Differential antagonism of the negative inotropic effect of gentamicin by calcium ions, Bay K 8644 and isoprenaline in canine ventricular muscle: comparison with cobalt ions

Hiroo Hashimoto, Teruyuki Yanagisawa & ¹Norio Taira

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

1 Gentamicin (10^{-4} – 10^{-2} M) and Co^{2+} (10^{-4} – 10^{-2} M) produced a decrease in developed tension of canine isolated ventricular muscles leading to near abolition at 10^{-2} M. Their negative inotropic effects developed rapidly and wore off shortly after wash-out.

2 The concentration-negative inotropic effect curves for gentamicin were shifted to the right in a parallel manner by increasing external Ca^{2+} , or by the presence of Bay K 8644 (10^{-7} – 10^{-5} M) or isoprenaline (10^{-7} – 10^{-5} M). IC_{50} values for gentamicin increased about 3-fold with about a 6 fold increase in external Ca^{2+} . The Schild plot yielded a pA_2 of 2.29 for Ca^{2+} and its slope was -1.17 ($r = -0.79$).

3 The concentration-negative inotropic effect curves for Co^{2+} were shifted to the right in a parallel manner by increasing external Ca^{2+} , or by the presence of isoprenaline (10^{-7} – 10^{-5} M). IC_{50} values for Co^{2+} increased about 5 fold with about a 6 fold increase in external Ca^{2+} . The Schild plot gave a pA_2 value of 2.60 for Ca^{2+} and its slope was -1.11 ($r = -0.86$).

4 The concentration-positive inotropic effect curves for Ca^{2+} , which were computer-fitted to a logistic equation, gave 2.88×10^{-3} M for EC_{50} . This value was very close to the K_{Ca} calculated from pA_2 values for Ca^{2+} based on antagonism between gentamicin or Co^{2+} and Ca^{2+} (5.13×10^{-3} and 2.51×10^{-3} M).

5 It is concluded that like Co^{2+} , gentamicin molecules compete with Ca^{2+} for the same binding sites presumably located at the outer orifice of Ca-channels in the cardiac sarcolemma.

Introduction

Aminoglycoside antibiotics like streptomycin and neomycin have long been known to produce respiratory depression (by impairing neuromuscular transmission), hypotension (by relaxation of vascular smooth muscle) and myocardial depression. Since these effects were readily reversed by raising extracellular concentrations of Ca^{2+} , interference of aminoglycosides with the function of Ca^{2+} was suggested as the underlying mechanism (Swain *et al.*, 1956; Vital Brazil & Corrado, 1957; Corrado, 1963). The hypothesis has later been refined in such that at least at the prejunctional membrane of the motor

endplate, aminoglycoside molecules compete with Ca^{2+} for binding sites presumably located near the outer orifice of Ca-channels to impede the Ca^{2+} -influx (Vital Brazil & Prado-Franceschi, 1969; Pittinger & Adamson, 1972). In cardiac muscle the negative inotropic effect of gentamicin, an aminoglycoside antibiotic, has also been found to be antagonized by Ca^{2+} apparently in a competitive manner (Adams, 1975). Thus, the mechanism of action of gentamicin may be to affect adversely either the transport system responsible for Ca^{2+} movement through Ca-channels, the availability of Ca^{2+} for transportation to these sites, or both (Adams & Durrett, 1978).

¹ Author for correspondence.

The present experiments were designed to gain evidence for the hypothesis that aminoglycosides produce a negative inotropic effect by competing with Ca^{2+} for the same binding sites at the outer orifice of Ca-channels in the cardiac sarcolemma, as postulated at the motor endplate. For this purpose, gentamicin was chosen, because it has clearly been demonstrated to block the inward Ca^{2+} -current in guinea-pig papillary muscles (Hino *et al.*, 1982). The interaction of gentamicin with procedures that increase trans-sarcolemmal Ca^{2+} influx through different mechanisms (increase in extracellular Ca^{2+} concentrations, Bay K 8644 and isoprenaline) was investigated in canine isolated ventricular muscle. The positive inotropic effect of Bay K 8644 has been ascribed to the increased inward Ca^{2+} -current resulting from the prolonged open state of Ca-channels (Ochi *et al.*, 1984; Hess *et al.*, 1984) and that of isoprenaline to their increased opening probability (Reuter, 1983). For comparison, the antagonism of the negative inotropic effect of Co^{2+} by Ca^{2+} and isoprenaline was also investigated, because Co^{2+} is known to compete with Ca^{2+} for the same binding sites at the outer orifice of Ca-channels (Hagiwara & Byerly, 1981).

Methods

Mongrel dogs of either sex weighing 5 to 15 kg were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} , i.v.) and hearts were excised. Right ventricular trabeculae were dissected from the hearts and mounted in 20 ml organ baths containing Krebs-Henseleit solution or HEPES buffered solution. Krebs-Henseleit solution was gassed with 95% O_2 and 5% CO_2 and HEPES buffered solution with 100% O_2 at a temperature of 37°C . The composition (mm) of the solutions was as follows: (1) Krebs-Henseleit solution: NaCl 118, KCl 4.7, CaCl_2 2.55, MgSO_4 1.18, KH_2PO_4 1.18, NaHCO_3 24.9 and glucose 11.1; (2) HEPES buffered solution: HEPES 3, NaCl 140, KCl 4.7, CaCl_2 2.55, MgCl_2 2.5 and glucose 11.1 (pH 7.4). HEPES buffered solution was used to avoid the precipitation of insoluble salts in the experiments with Co^{2+} or to obtain the maximum developed tension by increasing Ca^{2+} concentrations.

Muscle preparations were stretched to a resting tension of about 500 mg and stimulated by square-wave pulses of a voltage of about 20% above threshold and 5 ms duration at a frequency of 0.5 Hz. Muscles were allowed to equilibrate for 1–1.5 h in Krebs-Henseleit solution and washed once or twice during the equilibration period. The developed

tension was recorded on a thermal pen writing oscillograph (NEC San-ei Instrument, 8K-23) by the use of force displacement transducers (Shinkoh, UL-10). Four to eight muscles were isolated from each heart and run in parallel. One of them always served as control; a cumulative concentration-negative inotropic effect curve for gentamicin was determined at $2.55 \times 10^{-3} \text{ M}$ external Ca^{2+} in the absence of Bay K 8644 or isoprenaline. In the remaining muscles, similar concentration-effect curves were determined at various concentrations of external Ca^{2+} or in the presence of Bay K 8644 or isoprenaline at $2.55 \times 10^{-3} \text{ M}$ external Ca^{2+} . In these muscles gentamicin was administered when the developed tension had reached a plateau after changing external Ca^{2+} concentrations or adding Bay K 8644 or isoprenaline. The time required for the developed tension to reach a plateau was about 20, 60 or 10 min after changing external Ca^{2+} concentrations or adding Bay K 8644 or isoprenaline, respectively. Concentrations of gentamicin were increased at 5 min intervals, because each concentration of gentamicin produced its full effect within 5 min after administration. Experiments with Bay K 8644 were done under a sodium lamp.

Essentially similar experiments were done with Co^{2+} in the place of gentamicin in HEPES buffered solution except that in the equilibration period muscles were bathed in Krebs-Henseleit solution. In experiments in which the concentration of Ca^{2+} that produced a half maximum positive inotropic effect (EC_{50}) was determined, after the equilibration period Krebs-Henseleit solution was replaced by HEPES buffered solution containing $6.4 \times 10^{-4} \text{ M}$ Ca^{2+} . Concentrations of Ca^{2+} were increased cumulatively up to $2.0 \times 10^{-2} \text{ M}$. The maximum developed tension was obtained at $1.0\text{--}1.25 \times 10^{-2} \text{ M}$ Ca^{2+} . In all experiments preparations showing unstable resting tension or less than 100 mg of the developed tension were not used.

The drugs used were gentamicin sulphate (Shionogi), cobalt chloride (Wako), methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay K 8644, Bayer) and (–)-isoprenaline hydrochloride (Sigma). Bay K 8644 was dissolved in 99.5% ethanol at a concentration of $2 \times 10^{-3} \text{ M}$ and this stock solution was diluted with distilled water to the desired concentrations. Other drugs were dissolved in distilled water in the desired concentrations. These drug solutions were added to the organ baths in a volume of 50 or 100 μl .

Experimental values are given in terms of mean $\pm$ s.e. mean or geometric mean with 95% confidence limits in parentheses. For cumulative concentration-negative inotropic effect curves for gentamicin or Co^{2+} , effects were expressed as per-

centage suppression of the developed tension just before administration of this agent, whether positive inotropic intervention was absent (control) or present. The concentrations of gentamicin or Co^{2+} that produced a half maximum negative inotropic effect (IC_{50}) were determined in the following way. Each concentration-negative inotropic effect curve was computer-fitted to a logistic equation:

$$E = 100 \times A^p / (K^p + A^p) \quad (1)$$

where E is an effect of gentamicin or Co^{2+} expressed as above at a concentration A , K is the IC_{50} value of gentamicin or Co^{2+} and p is a slope parameter (Parker & Waud, 1971). Gentamicin failed to produce a 100% suppression of the developed tension at 10^{-2} M which was the maximum concentration obtainable because of its limited solubility, and instead the tension remained about 10%. However, it was assumed that gentamicin would produce a 100% suppression if further higher concentrations were available, because the concentration-negative inotropic curves for gentamicin did not seem saturated at 10^{-2} M . The concentration-ratios (r) of the IC_{50} of gentamicin or Co^{2+} were determined based on the IC_{50} values at $1.25 \times 10^{-3} \text{ M}$ external Ca^{2+} . pA_2 values were calculated from the Schild equation (Arunlakshana & Schild, 1959):

$$\log(r - 1) = \text{pA}_2 - a \times \log B \quad (2)$$

where a is the slope of linear regression and B is the concentration of Ca^{2+} .

The EC_{50} of Ca^{2+} and the slope parameter p of the concentration-positive inotropic effect curves for Ca^{2+} were determined by computer-fitting the curves expressed as percentages of the maximum developed tension to the logistic equation (1), where E is an effect of Ca^{2+} at a concentration A , K is the EC_{50} of Ca^{2+} and p is a slope parameter (Parker & Waud, 1971).

The developed tension increased by 10^{-5} M isoprenaline gradually decayed with time (decreased to 92% of its maximum 30 min after administration). Therefore, the negative inotropic effect of each concentration of gentamicin or Co^{2+} in the presence of 10^{-5} M isoprenaline was expressed as percentage decrease from the averaged developed tension obtained from the muscles ($n = 6$) in the presence of only 10^{-5} M isoprenaline at the corresponding time. Each preparation was subjected to only one concentration of external Ca^{2+} , Bay K 8644 or isoprenaline.

Figure 1 Negative inotropic effect of gentamicin in canine ventricular muscle. Gentamicin was administered cumulatively (10^{-4} – 10^{-2} M). The negative inotropic effect of gentamicin developed rapidly and wore off shortly after wash-out.

Statistical significance between mean values was estimated by Student's t test. Analyses of parallelism of concentration-effect curves were carried out on the slope parameters described above. A P value less than 0.05 was considered significant.

Results

Effect of gentamicin on developed tension

Gentamicin (10^{-4} – 10^{-2} M) produced a concentration-dependent suppression of the developed tension of ventricular muscles amounting to about 90% at 10^{-2} M (Figure 1). Although the effect of gentamicin in further higher concentrations of gentamicin was not obtainable because of its limited solubility, it seemed that gentamicin would produce a 100% suppression of the developed tension if its further higher concentrations were available. The negative inotropic effect of gentamicin emerged rapidly to reach a peak within 5 min after administration, and wore off rapidly with wash-out.

Negative inotropic effect of gentamicin in the presence of various concentrations of external Ca^{2+}

A reduction of external Ca^{2+} from 2.55×10^{-3} to $1.25 \times 10^{-3} \text{ M}$ resulted in a decrease in developed tension to nearly half the basal value, whereas elevations of external Ca^{2+} (to 5.05×10^{-3} and $7.55 \times 10^{-3} \text{ M}$) increased developed tension; the increase was about 135% of the basal value at $7.55 \times 10^{-3} \text{ M}$ Ca^{2+} . The concentration-negative inotropic effect curves for gentamicin were shifted to the right in a concentration-dependent and parallel manner by increases in external Ca^{2+} (Figure 2). About a 6 fold increase in external Ca^{2+} resulted in about a 3 fold increase in the IC_{50} of gentamicin (Table 1). The Schild plot yielded the regression line within a slope of -1.17 ($r = -0.79$) and pA_2 value of 2.29 for Ca^{2+} (Figure 2, inset).

Figure 2 Concentration-negative inotropic effect curves for gentamicin in the Krebs-Henseleit solution containing 1.25 (○) ($n = 6$), 2.55 (●) ($n = 6$), 5.05 (□) ($n = 6$) and 7.55 (■) ($n = 6$) $\times 10^{-3}$ M Ca^{2+} . Effects are expressed as percentage suppression of the developed tension just before administration of gentamicin. Inset: Schild plot showing the competitive antagonism of the negative inotropic effect of gentamicin by Ca^{2+} . The pA_2 value was 2.29 and the slope of the regression line was -1.17 . The correlation coefficient was -0.79 .

Negative inotropic effect of gentamicin in the presence of Bay K 8644

As reported previously (Ishii *et al.*, 1985), Bay K 8644 (10^{-7} – 10^{-5} M) produced a concentration-dependent increase in developed tension which was about 100% of the basal value at 10^{-5} M. The concentration-negative inotropic effect curves for gentamicin in the presence of Bay K 8644 are shown in Figure 3. The curves for gentamicin were shifted by these concentrations of Bay K 8644 to the right in a concentration-dependent manner to a comparable extent with changing external Ca^{2+} concentrations. These curves were parallel except for that in the presence of 10^{-5} M Bay K 8644. As shown in Table 1, the IC_{50} values of gentamicin were increased about 3 fold by 10^{-5} M Bay K 8644.

Negative inotropic effect of gentamicin in the presence of isoprenaline

Isoprenaline (10^{-7} – 10^{-5} M) produced an increase in developed tension and at 10^{-5} M the developed tension nearly doubled. In the presence of isoprenaline the concentration-effect curves for gentamicin

Table 1 IC_{50} values of gentamicin and Co^{2+} in the absence (control) and in the presence of various concentrations of external Ca^{2+} , Bay K 8644 or isoprenaline

			Developed tension		Mean IC ₅₀ (10 ⁻³ M) (Confidence limits)
			Basal (mg)	Increase (%)	
Gentamicin					
Ca ²⁺ (10 ⁻³ M)	1.25	6	888 ± 223	-53.0 ± 3.7	1.86 (1.49-2.33)
	2.55	6	809 ± 161		2.88** (2.41-3.45)
	5.05	6	482 ± 159	53.5 ± 13.5	3.80** (3.17-4.55)
	7.55	6	419 ± 54	134.8 ± 40.8	5.50** (4.39-6.89)
Control		6	844 ± 143		2.51 (2.00-3.15)
Bay K 8644 (M)	10 ⁻⁷	7	627 ± 168	33.5 ± 7.5	3.55 (2.59-4.87)
	10 ⁻⁶	6	164 ± 9	91.1 ± 25.0	5.89** (3.92-8.84)
	10 ⁻⁵	6	346 ± 67	95.1 ± 30.1	8.13* (3.77-17.51)
Control		5	1008 ± 283		2.63 (2.10-3.30)
Isoprenaline (M)	10 ⁻⁷	6	1030 ± 175	53.6 ± 11.3	4.27 (3.25-5.59)
	10 ⁻⁶	5	1552 ± 199	65.3 ± 33.3	5.25* (4.19-6.58)
	10 ⁻⁵	6	748 ± 180	92.1 ± 19.3	6.46* (4.71-8.86)
Co²⁺					
Ca ²⁺ (10 ⁻³ M)	1.25	7	495 ± 95	-54.9 ± 5.3	0.63 (0.53-0.76)
	2.55	5	371 ± 83		1.29** (0.94-1.77)
	5.05	5	485 ± 137	47.0 ± 10.9	2.19** (1.67-2.87)
	7.55	5	238 ± 38	81.6 ± 12.0	3.24** (2.83-3.71)
Control		5	964 ± 393		1.32 (1.26-1.38)
Isoprenaline (M)	10 ⁻⁷	5	522 ± 129	48.5 ± 14.7	1.20 (1.00-1.44)
	10 ⁻⁶	5	1463 ± 355	66.2 ± 9.6	2.24* (1.49-3.36)
	10 ⁻⁵	5	590 ± 144	124.0 ± 17.1	2.00** (1.83-2.18)

* $P < 0.05$; ** $P < 0.01$ against IC_{50} values in the presence of 1.25×10^{-3} M Ca^{2+} or in the control conditions.

Figure 3 Concentration-negative inotropic effect curves for gentamicin in the absence (○) ($n = 6$) and presence of Bay K 8644 at 10^{-7} M (●) ($n = 7$), 10^{-6} M (□) ($n = 6$) or 10^{-5} M (■) ($n = 6$).

were shifted to the right in a concentration-dependent and parallel manner to a comparable extent with increasing external Ca^{2+} concentrations or Bay K 8644 (Figure 4). The IC_{50} values of gentamicin were increased about 2.5 fold by 10^{-5} M isoprenaline (Table 1).

Negative inotropic effect of Co^{2+} in the presence of different concentrations of external Ca^{2+}

In the presence of the normal concentration of external Ca^{2+} (2.55×10^{-3} M) Co^{2+} (10^{-4} – 10^{-2} M) pro-

Figure 4 Concentration-negative inotropic effect curves for gentamicin in the absence (○) ($n = 5$) and presence of isoprenaline at 10^{-7} M (●) ($n = 6$), 10^{-6} M (□) ($n = 5$) or 10^{-5} M (■) ($n = 6$).

duced a concentration-dependent negative inotropic effect and at 10^{-2} M the developed tension was nearly abolished. The onset and wearing off with wash-out of the negative inotropic effect of Co^{2+} were rapid as were those of gentamicin. With a reduction of external Ca^{2+} from 2.55×10^{-3} to 1.25×10^{-3} M the concentration-negative inotropic effect curve for Co^{2+} was shifted to the left, and with elevations of external Ca^{2+} to 7.55×10^{-3} M the concentration-effect curves for Co^{2+} were shifted to the right in a concentration-dependent and parallel manner (Figure 5). The IC_{50} values of Co^{2+} increased about 5 fold with an approximately 6 fold increase in external Ca^{2+} (Table 1). The Schild plot yielded the regression line with a slope of -1.11 ($r = -0.86$) and gave a pA_2 value of 2.60 for Ca^{2+} (Figure 5, inset).

Negative inotropic effect of Co^{2+} in the presence of isoprenaline

In the presence of isoprenaline (10^{-7} – 10^{-5} M) the concentration-effect curves for Co^{2+} were shifted to the right in a concentration-dependent and parallel manner to a similar extent to the shift of the concentration-effect curves for gentamicin. The IC_{50} values of Co^{2+} were increased about 1.5 fold by 10^{-5} M isoprenaline (Table 1).

Concentration of Ca^{2+} that produced a half maximum developed tension

The concentration-positive inotropic effect curves for external Ca^{2+} were obtained in HEPES buffered solution by increasing concentrations of Ca^{2+} from 6.4×10^{-4} to 1.25×10^{-2} M. The maximum developed tension obtained was $3,276 \pm 439$ mg ($n = 8$). Computer-fitting of each concentration-effect curve to the logistic equation (1) yielded 1.89 ± 0.10 as the slope parameter and gave 2.88 (2.51 – 3.31) $\times 10^{-3}$ M for the EC_{50} .

Discussion

In the present experiments gentamicin produced a negative inotropic effect with rapid onset and wearing off upon wash-out in canine ventricular muscle, as has been observed in rat atria (Adams, 1975). Co^{2+} produced a similar negative inotropic effect. In the presence of increasing external Ca^{2+} , the concentration-negative inotropic effect curves for gentamicin were shifted to the right in a parallel

Figure 5 Concentration-negative inotropic effect curves for Co^{2+} in the HEPES buffered solution containing 1.25 ($\circ$) ($n = 7$), 2.55 ($\bullet$) ($n = 5$), 5.05 ($\square$) ($n = 5$) and 7.55 ($\blacksquare$) ($n = 5$) $\times 10^{-3}$ M Ca^{2+} . Inset: Schild plot showing the competitive antagonism of negative inotropic effect of Co^{2+} by Ca^{2+} . The pA_2 value was 2.60 and the slope of the regression line was -1.11 . The correlation coefficient was -0.86 .

manner. The Schild plot of antagonism of the negative inotropic effect of gentamicin by Ca^{2+} yielded unity as the absolute value for the slope of the regression line. This antagonism was very similar to that seen with Co^{2+} and Ca^{2+} ; the concentration-negative inotropic effect curves for Co^{2+} were shifted to the right in a parallel manner by increasing external Ca^{2+} and the Schild plot gave unity as the absolute value of the slope of the regression line. Co^{2+} has been known to compete with Ca^{2+} for the same binding sites at the outer orifice of Ca-channels in excitable membrane (Hagiwara & Byerly, 1981). Thus, like Co^{2+} , gentamicin molecules as polyvalent cations may compete with Ca^{2+} for the same binding sites at the outer orifice of Ca-channels in the cardiac sarcolemma. This supposition is in line with that proposed at the prejunctional membrane in the motor endplate (Vital Brazil & Prado-Franceschi, 1969; Pittinger & Adamson, 1972). However, the present experiments give no direct evi-

dence that gentamicin and Co^{2+} are competing for the same site with Ca^{2+} and some other form of interaction remains a possibility.

The Schild plot of the antagonism of the negative inotropic effect of gentamicin by Ca^{2+} gave a pA_2 value of 2.29 for Ca^{2+} . The Schild plot of the antagonism of the negative inotropic effect of Co^{2+} also yielded a pA_2 value of 2.60 for Ca^{2+} . The close proximity of two pA_2 values for Ca^{2+} determined from antagonism against the different agonists (difference between two pA_2 values was less than 0.5) suggests that Ca^{2+} , gentamicin and Co^{2+} bind to the same type of binding sites (Furchgott, 1972). The pA_2 values for Ca^{2+} yielded 5.13×10^{-3} and 2.51×10^{-3} M for dissociation constants, K_{Ca} , between Ca^{2+} and its binding sites. The EC_{50} value for Ca^{2+} was 2.88×10^{-3} M. The close proximity of K_{Ca} and the EC_{50} value for Ca^{2+} suggests that when a half population of Ca^{2+} binding sites presumably located at the outer orifice of Ca-channels is occupied by Ca^{2+} , the Ca^{2+} -influx through Ca-channels occurring as a result would be enough to produce a half maximum positive inotropic effect.

The negative inotropic effect of gentamicin or Co^{2+} was far less efficiently antagonized by Bay K 8644 or isoprenaline than by Ca^{2+} , a 100 fold increase in concentration of Bay K 8644 or isoprenaline being required for about a 2 fold increase in the IC_{50} of gentamicin or Co^{2+} . This is understandable because it is unlikely that competition of gentamicin or Co^{2+} with Ca^{2+} for the same binding sites at the outer orifice of Ca-channels is influenced by whether the open state of Ca-channels is prolonged by Bay K 8644 (Ochi *et al.*, 1984; Hess *et al.*, 1984) or the opening probability of Ca-channels is increased by isoprenaline (Reuter, 1983).

This study was supported by Research Grant for Cardiovascular Disease (61-A-1) from the Ministry of Health and Welfare, Japan. The authors are grateful to Shionogi Pharmaceutical Co., Ltd., Osaka, Japan for a gift of gentamicin and Prof. Dr med. F. Hoffmeister, Bayer AG, Wuppertal, F.R.G. for Bay K 8644.

References

- ADAMS, H.R. (1975). Direct myocardial depressant effects of gentamicin. *Eur. J. Pharmacol.*, **30**, 272-279.
- ADAMS, H.R. & DURRETT, L.R. (1978). Gentamicin blockade of slow Ca^{++} channels in atrial myocardium of guinea pigs. *J. Clin. Invest.*, **80**, 241-247.
- ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48-58.
- CORRADO, A.P. (1963). Respiratory depression due to antibiotics. Calcium in treatment. *Anesth. Analg.*, **42**, 1-5.
- FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamines. Handb. Exp. Pharmacol.*, Vol. 33. ed. Blaschko, H. & Muscholl, E. pp. 283-335. Berlin & Heidelberg: Springer Verlag.

- HAGIWARA, S. & BYERLY, L. (1981). Calcium channel. *Ann. Rev. Neurosci.*, **4**, 69–125.
- HESS, P., LANSMAN, J.B. & TSIEN, R.W. (1984). Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature*, **311**, 538–544.
- HINO, N., OCHI, R. & YANAGISAWA, T. (1982). Inhibition of the slow inward current and the time-dependent outward current of mammalian ventricular muscle by gentamicin. *Pflügers Arch.*, **394**, 243–249.
- ISHII, K., TAIRA, N. & YANAGISAWA, T. (1985). Differential antagonism by Bay K 8644, a dihydropyridine calcium agonist, of the negative inotropic effects of nifedipine, verapamil, diltiazem and manganese ions in canine ventricular muscle. *Br. J. Pharmacol.*, **84**, 577–584.
- OCHI, R., HINO, N. & NIIMI, Y. (1984). Prolongation of calcium channel open time by the dihydropyridine derivative BAY K 8644 in cardiac myocytes. *Proc. Jpn. Acad.*, **60** (B), 153–156.
- PARKER, R.B. & WAUD, D.R. (1971). Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J. Pharmacol. Exp. Ther.*, **177**, 1–12.
- PITTINGER, C. & ADAMSON, R. (1972). Antibiotic blockade of neuromuscular function. *Ann. Rev. Pharmacol.*, **12**, 169–184.
- REUTER, H. (1983). Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature*, **301**, 569–574.
- SWAIN, H.H., KIPLINGER, G.F. & BRODY, T.M. (1956). Actions of certain antibiotics on the isolated dog heart. *J. Pharmacol. Exp. Ther.*, **117**, 151–159.
- VITAL BRAZIL, O. & CORRADO, A.P. (1957). The curariform action of streptomycin. *J. Pharmacol. Exp. Ther.*, **120**, 452–459.
- VITAL BRAZIL, O. & PRADO-FRANCESCHI, J. (1969). The nature of neuromuscular block produced by neomycin and gentamicin. *Arch. Int. Pharmacodyn.*, **179**, 78–85.

(Received January 12, 1988

Revised November 21, 1988

Accepted December 7, 1988)