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Effects of aminoglycoside antibiotics on bound calcium and contraction in guinea-pig atria

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1 We studied the effect of three aminoglycoside antiobiotics which have been shown to replace Ca from lipid monolayers on the superficially bound Ca of isolated beating left atria from guinea-pigs.

2 The cellular Ca content was determined by means of ^{45}Ca after having attained complete exchange.

3 The antibiotics dibekacin, sisomicin, and gentamicin, all reduced the cellular Ca content by 10–20% in a dose-dependent manner. The loss of superficially bound Ca was accompanied by a decline of the contractile force by 40–90%.

4 It is concluded that in isolated atrial muscle it is the amount of Ca bound to the outer surface of the cardiac plasmalemma, rather than the extracellular Ca^{2+} concentration, that determines contraction height.

Introduction

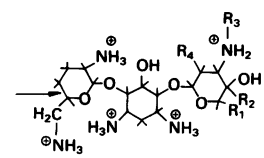
Aminoglycoside antibiotics are polybasic highly hydrophilic compounds (see formula Figure 1). According to these properties they do not penetrate membranes (Tulkens & Trouet, 1978) but interfere with the binding of calcium to anionic sites. This has been demonstrated by both Lohdi *et al.* (1980) and Lüllmann & Vollmer (1982) for phospholipid layers and by Lüllmann & Vollmer (1982) for isolated biomembranes. In both cases the molar concentrations of aminoglycoside antibiotics required to displace calcium were of the same order of magnitude as the Ca^{2+}-concentrations.

In the present study we have investigated whether aminoglycoside antibiotics also displace Ca from intact cardiac muscle tissue and if so, whether this affects the function of the muscle. Preliminary results have been previously communicated in short form elsewhere (Lüllmann & Schwarz, 1985).

Methods

The experiments were performed using isolated, electrically driven left atria of guinea-pigs. The tissue Ca content was determined by means of ^{45}Ca. In the case of cardiac tissue this is possible since the entire cellular Ca content exchanges in the course of 30 to 60 min thus approaching the same specific activity (ratio ^{45}Ca/Ca) as present in the medium. When this state is attained, the determination of the muscular ^{45}Ca content is a measure of its Ca content.

Guinea-pigs weighing 300–350 g were killed by a blow on the neck, the hearts were quickly removed and the left atria dissected. For recording the contractions single left atria were fixed between two stimulation electrodes (platinum wires) and connected to a strain gauge. The contractions were continuously recorded. The atria were stimulated by square impulses of 2 ms duration and by double threshold voltage at a frequency of 3 Hz.



	R_1	R_2	R_3	R_4	
Gentamicin	—H	—CH_3	—CH_3	—OH	
Dibekacin	—CH_2OH	—H	—H	—OH	
Sisomicin	—H	—CH_3	—CH_3	—H,	arrow indicates double bound in the sisomicin molecule

Figure 1 Chemical formulae of gentamicin, dibekacin and sisomicin demonstrating their polybasic and hydrophilic nature.

For determination of the cellular Ca content, the isolated left atria were suspended between two horizontal platinum electrodes 12 cm in length, fitting into baths which contained 500 ml of Tyrode solution.

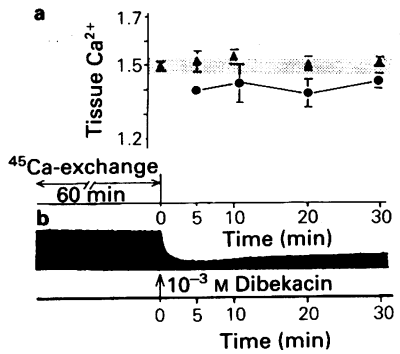


Figure 2 Effect of dibekacin 10^{-3} M on (a) the Ca content (▲, control; ●, dibekacin-treated) and (b) the contractile force of guinea-pig isolated left atria. (a) the Ca content was determined by ^{45}Ca after having attained a complete exchange within 60 min and is expressed as μmol Ca g^{-1} wet wt. The stippled horizontal bar indicates the mean ± s.e.mean of all controls. The symbols are means and vertical lines s.e.means of 6–8 atria. The decrease of the tissue Ca content induced by dibekacin is significant at the 0.05 level. (b) A typical trace demonstrating the reduction of the contractile force induced by dibekacin 10^{-3} M.

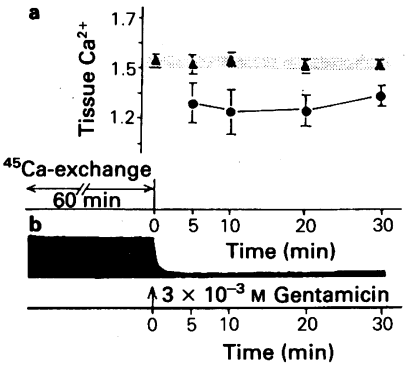


Figure 3 Effect of gentamicin C_{1a} 3×10^{-3} M on (a) the Ca content (▲, control; ●, gentamicin-treated) and (b) the contractile force of guinea-pig isolated left atria. For details see legend to Figure 2.

Each bath could simultaneously take 12–16 preparations which were stimulated as described above for single atria.

The Tyrode solution had the following composition (in mM): NaCl 137, KCl 2.7, $MgCl_2$ 1.1, $NaHPO_4$ 0.2, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, glucose 5.5. The temperature was kept at 32°C. The solution was gassed by 95% O_2 and 5% CO_2 resulting in a pH of 7.4.

To determine the Ca tissue content the following protocol was used. After an equilibration period of 30 min the regularly beating atria were exposed to ^{45}Ca added in trace amounts to the Tyrode solution. In atria beating at a frequency of 3 Hz the exchange process is completed within about 30 min. To ensure total exchange, the exchange period was prolonged to 60 min. As soon as the exchange process is completed the determination of ^{45}Ca is equivalent to a determination of the total tissue Ca content including the extracellular space. At the end of the exchange period the aminoglycoside antibiotics were added, and the atria removed after different times of incubation. The atria were gently blotted and dissolved in 2 ml Soluene-350 at 42°C for 6 h. Using the scintillation fluid Dimilume-30, the radioactivity was counted by a liquid-scintillation counter (Packard I Tricarb 460 C). The tissue Ca content was expressed as ± μmol g^{-1} wet wt. by converting the ^{45}Ca counts into Ca values according to the specific activity applied. The cellular Ca content is given as ± μmol Ca g^{-1} cells, having assumed an extracellular space of 0.3 ml per 1 g of tissue (Goodford & Lüllmann, 1962; Lüllmann & van Zwieten, 1967).

The following aminoglycoside antibiotics were used as sulphate salts: dibekacin, gentamicin and sisomicin. To check whether the sulphate concentrations resulting would have any effect on the atrial tissue Ca content and the contractile force, control experiments were performed using sodium sulphate in equivalent concentrations. Up to 3×10^{-3} M Na_2SO_4 had no detectable effects.

Results

The tissue Ca content at the end of the exchange period amounted to about 1.5 μmol g^{-1} wet wt. which is in accordance with results obtained by chemical methods (Haacke *et al.*, 1970). The tissue Ca content remained constant over the experimental period of 30 min as demonstrated by data for the control muscles depicted in Figures 2 and 3.

The aminoglycoside antibiotics studied reduced the contractile force of beating atria in a dose-dependent manner. To reduce the contractile force by 50% a concentration of about 0.3×10^{-3} M was required. It should be noted that raising the extracellular Ca^{2+} concentration improved the depressed contractile force immediately. Figure 2 illustrates the results of an experiment in which dibekacin 10^{-3} M depressed the contractile force by about 60%. The onset of action was fast, and the new equilibrium was attained in 2 to 3 min. The tissue Ca content was determined in atria incubated with the antibiotics for 5, 10, 20 and 30 min. After only a 5 min incubation the tissue Ca content was found to be lower than that of the control muscles. Similar results were obtained in muscles for 10 and

Table 1 The effect of aminoglycoside antibiotics on the contractile force of guinea-pig left atria and their tissue Ca content

Aminoglycoside antibiotic	*Conc.* (mM)	*% decrease contractile force*	*% reduction of tissue Ca content*	*% reduction of cellular Ca content*
Dibekacin	0.3	42	6	10
Dibekacin	1.0	64	8	12
Gentamicin	1.0	68	9	15
Gentamicin	3.0	85	12	20
Sisomicin	3.0	87	14	22

*Calculated assuming that the extracellular space is 0.3 ml per 1 g of tissue.
The values were obtained after 5–10 min incubation with each antibiotic.

20 min. After a 30 min incubation with dibekacin the tissue Ca content seemed to have recovered slightly, which is also reflected in the mechanical trace. The loss of tissue Ca induced by 10^{-3} M dibekacin amounted to about 0.1 μmol g^{-1} wet wt. within 5–10 min. When the cellular Ca content was calculated, this loss of tissue Ca corresponded to about 12% of cellular Ca.

An example of the effects of a higher concentration of antibiotics e.g. 3×10^{-3} M, is given in Figure 3. Gentamicin reduced the contractile force by about 85%, the onset of action being rapid. In addition the loss of tissue Ca was more pronounced than at the lower drug concentration, the decrease being from 1.52 μmol g^{-1} wet wt. in the controls to 1.33 μmol g^{-1} wet wt. in treated tissue. This corresponds to a loss of about 20% of cellular Ca. Again there was a slight recovery during continued exposure to gentamicin.

The results obtained with the three aminoglycosides antibiotics are summarized in Table 1. As can be seen, dibekacin, gentamicin, and sisomicin are similarly effective in reducing the contractile force of beating atria and in decreasing the tissue Ca content. A loss of about 20% of cellular Ca corresponded to a reduction of the contractile force by more than 80%.

Discussion

The only cellular structure of cardiac muscles which is exposed to the high extracellular Ca^{2+} concentration is the outer leaflet of the plasmalemma. All other structures are in contact with the much lower cytosolic Ca^{2+} concentration. The aminoglycoside antibiotics provide a unique opportunity to interfere selectively with the superficially bound Ca, and to investigate the consequences induced by a displacement of this Ca, since these organic compounds become neither incorporated into nor do they penetrate through lipid membranes. As shown by the results, dibekacin, gentamicin and sisomicin decreased the cellular Ca content in a dose-dependent manner. The highest concentrations investigated, i.e., 3×10^{-3} M reduced the cellular Ca content by about 20% , which suggests that a considerable fraction of the cellular Ca is located at the outer surface of the cells. This fraction is thought to be in equilibrium with the actual extracellular Ca^{2+} concentration.

Similar results have been obtained by Langer (1984), who used polymyxin B as a tool for interfering with the Ca binding of heart muscle cells. Polymyxin B is a compound which becomes integrated into the membrane due to its hydrophobic moiety. Results obtained from skeletal muscles cannot be compared with the present findings, since aminoglycoside antibiotics interfere with neuromuscular transmission rather than the contractile response of skeletal muscles (see Lüllmann & Reuter, 1960).

The reduction of the cellular Ca content by 20% induced by gentamicin or sisomicin (3×10^{-3} M) was accompanied by a depression of the contractile force by more than 80%. A comparable diminution of the contractile force from the control level at 1.8 mM Ca can also be induced by lowering the extracellular Ca^{2+} concentration to about 5×10^{-4} M, as can be deduced from the Ca^{2+} concentration-response relationship which covered the range from 0.15 to 1.8 mM under the present conditions. Since the actual extracellular Ca^{2+} concentration was unchanged in the presence of aminoglycoside antibiotics, the conclusion can be drawn that it is the amount of Ca bound to the surface, rather than the interstitial Ca^{2+} concentration, that determines the contractility.

This conclusion is also supported by the findings of Hino *et al.* (1982), who found by means of the sucrose gap technique, that the slow Ca inward current was decreased in the presence of gentamicin without any change in the characteristics of the 'Ca channel'. Again, it was the superficially bound Ca rather than the actual Ca^{2+} concentration which determined the cellular event.

The results obtained with aminoglycoside antibiotics concerning contractility and Ca fluxes can be depicted as shown in Figure 4: the superficially

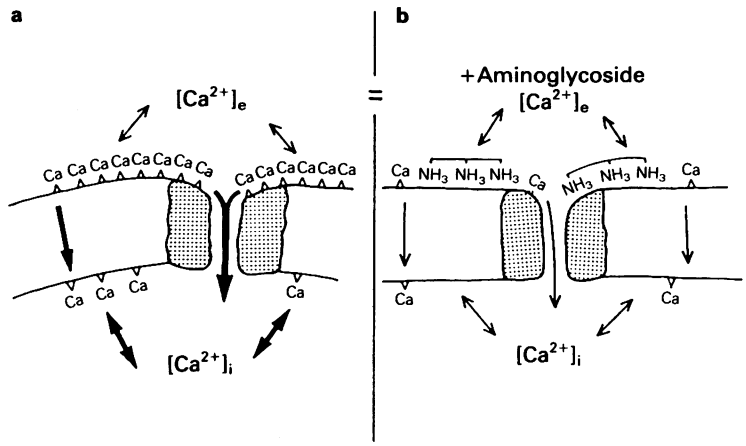


Figure 4 Schematic representation of the effect of aminoglycoside antibiotics upon the binding of Ca^{2+} at the outer surface of the cardiac sarcolemma, and on the consequences of excitation-contraction coupling. (a) Control condition: the extracellular Ca^{2+} concentration is in equilibrium with numerous anionic binding sites at the outer surface. On stimulation (1) Ca will be released from a voltage-dependent Ca store situated at the inner surface which is in equilibrium with the surface bound Ca, and (2) Ca primarily attached to the surface will flow through a channel into the cytosol. (b) The situation is schematically depicted in the presence of aminoglycoside antibiotics which compete for anionic binding sites. On stimulation both the release and the flow will be reduced resulting in impaired excitation-contraction coupling.

bound Ca fraction which is, under normal conditions, in equilibrium with the interstitial Ca^{2+} concentration supplies both a deeper Ca compartment participating in the excitation-contracting coupling (Lüllmann *et al.*, 1983) and the slow Ca inward current. When the superficially bound Ca is partially replaced by an aminoglycoside antibiotic the contractile force as well as the Ca inward current are decreased although the extracellular Ca^{2+} concentration remains unaltered. Thus a partial displacement of Ca from the outer leaflet of the cardiac plasmalemma imitates the effect of a decrease of the extracellular Ca^{2+} concentrations.

On the basis of the present experiments we cannot state conclusively where the superficially bound Ca is exactly located. In our view the acidic groups of phospholipids belonging to the outer leaflet of the plasmalemma and the acidic groups of glycoproteins forming the glycocalyx represent the anionic binding sites for the Ca binding.

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