Brazilian Journal of Medical and Biological Research (2002) 35: 395-403
ISSN 0100-879X

Antinociceptive potency of aminoglycoside antibiotics and magnesium chloride: a comparative study on models of phasic and incisional pain in rats

W.A. Prado and E.B. Machado Filho

Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

A close relationship exists between calcium concentration in the central nervous system and nociceptive processing. Aminoglycoside antibiotics and magnesium interact with N- and P/Q-type voltage-operated calcium channels. In the present study we compare the antinociceptive potency of intrathecal administration of aminoglycoside antibiotics and magnesium chloride in the tail-flick test and on incisional pain in rats, taken as models of phasic and persistent post-surgical pain, respectively. The order of potency in the tail-flick test was gentamicin (ED_{50} = 3.34 µg; confidence limits 2.65 and 4.2) > streptomycin (5.68 µg; 3.76 and 8.57) = neomycin (9.22 µg; 6.98 and 12.17) > magnesium (19.49 µg; 11.46 and 33.13). The order of potency to reduce incisional pain was gentamicin (ED_{50} = 2.06 µg; confidence limits 1.46 and 2.9) > streptomycin (47.86 µg; 26.3 and 87.1) = neomycin (83.17 µg; 51.6 and 133.9). The dose-response curves for each test did not deviate significantly from parallelism. We conclude that neomycin and streptomycin are more potent against phasic pain than against persistent pain, whereas gentamicin is equipotent against both types of pain. Magnesium was less potent than the antibiotics and effective in the tail-flick test only.

Correspondence
W.A. Prado
Departamento de Farmacologia
FMRP, USP
Av. Bandeirantes, 3900
14049-900 Ribeirão Preto, SP
Brasil
Fax: +55-16-633-2301
E-mail: wadprado@fmrp.usp.br

Research supported by FAPESP and CNPq. E.B. Machado Filho was the recipient of a FAPESP fellowship (No. 00/02742-4).

Received August 3, 2001
Accepted February 4, 2002

Key words
- Gentamicin
- Neomycin
- Streptomycin
- Magnesium
- Antinociception
- Aminoglycosides
- Pain

Introduction

Calcium ions (Ca^{2+}) play an important role in nociception regulation (for a review, see 1). Elevation of Ca^{2+} concentration in the brain produces hyperalgesia (2,3) and antagonizes opiate analgesia (4,5). In contrast, intracerebral administration of Ca^{2+} chelators causes antinociception (5,6) and potentiates opiate analgesia (3,7,8).

A transient increase in cytosolic Ca^{2+} levels represents a key step for neurotransmitter release (for a review, see 9). Ca^{2+} influx occurs mainly through L-, N-, P/Q-, and T-type voltage-operated calcium channels (VOCC) (for a review, see 1,10). Antagonists of L-type VOCC potentiate opiate analgesia in rodents, but this effect depends on the route of administration and the algesimetric test used (for a review, see 11). Mibefredil (12), a selective T-type VOCC antagonist, does not alter the response of mice in the tail-flick test. Intrathecal administration of ω-agatoxin IVA, a selective an-

tagonist of P/Q-type VOCC, reduces the first phase and blocks the second phase of the rat response to formalin (13). In contrast, N-type VOCC antagonists are analgesic in several models of pain (for a review, see 11). According to Malmberg and Yaksh (13), N-type, and possibly P/Q-type, but not L-type VOCC antagonists have a selective inhibitory effect on nociceptive transmission at the spinal cord level.

Aminoglycoside antibiotics such as streptomycin, gentamicin, kanamycin, neomycin and amikacin act as competitive antagonists of the cellular influx of Ca^{2+} in several biological processes (for a review, see 14). These drugs act as antagonists on N- (15,16) and P/Q-type (17) VOCC. A marked similarity between the effects of aminoglycoside antibiotics and magnesium ions, as well as a prompt antagonism of their effects when the extracellular Ca^{2+} concentration is increased, have been demonstrated earlier in neuromuscular transmission (for a review, see 11), and confirmed in models of artificial membranes, in rodent cardiac and smooth muscles, and isolated mast cells (for a review, see 18). Antinociception following gentamicin has been demonstrated in rodent models of phasic or persistent pain (19-22). Intrathecal neomycin (22), or subcutaneous or intraperitoneal amikacin (23) also produced antinociception in the mouse writhing test.

The present study compares the ED_{50} of intrathecal administration of streptomycin, neomycin, gentamicin, and magnesium chloride needed to change the nociceptive thresholds of rats in models of phasic (tail-flick test) and persistent (incisional) pain.

Material and Methods

Animals

Adult male Wistar rats (220-250 g) were individually housed in cages with water and food available *ad libitum*, under controlled light and temperature conditions (12-h light-dark cycle, with the dark cycle beginning at 7:00 h). All tests were performed in the morning. The study was conducted in accordance with the IASP guidelines on the use of laboratory animals. Each rat was used in only one test and on only one occasion.

Intrathecal catheterization

Each animal was placed in a closed box containing 3% halothane in oxygen for induction of anesthesia. Anesthesia was then maintained via a loose-fitting, cone-shaped mask through which 1.5% halothane in oxygen was passed. With the rat in the prone position, the back was shaved and a 5.8-cm long, 20-gauge Weiss needle was introduced through the skin into the L5-L6 intervertebral space. The correct subarachnoid positioning of the needle was assured by a typical flick of the tail or hind paw. A 9-cm long catheter (PE tubing, OD = 0.4 mm, dead space = 10 µl) was then introduced through the needle to protrude 1.5 cm into the subarachnoid space in a cranial direction. The needle was carefully removed and the catheter was gently fixed to the paravertebral musculature with a cotton thread ligature. Anesthesia was discontinued and the animal allowed to recover. Only animals showing no sign of motor impairment were utilized for further experimentation. Each experiment was then initiated 2 h after these procedures.

Tail-flick test

The animal was placed in a glass tube for about 15 s, with its tail lying across a nichrome wire coil maintained at room temperature (23 ± 2ºC). The coil temperature was then raised by the passage of an electric current which was adjusted to ensure a tail withdrawal reflex within 2.5-3.5 s. A cut-off time of 6 s was established to minimize the probability of skin damage. Animals were tested every 5 min until a stable baseline was ob-

tained over three consecutive trials. Only rats showing a stable baseline latency after six trials were used in the experiments. After these procedures, saline or a calcium antagonist was injected intrathecally and tail-flick latency was measured at 5-min intervals for up to 60 min.

Model of incisional pain

Soon after the procedures for intrathecal catheterization and still under halothane anesthesia, the plantar aspect of the right hind paw of each rat was sterilized with a 10% povidone-iodine solution. A 1-cm longitudinal incision was made with a surgical blade through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel. The plantaris muscle was elevated, but its origin and insertion were left intact. After hemostasis, the skin incision was closed with two 5-0 nylon sutures. After this procedure, anesthesia was discontinued and the animal allowed to recover in its home cage for a period of 2 h. Details of this model have been described elsewhere (24).

Nociceptive testing of post-incisional mechanical threshold

Mechanical threshold was measured with von Frey filaments (Stoelting). Rats were placed in an elevated clear plastic cage with a nylon mesh bottom, which allowed easy access to the paw plantar surface. Before the experiment, the animals remained in the cage for approximately 15 min for behavioral accommodation. The area tested was the mid-plantar right hind paw and the baseline mechanical threshold was determined 2 h after the incision. The paw was touched with one of a series of 15 von Frey filaments with logarithmically incremental stiffness (0.0275 and 75.858 g, lower and upper limit of the test, respectively). Each filament was applied from underneath the nylon mesh floor, through the mesh, vertically to the plantar surface with sufficient force to bend the filament a little. A single trial consisted of six applications of a particular filament, applied once every 3-4 s. Testing was initiated with the 2.041 g filament which is in the middle of the series. A response was defined as a withdrawal of the stimulated paw. In the absence of a response to a particular filament, the next stronger filament was used; in the case of a response, the next weaker filament was applied. The up-down method was used to record the threshold (25). Five minutes later, saline or a calcium antagonist was injected intrathecally and the thresholds were determined shortly after the injection and then at 10-min intervals for up to 40 min.

Drugs

The following drugs were used: gentamicin, neomycin, and streptomycin sulfates, kindly supplied by Fontoura-Wyeth (São Paulo, SP, Brazil), and magnesium chloride from Merck (Rio de Janeiro, RJ, Brazil). All doses refer to the salt. Drugs were diluted in saline (10 µl) and injected intrathecally at a constant rate (1 µl/10 s). The catheter was then flushed with 10 µl of drug-free saline delivered at the same rate.

Data analysis and statistics

The tail-flick latencies were plotted as means ± SEM. The time course of the effects of different drug doses was analyzed by multivariate analysis of variance (MANOVA). The factors analyzed were treatment, time and treatment x time interactions. In the case of significant treatment x time interactions, one-way ANOVA followed by the Duncan test was performed for each time point. The level of significance was set at $P<0.05$.

The mechanical thresholds (in g) are reported as medians and corresponding confidence limits (95%) and are presented in graphs as log of mean ± SEM. The experi-

mental groups were compared by the non-parametric Kruskal-Wallis test and differences between the data at each time point were compared by the Mann-Whitney test. The level of significance was set at $P<0.05$.

ED_{50}, i.e., the dose of drug producing an antinociceptive effect in 50% of the animals in an experimental group, was estimated by the method of Litchfield Jr. and Wilcoxon (26). For calculation, antinociception was arbitrarily considered to occur whenever latency ≥4.0 s (mean baseline tail-flick latency + 7 SD) or mechanical threshold >5.72 g (mean baseline threshold + 7 SD; incisional pain test) was obtained. Dose-response curves were constructed for each drug in each experimental model, and the slope function ratio and corresponding factor for the slope ratio for each curve were calculated by the method of Litchfield Jr. and Wilcoxon (26). The level of significance was also set at $P<0.05$.

Results

The intrathecal administration of increasing doses of gentamicin (Figure 1A), streptomycin (Figure 1B), neomycin (Figure 1C), or magnesium chloride (Figure 1D) produced a slow onset and dose-dependent increase in the latency for tail withdrawal due to noxious heat on the skin. The peak effect was

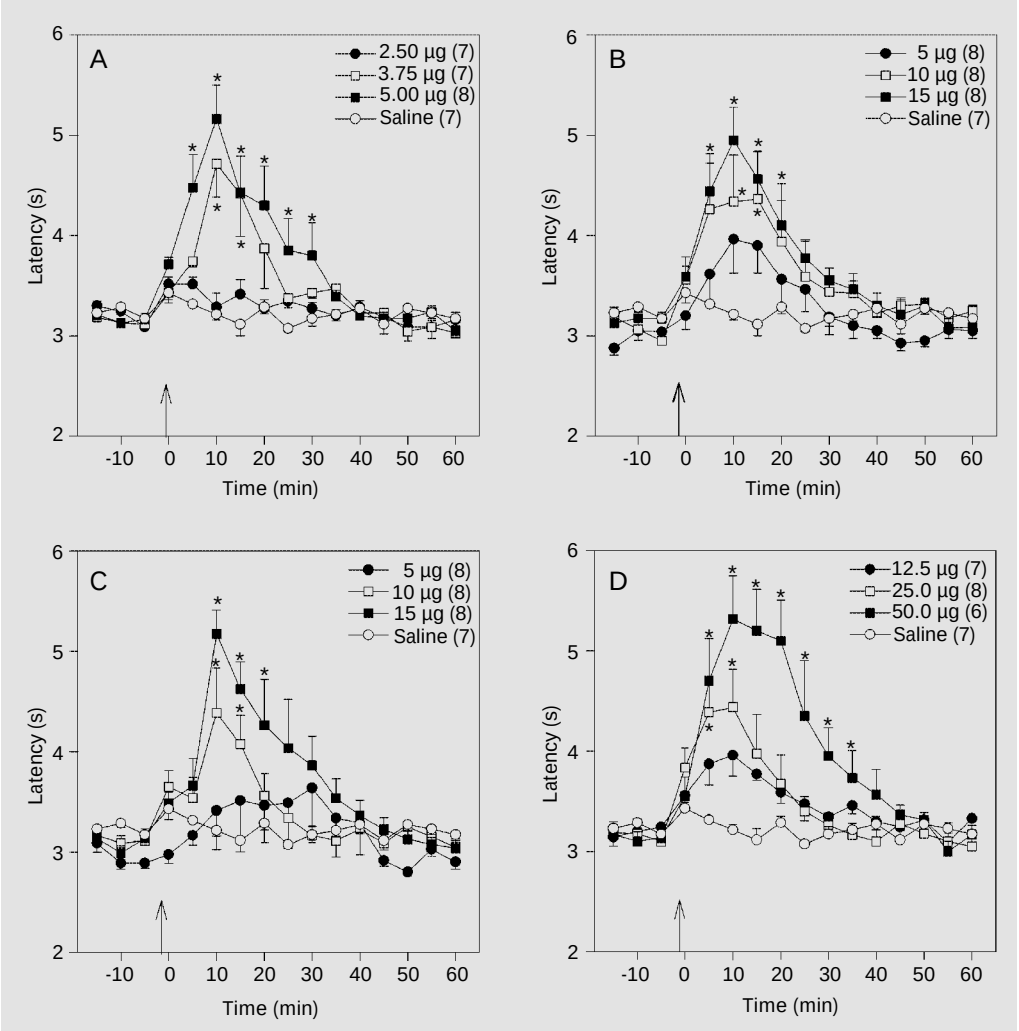

Figure 1. Time course of the effects of intrathecal administration (arrow) of gentamicin (A), streptomycin (B), neomycin (C) and magnesium chloride (D) on latency in the tail-flick reflex of rats. Points are means ± SEM. Doses and number of rats are indicated in the upper right corner. *P<0.05 compared to saline (Duncan test).

observed 10 min after drug administration in all cases. MANOVA applied to the data in Figure 1 revealed significant differences between treatments ($F_{3,25} = 7.7$, $P = 0.001$, in A; $F_{3,27} = 2.98$, $P = 0.049$, in B; $F_{3,27} = 3.02$, $P = 0.04$, in C; $F_{3,24} = 6.44$, $P = 0.002$, in D) and significant time x treatment interaction ($F_{45,375} = 5.21$, $P<0.001$, in A; $F_{45,405} = 2.52$, $P<0.001$, in B; $F_{45,405} = 3.39$, $P<0.001$, in C; $F_{45,360} = 5.39$, $P<0.001$, in D).

The intrathecal administration of increasing doses of gentamicin (Figure 2A), streptomycin (Figure 2B), or neomycin (Figure 2C) reduced incisional allodynia in a dose-dependent manner. The onset of this effect was slower than in the tail-flick test so that peak effects were obtained 20 min after drug administration. The Kruskal-Wallis test revealed significant differences between treatments and the corresponding control (saline) in all cases. The incisional allodynia was not modified significantly by the intrathecal administration of magnesium chloride (50 to 400 µg). For this reason, a time-course curve for the effects of this salt in the model of incisional pain was not constructed. A discrete motor deficit was observed only following the higher dose of magnesium.

The antibiotics and magnesium chloride also dose-dependently increased the percentage of rats showing antinociception in the tail-flick test at 10 min after drug administration (Figure 3A). The ED_{50} (corresponding confidence limits in parentheses) was 3.34 µg (2.65 and 4.2) for gentamicin, 9.22 µg (6.98 and 12.17) for neomycin, 5.68 µg (3.76

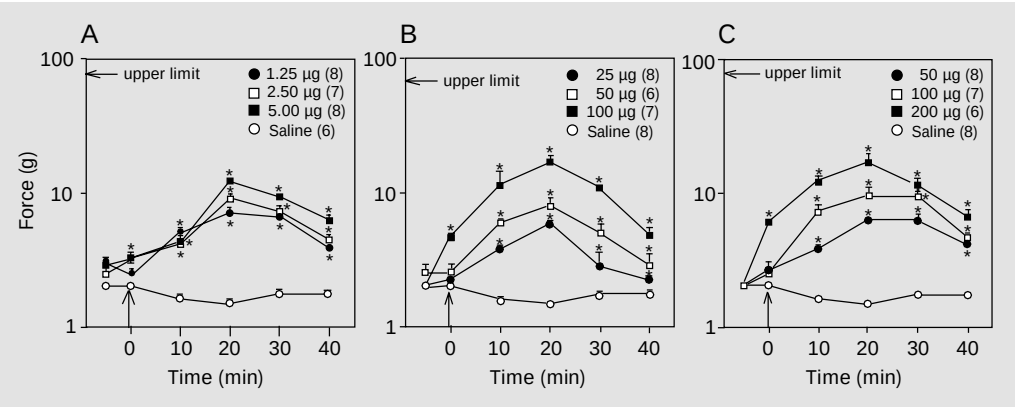

Figure 2. Time course of the effects of intrathecal administration (vertical arrow) of gentamicin (A), streptomycin (B) and neomycin (C) on the incisional paw withdrawal threshold to mechanical stimuli in rats. The upper limit (horizontal arrow) representing the maximum force (75.858 g) used in the test is indicated in the upper left corner. Points are means ± SEM. Doses and number of rats are indicated in the upper right corner. *P<0.05 compared to saline (Mann-Whitney test).

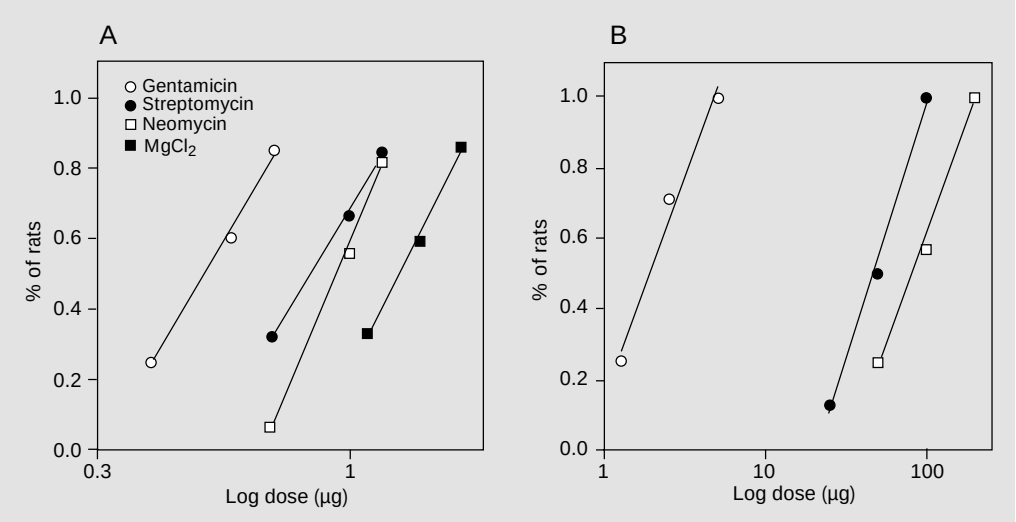

Figure 3. Dose-response regression lines obtained from the experiments in the tail-flick test (A) and incisional pain model (B). Responses are given as percentage of rats showing antinociception recorded 10 and 20 min after drug administration in the tail-flick test and incisional pain model, respectively.

and 8.57) for streptomycin, and 19.49 µg (11.46 and 33.13) for magnesium chloride. The straight lines obtained for gentamicin and neomycin had a smaller slope function ratio (S.R. = 1.1) than the corresponding factor for slope ratio ($f_{S.R.}$ = 1.48), thus indicating that they do not deviate significantly from parallelism. A similar conclusion was reached when comparing gentamicin with streptomycin (S.R. = 1.42, $f_{S.R.}$ = 2.2) or magnesium chloride (S.R. = 1.63, $f_{S.R.}$ = 2.5), when comparing neomycin with streptomycin (S.R. = 1.28, $f_{S.R.}$ = 2.25) or magnesium chloride (S.R. = 1.47, $f_{S.R.}$ = 3.7), or when comparing streptomycin with magnesium chloride (S.R. = 1.14, $f_{S.R.}$ = 3.2). Gentamicin was 2.76 times more potent than neomycin (1.9 and 4.0), 1.7 times more potent than streptomycin (1.06 and 2.72), and 5.83 times more potent than magnesium chloride (3.23 and 10.5). Magnesium chloride was 2.11 times less potent than neomycin (1.14 and 3.9) and 3.43 times less potent than streptomycin (1.75 and 6.7). Streptomycin was more potent than neomycin but the difference was not statistically significant (potency ratio = 1.62; confidence limits 0.98 and 2.67).

The antibiotics also increased in a dose-dependent manner the percent of rats showing antinociception in the model of incisional pain at 20 min after drug administration (Figure 3B). The ED_{50} (confidence limits in parentheses) was 2.06 µg (1.46 and 2.9) for gentamicin, 83.17 µg (51.6 and 133.9) for neomycin, and 47.86 µg (26.3 and 87.1) for streptomycin. The straight lines obtained for gentamicin and neomycin had a smaller slope function ratio (S.R. = 1.13) than the corresponding factor for the slope ratio ($f_{S.R.}$ = 1.95), thus indicating that the lines did not deviate significantly from parallelism. A similar conclusion was reached when comparing gentamicin with streptomycin (S.R. = 1.04, $f_{S.R.}$ = 2.48) or when comparing neomycin with streptomycin (S.R. = 1.1, $f_{S.R.}$ = 3.1). Gentamicin was 40.37 times more potent than neomycin (confidence limits 22.42 and 72.6) and 23.23 times more potent than streptomycin (11.73 and 45.99). Streptomycin was more potent than neomycin but the difference did not reach statistical significance (potency ratio = 1.73; confidence limits 0.8 and 3.72).

Discussion

The present results confirm the antinociceptive effect of spinal administration of gentamicin in the rat tail-flick test and demonstrate that neomycin and streptomycin have similar properties. The study also demonstrates that the antibiotics reduce the mechanical allodynia in a rat model of incisional pain. Incisional pain is characterized by a rapid and long-lasting mechanical allodynia and thermal hyperalgesia that correspond to those obtained during postsurgical states in humans (24). Therefore, the spinal administration of aminoglycoside antibiotics is effective against both phasic thermal and persistent incisional types of pain.

The present study (using lumbar puncture) differs from a previous report (using cisterna magna puncture) on the antinociceptive effects of gentamicin regarding the technique of spinal catheterization (20). In spite of this difference, the ED_{50} for gentamicin obtained on that occasion (3.98 µg; confidence limits 2.55 and 6.2) was within the range of that obtained in the present experiment (3.34 µg; 2.65 and 4.2).

The order of potency calculated here for the antibiotics was gentamicin > neomycin = streptomycin in both models of pain. Magnesium chloride was much less potent than the antibiotics in the tail-flick test and ineffective in the model of incisional pain. Aminoglycoside antibiotics displace the [^{125}I]-ω-conotoxin GVIA, but not tritiated L-type VOCC antagonists from their binding sites in rat brain homogenates (16) or guinea pig brain cortex membranes (15), and inhibit synaptosomal [^{45}Ca] uptake (16). The IC_{50} of the antibiotics for both effects was within

the micromolar range and the order of potency found was neomycin > gentamicin > streptomycin > kanamycin. Also, the antibiotics were all more potent than magnesium (IC_{50} = 3.8 mM) and less potent than ω-conotoxin GVIA (IC_{50} = 21 pM) (15). More recently, Pichler et al. (17) found that nanomolar concentrations of antibiotics were effective in inhibiting the binding of [^{125}I]-ω-conotoxin GVIA to guinea pig cerebellum membranes. In this case, the order of potency was neomycin > kanamycin > gentamicin > streptomycin. A different order of potency (neomycin > kanamycin > streptomycin > gentamicin) was demonstrated at the nanomolar level for concentrations of antibiotics in displacing the binding of [^{125}I]-ω-conotoxin MVIIC, a P/Q-type VOCC antagonist, in guinea pig cerebellum membranes.

The relative potency of aminoglycoside antibiotics as calcium antagonists in smooth muscles and artificial membranes was similar to that obtained in the present study (for a review, see 18). However, a neomycin > gentamicin > kanamycin order was found in the tail-flick and hot-plate tests following intracerebroventricular administration to mice (21). A similar order was obtained for the antibiotics as antagonists of naloxone-precipitated withdrawal in mice acutely dependent on morphine (27), or as antagonists against the calcium-dependent release of histamine from isolated rat mast cells (28). The differences between the potencies of neomycin, gentamicin and streptomycin were very narrow in all cases, probably because *in vitro* models allow better control of the final drug concentration than *in vivo* experiments. The presence of divalent cations in the medium decreases the apparent affinity of antibiotics (17) and may somehow be the reason for the discrepancies.

Another result of the present study was the observation that the ED_{50} for gentamicin in the tail-flick test (3.34 μg; confidence limits 2.65 and 4.2) did not differ from that obtained in the model of incisional pain (2.06 μg; 1.46 and 2.9). In contrast, neomycin and streptomycin were much weaker in reducing the incisional allodynia. Our experiments, however, do not allow us to speculate on the cause(s) for such difference.

Takano et al. (29) have reported that intrathecal magnesium sulfate reduces the second, but not the first phase of the behavioral responses of rats to formalin, and does not change the nociceptive threshold in the rat paw pressure and hot-plate tests. The antinociceptive effect was assumed to be dependent on an inhibitory activity of magnesium ions on NMDA receptors, normally activated in hyperalgesic states. In contrast to the present study, the effectiveness of magnesium sulfate was demonstrated for a dose of 300 μg/rat, but not confirmed for smaller doses. Even very high doses of magnesium chloride were ineffective in reducing the mechanical incisional allodynia, a model of persistent pain in which NMDA receptor activation is expected (30). Therefore, interference with NMDA receptors is unlikely to be the reason for the antinociceptive efficacy of magnesium ions in the tail-flick test, a model of phasic pain in which NMDA receptors are not supposed to be activated.

Aminoglycoside antibiotics act primarily at the presynaptic level to decrease the release of acetylcholine at the neuromuscular junction (31-33), but higher doses may also act postjunctionally, blocking cholinergic receptors or interacting with ionic channels of the cholinergic receptors (34). Therefore, the possibility of higher doses of neomycin or streptomycin acting postsynaptically on the spinal cord to reduce incisional pain cannot be ruled out by the present experiments.

Aminoglycoside antibiotics have long been known to act as inhibitors of phospholipase C (35), but this effect requires very high concentrations of antibiotics and depends on the tissue studied. In the rat kidney proximal tubule brush border membrane,

gentamicin (1.5 mM) inhibits the activity of phospholipase C, an effect independent of the Ca^{2+} concentration but reduced when the pH of the medium is increased (36). The activity of phospholipase C in human amnion tissue may be increased or inhibited by aminoglycoside antibiotics depending on the presence of a high or low Ca^{2+} concentration in the medium, respectively (37). Therefore, aminoglycoside antibiotics acting as inhibitors of phospholipase C are unlikely to be the reason for the antinociceptive effects shown here.

Acknowledgments

We thank Mr. P.R. Castania and Mr. M.A. de Carvalho for valuable technical assistance.

References

1. Venegas H & Schaible HG (2000). Effects of antagonists to high-threshold calcium channels upon spinal mechanisms of pain, hyperalgesia and allodynia. *Pain*, 85: 9-18.
2. Chapman DB & Way EL (1982). Modification of endorphin/enkephalin analgesia and stress-induced analgesia by divalent cations, a cation chelator and an ionophore. *British Journal of Pharmacology*, 75: 389-396.
3. Ben Sreti MM, Gonzalez JP & Sewell RDE (1983). Effects of elevated calcium and calcium antagonists on 6,7-benzomorphan-induced analgesia. *European Journal of Pharmacology*, 90: 385-391.
4. Kakunaga T, Kaneko H & Hano K (1966). Pharmacologic studies on analgesics. VII. Significance of the calcium ion in morphine analgesia. *Journal of Pharmacology and Experimental Therapeutics*, 153: 134-141.
5. Harris RA, Loh HH & Way EL (1975). Effects of divalent cations, cation chelators and an ionophore on morphine analgesia and tolerance. *Journal of Pharmacology and Experimental Therapeutics*, 195: 488-498.
6. Hano K, Kaneto H & Kakunaga T (1964). Significance of calcium ion in the morphine analgesia. *Japanese Journal of Pharmacology*, 14: 227-229.
7. Benedek G & Sziksay M (1984). Potentiation of thermoregulatory and analgesic effects of morphine by calcium antagonists. *Pharmacological Research Communications*, 16: 1009-1018.
8. Hoffmeister F & Tettenborn D (1986). Calcium agonists and antagonists of the dihydropyridine type: antinociceptive effects, interference with opiate-μ-receptor agonists and neuropharmacological actions in rodents. *Psychopharmacology*, 90: 299-307.
9. Zamponi GW & Snutch TP (1998). Modulation of voltage-dependent calcium channels by G proteins. *Current Opinion in Neurobiology*, 8: 351-356.
10. Bertolino M & Llinás RR (1992). The central role of voltage-activated and receptor-operated calcium channels in neuronal cells. *Annual Review of Pharmacology and Toxicology*, 32: 399-421.
11. Prado WA (2001). Involvement of calcium in pain and antinociception. *Brazilian Journal of Medical and Biological Research*, 34: 449-461.
12. Dogrul A, Yesilyurt Ö, Isimer A & Güzeldemir ME (2001). L-type and T-type calcium channel blockade potentiates the analgesic effects of morphine and selective μ opioid agonist, but not to selective δ and κ agonist at the level of the spinal cord in mice. *Pain*, 93: 61-68.
13. Malmberg AB & Yaksh TL (1994). Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception. *Journal of Neuroscience*, 14: 4882-4890.
14. Corrado AP, Pimenta de Morais I & Prado WA (1989). Aminoglycoside antibiotics as a tool for the study of the biological role of calcium ions. *Acta Physiologica et Pharmacologica Latinoamericana*, 39: 419-430.
15. Knauss HG, 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 Archives of Pharmacology*, 336: 583-586.
16. Wagner JA, Olivera BM & Snyder SH (1989). Aminoglycoside effects on voltage-sensitive calcium channels and neurotoxicity. *New England Journal of Medicine*, 317: 1669.
17. Pichler M, Wang ZY, GrabnerWeiss 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.
18. Corrado AP, Prado WA & Pimenta de Morais I (1982). Competitive antagonism between calcium and antibiotics. In: Anghileri LT & Tuffet-Anghileri AM (Editors), *The Role of Calcium in Biological Systems*. CRC Press, Boca Raton, FL, USA, 209-222.
19. Prado WA, Tonussi CR, Rego EM & Corrado AP (1990). Antinociception induced by intraperitoneal injection of gentamicin in rats and mice. *Pain*, 41: 365-371.
20. Rego EM, Corrado AP & Prado WA (1992). Antinociception induced by intracerebroventricular or intrathecal administration of gentamicin in rats. *General Pharmacology*, 23: 481-485.
21. Ocaña M & Baeyens JM (1991). Analgesic effects of centrally administered aminoglycoside antibiotics in mice. *Neuroscience Letters*, 126: 67-70.
22. Dogrul A & Yesilyurt Ö (1998). Effects of intrathecally administered aminoglycoside antibiotics, calcium-channel blockers, nickel and calcium on acetic acid-induced writhing test in mice. *General Pharmacology*, 30: 613-616.
23. Atamer-Simsek S, Ölmez-Salvarli H, Güç O & Eroglu L (2000). Antinociceptive effect of amikacin and its interaction with morphine and naloxone. *Pharmacological Research*, 41: 355-360.
24. Brennan TJ, Vandermeulen EP & Gebhart GF (1996). Characterization of a rat model of incisional pain. *Pain*, 64: 493-501.
25. Chaplan SR, Bach FW, Pogrel JW, Chung JM & Yaksh TL (1994). Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods*, 53: 55-63.

26. Litchfield Jr JT & Wilcoxon F (1949). A simplified method of evaluating dose-effect experiments. *Journal of Pharmacology and Experimental Therapeutics*, 96: 99-113.
27. Robles I, Barrios M & Baeyens JM (1992). Centrally-administered aminoglycoside antibiotics antagonize naloxone-precipitated withdrawal in mice acutely dependent on morphine. *Neuroscience Letters*, 145: 189-192.
28. Wenzel AM, Corrado AP & Prado WA (1985). The rat isolated mast cell as a model for the study of calcium antagonists. *Archives Internationales de Pharmacodynamie et de Therapie*, 275: 259-266.
29. Takano Y, Sato E, Kaneko T & Sato I (2000). Antihyperalgesic effects of intrathecally administered magnesium sulfate in rats. *Pain*, 84: 175-179.
30. Brennan TJ, Umali EF & Zahn PK (1997). Comparison of pre- versus post-incision administration of intrathecal bupivacaine and intrathecal morphine in a rat model of postoperative pain. *Anesthesiology*, 87: 1517-1528.
31. Molgo J, Lemeignan M, Uchiyama T & Lechat P (1979). Inhibitory effect of kanamycin on evoked transmitter release. Reversal by 3,4 diaminopyridine. *European Journal of Pharmacology*, 57: 93-97.
32. Torda T (1980). The nature of gentamicin-induced neuromuscular block. *British Journal of Anaesthesia*, 52: 325-329.
33. Fieckers JF (1983). Effects of aminoglycoside antibiotics, streptomycin and neomycin, on neuromuscular transmission. I. Presynaptic considerations. *Journal of Pharmacology and Experimental Therapeutics*, 225: 487-495.
34. Fieckers JF (1983). Effects of aminoglycoside antibiotics, streptomycin and neomycin, on neuromuscular transmission. II. Postsynaptic considerations. *Journal of Pharmacology and Experimental Therapeutics*, 225: 496-502.
35. Schacht J (1974). Interaction of neomycin with phosphoinositide metabolism in guinea pig inner ear and brain tissues. *Annals of Otology, Rhinology and Laryngology*, 83: 613-618.
36. Schwertz DW, Kreisberg JI & Venkatachalam MA (1984). Effects of aminoglycosides on proximal tubule brush border membrane phosphatidylinositol-specific phospholipase C. *Journal of Pharmacology and Experimental Therapeutics*, 231: 48-55.
37. Segawa N, Bleasdale JE & DiRenzo GC (1983). The effects of polyamines and aminoglycosides on phosphatidylinositol-specific phospholipase C from human amnion. *Biochimica et Biophysica Acta*, 752: 153-161.