type Ca^{2+} channels to the effects of low micromolar concentrations of neomycin.

Veratridine-evoked [^{3}H]dopamine release was not affected by neomycin concentrations up to 100 μM and was reduced by ~ 35% only with 1 mM of neomycin (Fig. 2), providing further evidence for a preferential block of P-type Ca^{2+} channels at low micromolar concentrations of neomycin (≤ 100 μM).

3.4. Involvement of intracellular Ca^{2+} stores in neomycin-induced inhibition of [^{3}H]dopamine release

To exclude contribution of intracellular Ca^{2+} stores to neomycin-induced inhibition of [^{3}H]dopamine release, we used thapsigargin to deplete intracellular Ca^{2+} stores by

selective inhibition of the sarcoplasmic reticulum Ca^{2+}-ATPase (Thastrup et al., 1990). In this set of experiments, we used veratridine-induced [^{3}H]dopamine release because veratridine is reported to increase 1,4,5-trisphosphate synthesis in the brain (Myles and Fain, 1994). Thapsigargin (500 nM) had no effect on [^{3}H]dopamine release (Table 1). Next we applied ryanodine to block Ca^{2+}-induced Ca^{2+} release channels (ryanodine receptors, Jenden and Fairhurst, 1969). Incubation of the slices with 30 μM of ryanodine was without effect on [^{3}H]dopamine release (Table 1). Thus, store-operated capacitative Ca^{2+} entry did not contribute significantly to the inhibitory effects of neomycin on [^{3}H]dopamine release.

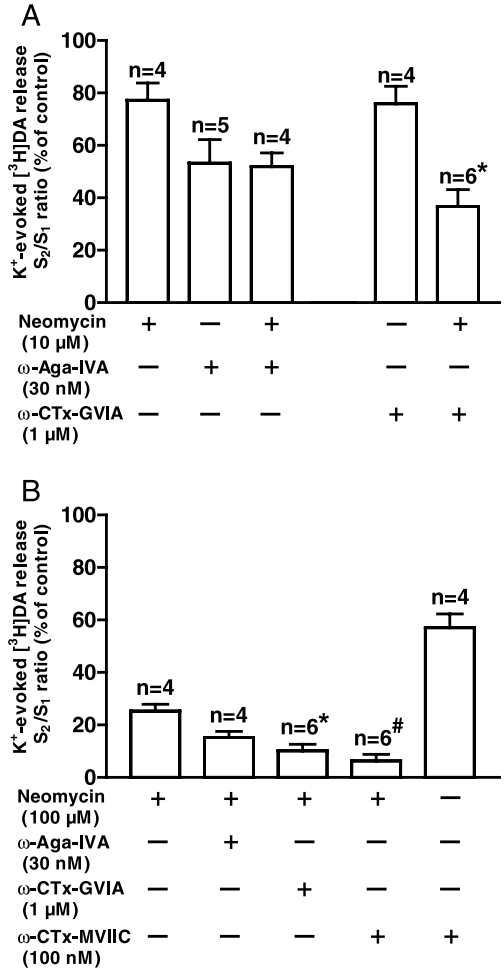

Fig. 3. (A) Inhibitory effects of ω-conotoxin-GVIA (ω-CTx-GVIA, 1 μM) and ω-agatoxin-IVA (ω-Aga-IVA, 30 nM) alone and in combination with 10 μM of neomycin on K^+-evoked [^{3}H]dopamine release. Neomycin and ω-CTx-GVIA were simultaneously applied 12 min before S_2, whereas neomycin and ω-Aga-IVA were applied sequentially. In these experiments, neomycin was added 12 min before S_2; 6 min later, the superfusion buffer was switched to medium containing both neomycin and ω-Aga-IVA. The control S_2/S_1 ratio was 0.80 ± 0.02 (n = 10) and is considered as 100%. Each column represents mean ± S.E.M. (bars) values from n independent experiments. *P < 0.05 compared with ω-CTx-GVIA only. (B) Effect of the combination of 100 μM of neomycin and ω-CTx-GVIA (1 μM), ω-Aga-IVA (30 nM) or ω-conotoxin-MVIIC (ω-CTx-MVIIC, 100 nM) on K^+-evoked [^{3}H]dopamine release. Statistical analysis was performed on untransformed data (S_2/S_1) by one-way ANOVA and Student–Newman–Keuls multiple comparison test. *P < 0.05 compared with neomycin and with ω-CTx-GVIA only (A). $^{\#}P$ < 0.05 compared with neomycin and with ω-CTx-MVIIC only.

Table 1
Effects of thapsigargin and ryanodine on veratridine-evoked [^{3}H]dopamine release from rat striatal slices

Drugs	Concentration (μM)	Percentage of control S_1
Thapsigargin	0.5	100.8 ± 6.2 (n = 4)
Ryanodine	30	105.4 ± 6.3 (n = 4)

Striatal slices were stimulated once with 25 μM of veratridine (S_1) and the release of [^{3}H]dopamine was expressed as a percentage of the total radioactivity present in the slices before the stimulus. Drugs were added 12 min before S_1 (e.g., 6 min before fraction collection, see Methods). Results are mean ± S.E.M. of n experiments. The control S_1 is considered as 100%. Statistical analysis was performed on untransformed data (S_1) by one-way ANOVA (P = N.S.).

4. Discussion

In the present study, clinically relevant concentrations of neomycin preferentially block P-type Ca^{2+} channels. This finding is further supported by the lack of effect of low concentrations of neomycin on veratridine-evoked [^{3}H]dopamine release, a process independent of Ca^{2+} influx through P-type Ca^{2+} channels. However, with high concentrations of neomycin (1 mM), involvement of N- and Q-type Ca^{2+} channels cannot be ruled out.

4.1. Effects of neomycin on K^+- and veratridine-evoked [^{3}H]dopamine release

Neomycin inhibits ω-conotoxin-GVIA-sensitive N-type Ca^{2+} channels (Knaus et al., 1987; Stumpo et al., 1991; Pichler et al., 1996) and blocks ω-conotoxin-GVIA-resistant Ca^{2+} channels in a concentration-dependent manner (Carvalho et al., 1995; Pichler et al., 1996; Elverfors et al., 1997; Toner and Stamford, 1997; Bergquist et al., 1998).

In the present study, neomycin (1–1000 μM) concentration dependently inhibited K^+-evoked [^{3}H]dopamine release, producing maximum inhibition of release of ~ 98% at 1 mM. An IC_{50} of ~ 25 μM reported here is in good agreement with the IC_{50} for neomycin-induced inhibition of "fast phase" Ca^{2+} uptake in brain synaptosomes (~ 25 μM; Atchison et al., 1988) and with the IC_{50} for block of voltage-activated Ca^{2+} currents in rat arterial myocytes (~ 32 μM; Langton et al., 1996). Interestingly, some VACCs of rat arterial vessels have been recently identified to belong to the ω-agatoxin-IVA-sensitive P/Q-type Ca^{2+} channels (Hansen et al., 2000). In other studies, inhibition of transmitter release by neomycin required much higher concentrations. Neomycin inhibited K^+-evoked [^{3}H]noradrenaline release from rat brain slices with IC_{50} values of 500 (Diamant et al., 1987) and 390 μM (Keith et al., 1992). Similarly, neomycin blocked Ca^{2+} uptake in rat synaptosomes with IC_{50} values of 340 (Keith et

al., 1992) and 670 μM (Perrier et al., 1992). The differences have probably arisen because these experiments were performed at different concentrations of free Ca^{2+}. In fact, IC_{50} values for neomycin are larger in the presence of millimolar than of micromolar concentrations of extracellular-free Ca^{2+} (Atchison et al., 1988; Pichler et al., 1996). Thus, in the present study, the strong efficacy of neomycin might be due to the low free Ca^{2+} concentration in the superfusion buffer (calculated free Ca^{2+} levels of ~ 0.6 mM, see Dobrev et al., 1998). However, it should be noted that in humans, normal free Ca^{2+} concentration is 0.9–1.3 mM (Moore, 1970), indicating that the Ca^{2+} channel blocking effects of neomycin may be operative in vivo.

4.2. Involvement of different types of VACCs in neomycin-induced inhibition of [^{3}H]dopamine release

In previous studies in which neomycin concentrations of less than 300 μM were used to block non-L- and non-N-type Ca^{2+} channels in rat striatum (Carvalho et al., 1995; Duarte et al., 1996), the nature of the VACCs was not identified. We and others (Pichler et al., 1996) provide evidence that neomycin interacts selectively with P/Q-type VACCs. In the present study, the combined inhibitory effects of neomycin (10–100 μM) and ω-agatoxin-IVA (30 nM) were not additive (Fig. 3A and B). Because 30 nM of ω-agatoxin-IVA maximally blocks P-type VACCs (Mintz et al., 1992; Wheeler et al., 1994), our findings suggest that low micromolar concentrations of neomycin (10–100 μM) selectively inhibit P-type VACCs. However, because 1 mM of neomycin abolished K^+-evoked [^{3}H]dopamine release, higher concentrations of neomycin may also inhibit N-type VACCs.

The possible role of Q-type Ca^{2+} channels in neomycin-mediated inhibition of K^+-evoked [^{3}H]dopamine release was tested with the selective Q-type Ca^{2+} channel blocker ω-conotoxin-MVIIC (Wheeler et al., 1994). The combined effects of 100 μM of neomycin (mediated by P-type Ca^{2+} channels, see above) and 100 nM of ω-conotoxin-MVIIC (selectively blocking Q-type Ca^{2+} channels, see below) were additive. Thus, Q-type Ca^{2+} channels must have remained unaffected in the presence of this neomycin concentration. Higher concentrations of ω-conotoxin-MVIIC were not used because this toxin loses its selectivity for Q-type Ca^{2+} channels when concentrations exceed 100 nM (Wheeler et al., 1994).

The preferential inhibition of P-type channels by low micromolar concentrations of neomycin ($\leq$ 100 μM) was further supported by employing veratridine-evoked [^{3}H]dopamine release, which is mediated predominantly by Q-type Ca^{2+} channels (Dobrev et al., 1998). Up to 100 μM of neomycin did not affect veratridine-evoked [^{3}H]dopamine release, excluding significant interaction between neomycin and Q-type Ca^{2+} channels at these concentrations. In the presence of 1 mM of neomycin, however, veratridine-evoked [^{3}H]dopamine release was blocked by ~ 35%, suggesting possible interaction with Q-type channels at higher concentrations. Thus, P-type VACCs appear to be the only type of VACCs preferentially inhibited by therapeutically relevant concentrations of neomycin. Finally, it should be considered that dopamine itself is able to inhibit P-type Ca^{2+} channels (Mansvelder et al., 2002), indicating that the reported effects of neomycin on these channels may be underestimated.

4.3. Involvement of intracellular Ca^{2+} release mechanisms in neomycin-induced release inhibition

Modulation of inositol 1,4,5-trisphosphate- and ryanodine-induced intracellular Ca^{2+} release mechanisms may also contribute to neomycin-induced effects (Mack et al., 1992; Sayers and Michelangeli, 1993). Due to its polycationic chemical nature, neomycin does not readily penetrate the intracellular space (Chambers, 2001). However, there is convincing evidence that neomycin can bind to the inositol 1,4,5-trisphosphate precursor inositol 4,5-bisphosphate located in the plasma membrane and thus may block its synthesis without entering the cell (Gabev et al., 1989). Because veratridine is known to increase inositol 1,4,5-trisphosphate synthesis in brain cortical slices (Myles and Fain, 1994), we investigated whether Ca^{2+} release from intracellular stores contributes to veratridine-evoked [^{3}H]dopamine release. Depletion of internal stores with thapsigargin (500 nM) or block of Ca^{2+}-induced Ca^{2+} release with ryanodine (30 μM) had no effect on veratridine-evoked [^{3}H]dopamine release. Furthermore, neomycin (1 mM) did not affect the ionomycin-evoked [^{3}H]D-aspartate release from rat hippocampal slices (Keith et al., 1992) and did not inhibit activity of plasma membrane Ca^{2+}-ATPase and Na^+–Ca^{2+}-exchanger (Lehotsky et al., 1992; Canzoniero et al., 1993). Therefore, it is unlikely that intracellular Ca^{2+} release channels contribute to the effects of neomycin. However, because we did not study the contribution of intracellular Ca^{2+} stores to K^+-evoked [^{3}H]dopamine release, possible involvement of internal Ca^{2+} stores to this release mechanism cannot be fully excluded.

4.4. Clinical implications

The molecular mechanisms of the toxic effects of aminoglycosides are poorly understood (Fischel-Ghodsian, 1999; Chambers, 2001). Here we demonstrate that therapeutically relevant concentrations (10 μM) of neomycin selectively block P-type Ca^{2+} channels. Our findings are of potential clinical importance because P-type VACCs are distributed also outside the nervous system (kidney and arterial vessels, Hansen et al., 2000). Interestingly, mRNA for the α_{1A} subunit encoding the neuronal P/Q-type Ca^{2+} channel has been found in high density in the kidney cortex (Yu et al., 1992), where high concentrations of aminoglycosides accumulate (Davis et al., 1984). Thus, the block of P-type Ca^{2+} channels caused by these drugs may in part

mediate aminoglycoside-induced nephrotoxicity. In addition, blockade of P/Q-type VACCs by aminoglycosides may also contribute to their vestibular toxicity and ototoxicity (Pichler et al., 1996) because neomycin blocks cochlear dopamine release (Gaborjan et al., 2001). Finally, blockade of P-type Ca^{2+} channels may be involved in neuromuscular paralysis associated with aminoglycosides because transmitter release from human motor nerve terminals is mediated mainly by this type of VACCs (Protti et al., 1996).

In conclusion, we provide evidence that therapeutically relevant concentrations of neomycin preferentially block P-type Ca^{2+} channels which regulate dopamine release in the rat striatum. The blockade of P-type VACCs may be one possible mechanism contributing to renal, oto- and neuromuscular toxicity of aminoglycoside antibiotics.

Acknowledgements

The authors thank Ms. Martina Rossmann for her excellent technical assistance in preparing the striatal slices and performing part of the neurotransmitter studies.

References

Atchison, W.D., Adgate, L., Beaman, C.M., 1988. Effects of antibiotics on uptake of calcium into isolated nerve terminals. J. Pharmacol. Exp. Ther. 245, 394–401.

Bergquist, F., Jonason, J., Pileblad, E., Nissbrandt, H., 1998. Effects of local administration of L-, N-, and P/Q-type calcium channel blockers on spontaneous dopamine release in the striatum and the substantia nigra: a microdialysis study in rat. J. Neurochem. 70, 1532–1540.

Canzoniero, L.M., Taglialatela, M., Di Renzo, G., Annunziato, L., 1993. Gadolinium and neomycin block voltage-sensitive Ca^{2+} channels without interfering with the Na^{+}–Ca^{2+} antiporter in brain nerve endings. Eur. J. Pharmacol. 245, 97–103.

Carvalho, C.M., Ferreira, I.L., Duarte, C.B., Malva, J.O., Tretter, L., Adam-Vizi, V., Carvalho, A.P., 1995. Relation of $[Ca^{2+}]_i$ to dopamine release in striatal synaptosomes: role of Ca^{2+} channels. Brain Res. 669, 234–244.

Chambers, H.F., 2001. The aminoglycosides. In: Hardman, J.G., Limbird, L.E., Goodman Gilman, A. (Eds.), The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 1219–1238.

Davis, R.R., Brummett, R.E., Bendrick, T.W., Himes, D.L., 1984. Dissociation of maximum concentration of kanamycin in plasma and perilymph from ototoxic effect. J. Antimicrob. Chemother. 14, 291–302.

Diamant, S., Avraham, B., Atlas, D., 1987. Neomycin inhibits K^{+}- and veratridine-stimulated noradrenaline release in rat brain slices and rat brain synaptosomes. FEBS Lett. 219, 445–450.

Dobrev, D., Andreas, K., 1997. Modulation of potassium-evoked [^{3}H]dopamine release from rat striatal slices by voltage-activated calcium channel ligands: effects of ω-conotoxin-MVIIC. Neurochem. Res. 22, 1085–1093.

Dobrev, D., Milde, A.S., Andreas, K., Ravens, U., 1998. Voltage-activated calcium channels involved in veratridine-evoked [^{3}H]dopamine in rat striatal slices. Neuropharmacology 37, 973–982.

Dobrev, D., Milde, A.S., Andreas, K., Ravens, U., 1999. The effects of verapamil and diltiazem on N-, P- and Q-type calcium channels mediating dopamine release in rat striatum. Br. J. Pharmacol. 127, 576–582.

Duarte, C.B., Cristovao, A.J., Carvalho, A.P., Carvalho, C.M., 1996. Voltage-sensitive Ca^{2+} channels in rat striatal synaptosomes: role on the $[Ca^{2+}]_i$ responses to membrane depolarization. Neurochem. Int. 28, 67–75.

Elverfors, A., Jonason, J., Jonason, G., Nissbrandt, H., 1997. Effects of drugs interfering with sodium channels and calcium channels on the release of endogenous dopamine from superfused substantia nigra slices. Synapse 26, 359–369.

Fischel-Ghodsian, N., 1999. Genetic factors in aminoglycoside toxicity. Ann. N.Y. Acad. Sci. 884, 99–109.

Gabev, E., Kasianowicz, J., Abbott, T., McLaughlin, S., 1989. Binding of neomycin to phosphatidylinositol 4,5-bisphosphate (PIP2). Biochim. Biophys. Acta 979, 105–112.

Gaborjan, A., Halmos, G., Repassy, G., Vizi, E.S., 2001. A new aspect of aminoglycoside ototoxicity: impairment of cochlear dopamine release. NeuroReport 12, 3327–3330.

Hansen, P.B., Jensen, B.L., Andreasen, D., Friis, U.G., Skott, O., 2000. Vascular smooth muscle cells express the $alpha_{1A}$ subunit of a P-/Q-type voltage-dependent Ca^{2+} channel, and it is functionally important in renal afferent arterioles. Circ. Res. 87, 896–902.

Jenden, D.J., Fairhurst, A.S., 1969. The pharmacology of ryanodine. Pharmacol. Rev. 21, 1–25.

Keith, R.A., Mangano, T.J., DeFeo, P.A., Horn, M.B., Salama, A.I., 1992. Actions of neomycin on neuronal L-, N-, and non-L/non-N-type voltage-sensitive calcium channel responses. J. Mol. Neurosci. 3, 147–154.

Knaus, H.G., Striessnig, J., Koza, A., Glossmann, H., 1987. Neurotoxic aminoglycoside antibiotics are potent inhibitors of [^{125}I]-Omega-Conotoxin GVIA binding to guinea-pig cerebral cortex membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 583–586.

Langton, P.D., Farley, R., Everitt, D.E., 1996. Neomycin inhibits K^{+}-induced force and Ca^{2+} channel current in rat arterial smooth muscle. Pflugers Arch. 433, 188–193.

Lehotsky, J., Raeymaekers, L., Casteels, R., 1992. Effects of dimethyl sulfoxide and polycationic neomycin on stimulation of purified plasma membrane Ca^{2+}-pump by negatively charged phospholipids. Gen. Physiol. Biophys. 11, 567–577.

Mack, W.M., Zimanyi, I., Pessah, I.N., 1992. Discrimination of multiple binding sites for antagonists of the calcium release channel complex of skeletal and cardiac sarcoplasmic reticulum. J. Pharmacol. Exp. Ther. 262, 1028–1037.

Mansvelder, H.D., Lodder, J.C., Sons, M.S., Kits, K.S., 2002. Dopamine modulates exocytosis independent of Ca^{2+} entry in melanotropic cells. J. Neurophysiol. 87, 793–801.

Mermelstein, P.G., Foehring, R.C., Tkatch, T., Song, W.J., Baranauskas, G., Surmeier, D.J., 1999. Properties of Q-type calcium channels in neostriatal and cortical neurons are correlated with beta subunit expression. J. Neurosci., 7268–7277.

Mintz, I.M., Adams, M.E., Bean, B.P., 1992. P-type calcium channels in rat central and peripheral neurons. Neuron 9, 85–95.

Moore, E.W., 1970. Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. J. Clin. Invest. 49, 318–334.

Myles, M.E., Fain, J.N., 1994. Carbachol, but not norepinephrine, NMDA, ionomycin, ouabain, or phorbol myristate acetate, increases inositol 1,3,4,5-tetrakisphosphate accumulation in rat brain cortical slices. J. Neurochem. 62, 2333–2339.

Olivera, B.M., Miljanich, G.P., Ramachandran, J., Adams, M.E., 1994. Calcium channel diversity and neurotransmitter release: the ω-conotoxins and ω-agatoxins. Ann. Rev. Biochem. 63, 823–867.

Perrier, M.L., Scatton, B., Benavides, J., 1992. Dihydropyridine- and omega-conotoxin-resistant, neomycin-sensitive calcium channels mediate the depolarization-induced increase in internal calcium levels in cortical slices from immature rat brain. J. Pharmacol. Exp. Ther. 261, 324–330.

Pichler, M., Wang, Z., Grabner-Weiss, C., Reimer, D., Hering, S., Grabner, M., Glossmann, H., Striessnig, J., 1996. Block of P/Q-type calcium channels by therapeutic concentrations of aminoglycoside antibiotics. Biochemistry 35, 14659–14664.

Protti, D.A., Reisin, R., Mackinley, T.A., Uchitel, O.D., 1996. Calcium

channel blockers and transmitter release at the normal human neuromuscular junction. Neurology 46, 1391–1396.

Sayers, L.G., Michelangeli, F., 1993. The inhibition of the inositol 1,4,5-trisphosphate receptor from rat cerebellum by spermine and other polyamines. Biochem. Biophys. Res. Commun. 197, 1203–1208.

Soliakov, L., Wonnacott, S., 1996. Voltage-sensitive Ca^{2+} channels involved in nicotinic receptor-mediated [^{3}H]dopamine release from rat striatal synaptosomes. J. Neurochem. 67, 163–170.

Stumpo, R.J., Pullan, L.M., Salama, A.I., 1991. The inhibition of [^{125}I]omega-conotoxin GVIA binding to neuronal membranes by neomycin may be mediated by a GTP-binding protein. Eur. J. Pharmacol. 206, 155–158.

Thastrup, O., Cullen, P.J., Drobak, B.K., Hanley, M.R., Dawson, A.P., 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. U. S. A. 87, 2466–2470.

Toner, C.C., Stamford, J.A., 1997. Involvement of N- and P/Q- but not L- or T-type voltage-gated calcium channels in ischaemia-induced striatal dopamine release in vitro. Brain Res. 748, 85–92.

Wheeler, D.B., Randall, A., Tsien, R.W., 1994. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. Science 264, 107–111.

Yu, A.S., Hebert, S.C., Brenner, B.M., Lytton, J., 1992. Molecular characterization and nephron distribution of a family of transcripts encoding the pore-forming subunit of Ca^{2+} channels in the kidney. Proc. Natl. Acad. Sci. U. S. A. 89, 10494–10498.