ELSEVIER

European Journal of Pharmacology 412 (2001) 121–126

ejp

www.elsevier.nl/locate/ejphar

Effects of antibiotics on the contractility and Ca^{2+} transients of rat cardiac myocytes

Alexandra Belus, Ed White *

School of Biomedical Sciences, University of Leeds, Leeds, LS2 9JT, UK

Received 28 September 2000; received in revised form 15 December 2000; accepted 22 December 2000

Abstract

We have compared the effects of streptomycin sulphate, gentamicin sulphate and neomycin sulphate on cell shortening (our index of contractility) and intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients of rat ventricular myocytes. All three agents abolished shortening and $[Ca^{2+}]_i$, transients but streptomycin was significantly less potent than the other agents. The IC_{50} of streptomycin was 0.37 mM for shortening and 0.78 mM for $[Ca^{2+}]_i$, approximately an order of magnitude greater than equivalent values for gentamicin and neomycin. Gentamicin and streptomycin shortened the action potential duration of most cells but prolonged the action potential duration of others. We therefore conclude that multiple ionic mechanisms affecting action potential duration are modulated by these antibiotics. Our observations are consistent with the negative inotropic effect of antibiotics being caused by a decrease in Ca^{2+} influx causing a reduction in the $[Ca^{2+}]_i$ transient. © 2001 Published by Elsevier Science B.V.

Keywords: Cardiac myocyte; Aminoglycosidic antibiotic; Ca^{2+} intracellular

1. Introduction

Aminoglycosidic antibiotics have multiple effects on muscle. For example, they have been shown to block L-type Ca^{2+} channels (I_{Ca}) in vascular smooth muscle (streptomycin, Miller and Langton, 1998) in cardiac muscle (gentamicin, Hino et al., 1982) and in skeletal muscle (several agents, Haws et al., 1996). Possibly as a consequence of this effect on Ca^{2+} influx, they have been shown to decrease the contractility of cardiac muscle (e.g. gentamicin, Adams, 1975; streptomycin; Slinker and Tobias, 1996).

Antibiotics have also been reported to block stretch-activated channels (Winegar et al., 1996; see Hamill and McBride, 1996; Pascarel et al., 1997a for reviews). This ability has led to their use in the study of stretch-activated events. In the heart, these studies have concentrated on streptomycin, which has been shown to reduce the occurrence of stretch-activated arrhythmia in whole hearts (Nazir et al., 1995; Salmon et al., 1997) and reverse stretch-induced increases in $[Ca^{2+}]_i$ in single myocytes (Gannier et al., 1994).

Because agents that block stretch-activated channels are not specific (Hamill and McBride, 1996; Pascarel et al., 1997a) it is important to characterise their actions in unstretched preparations. Effects upon myocyte contractility, $[Ca^{2+}]_i$ transients and action potentials are useful indicators of stretch-independent actions that have been used previously with other stretch-activated channel blockers (Gd^{3+}, Ward and White, 1994; Hongo et al., 1997; tarantula venom, Pascarel et al., 1997b). To date, there has been no comparison of the contractile and electrical effects of neomycin, gentamicin and streptomycin under a given set of conditions, nor has any study looked at the effect of these agents on $[Ca^{2+}]_i$ transients. The purpose of this study therefore, was to carry out this comparison and test the hypothesis that the negative inotropic effect of these agents is associated with a reduction in the $[Ca^{2+}]_i$ transient. Preliminary observations have been presented in abstract form (Belus and White, 1999).

* Corresponding author. Tel.: +44-113-233-4248; fax: +44-113-233-4228.

E-mail address: phsew@leeds.ac.uk (E. White).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00717-8

2. Materials and methods

2.1. Isolation of rat cardiac ventricular myocytes

Single rat ventricular myocytes were isolated according to the method of Frampton et al. (1991). Briefly, male Wistar rats (200–250 g) were killed by Home Office Schedule 1 methods. Hearts were removed and mounted on a Langendorff apparatus and perfused retrogradely with a HEPES-based isolation solution of the following composition (mM): NaCl 130; KCl 5.4; NaH_2PO_4 0.4; $MgCl_2 \cdot 6H_2O$ 1.4; $CaCl_2$ 0.75; HEPES 10; glucose 10; taurine 20; creatine 10 (pH 7.3). When the coronary circulation had cleared of blood, perfusion was continued with Ca^{2+}-free isolation solution (in which 0.75 mM $CaCl_2$ had been replaced with 0.1 mM EGTA) for 5 min. This was followed by 10 min perfusion with isolation solution containing 50 μM Ca^{2+}, 1 mg ml^{-1} collagenase (type I; Worthington Biochemical, NJ) and 0.1 mg ml^{-1} protease (type XIV; Sigma). The ventricles were then separated from the rest of the heart, minced, and gently shaken at 37°C in collagenase containing isolation solution supplemented with 1% bovine serum albumin. Cells were filtered from this solution at 5-min intervals and re-suspended in isolation solution containing 0.75 mM Ca^{2+}.

Isolated cells were placed in an experimental chamber on the stage of an inverted microscope. The chamber was continuously superfused with a HEPES-based physiological solution containing (mM): NaCl 113; KCl 5; $MgSO_4 \cdot 7H_2O$ 1; NaH_2PO_4 1; CH_3COONa 20; $CaCl_2$ 1; HEPES 5; glucose 10; insulin 5 units l^{-1}; pH adjusted to 7.4 with NaOH. Aliquots of stock antibiotic solutions were added to this solution. Cells were field-stimulated by external platinum electrodes at a frequency of 0.5 Hz. All experiments were performed at ambient temperature (22–24°C).

2.2. Measurement of cell shortening and $[Ca^{2+}]_i$

When cell shortening and $[Ca^{2+}]_i$ were measured simultaneously, myocytes were illuminated with red light (> 610 nm) to generate an image of the cell detected by a camera mounted on the microscope and displayed on a monitor. This image was measured using an edge detection system (Crescent Electronics, UT). Cell shortening, expressed as the % change in cell length following stimulation was our index of contractility (see White et al., 1995). Cells were loaded with the Ca^{2+}-sensitive fluorescent indicator fura-2 AM (acetoxymethyl ester form of fura-2, Molecular Probes, OR) by incubation in 0.75 mM Ca^{2+} isolation solution containing 3–5 μM fura-2 AM for 10 min at room temperature. The ratio of fluorescence emitted at 510 nm in response to alternate excitation with light of 340 and 380 nm (340/380 ratio) was used as an index of $[Ca^{2+}]_i$.

2.3. Measurement of action potentials

Action potentials were measured using sharp microelectrodes containing 600 mM KCl, (resistance of 30–60 MΩ) in conjunction with an Axopatch 2B amplifier (Axon Instruments). Action potentials were elicited by 2 ms current pulses, just above threshold amplitude.

2.4. Statistical analysis

Data were expressed as mean ± S.E.M. of *n* observations. Statistical significance was tested using either one-way Analysis of Variance (ANOVA) followed by *t*-tests,

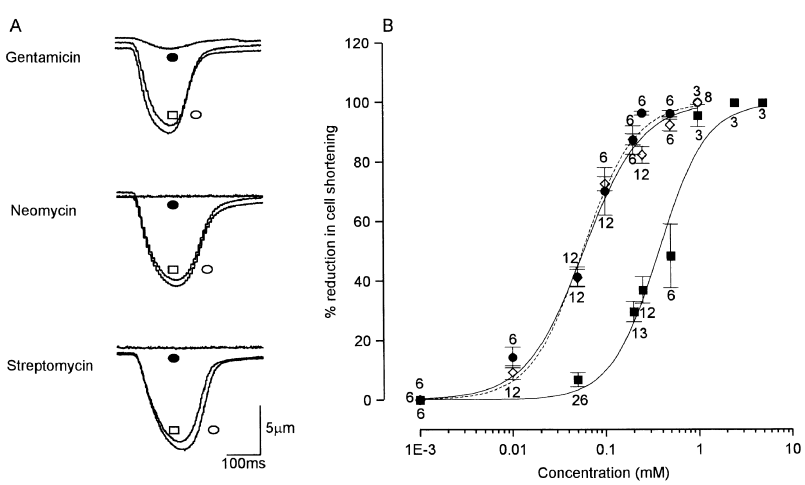

Fig. 1. (A) Representative traces of cell shortening in single rat myocytes before (○), during (●) and following (□) wash of 0.5 mM gentamicin (upper traces); 0.5 mM neomycin (middle traces) and 1 mM streptomycin (lower traces). (B) dose–inhibition curves for the effect of gentamicin (◇), neomycin ("●") and streptomycin (■). Data were fitted to a Hill equation (see Table 1). Mean ± S.E.M. (where larger than symbol) values for number of cells given in figure.

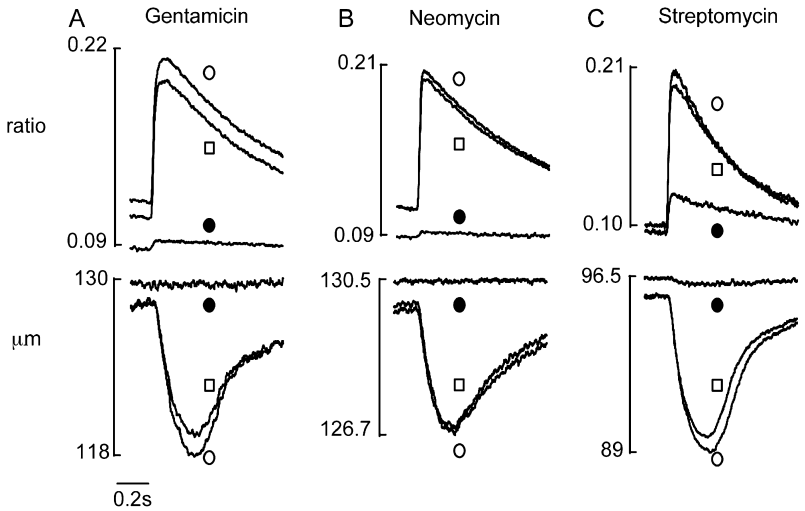

Fig. 2. Representative traces of $[Ca^{2+}]_i$ (upper traces) and simultaneously recorded cell shortening (lower traces) before (○), during (●) and following (□) wash of (A) 1 mM gentamicin; (B) 1 mM neomycin and (C) 2.5 mM streptomycin.

Tukey corrected for multiple comparisons; Student's paired or unpaired *t*-test as appropriate.

3. Results

Intracellular Ca^{2+} indicators, such as fura-2, can act as buffers of $[Ca^{2+}]_i$ and modulate contraction (see Frampton et al., 1991). Therefore, our initial experiments looked at the effect of the antibiotics on cell shortening in the absence of fura-2. Fig. 1A shows the effect of 0.5 mM gentamicin sulphate (upper traces); and 0.5 mM neomycin sulphate (middle traces) and 1 mM streptomycin sulphate (lower traces) on the shortening of single ventricular myocytes. Each antibiotic was able to rapidly reduce contractility and steady state inhibition of shortening was seen within 30 s of exposure. The reduction in shortening was readily reversible upon wash.

Fig. 1B shows the dose–inhibition relationships of the three agents for cell shortening. The data were fitted to the Hill equation

$$\%\,\text{inhibition} = 100[\text{A}]^{n_H}/\text{IC}_{50}^{n_H} + [\text{A}]^{n_H},$$

where 100 was the maximal percentage inhibition, [A] the antibiotic concentration, n_H the slope of the relationship and IC_{50} the concentration of drug at half-maximal inhibition. It can be seen from Fig. 1B that the negative inotropic effects of gentamicin and neomycin were similar, with effects beginning at approximately 10 μM. In contrast the curve for streptomycin is shifted to the right, and the threshold concentration is approximately 50–100 μM.

Fig. 2 shows the effects of the antibiotics on fura-2 loaded cells. Upper traces show cell shortening recorded simultaneously with $[Ca^{2+}]_i$ transients (lower traces) for gentamicin (Fig. 2A), neomycin (Fig. 2B) and strepto-

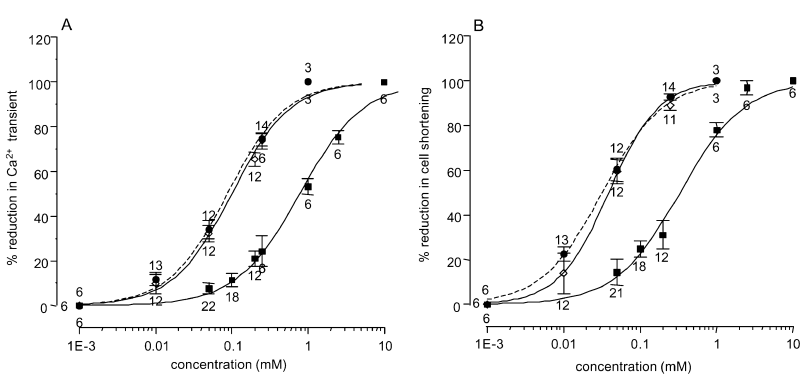

Fig. 3. Dose–inhibition curves for the effect of gentamicin (◇), neomycin ("●") and streptomycin (■) for (A) reduction in $[Ca^{2+}]_i$ transients and (B) cell shortening recorded in the same cells. Data were fitted to a Hill equation (see Table 1). Mean ± S.E.M. (where larger than symbol) values for number of cells given in figure.

Table 1
Characteristics of the dose–inhibition relationships for antibiotics on cell shortening in the presence and absence of fura-2 and on $[Ca^{2+}]_i$ transient (see Figs. 1 and 3)

	Cell shortening		$[Ca^{2+}]_i$ transient		Cell shortening fura-loaded cells	
	IC_{50} (mM)	n_H	IC_{50} (mM)	n_H	IC_{50} (mM)	n_H
Gentamicin	0.06 ± 0.004	1.38 ± 0.14	0.10 ± 0.01	1.13 ± 0.11	0.04 ± 0.001	1.32 ± 0.05
Neomycin	0.06 ± 0.004	1.54 ± 0.18	0.09 ± 0.01	1.12 ± 0.12	0.03 ± 0.002	1.09 ± 0.06
Streptomycin	0.37 ± 0.04	1.68 ± 0.34	0.78 ± 0.06	1.04 ± 0.07	0.33 ± 0.04	1.03 ± 0.11

IC_{50}, is the concentration giving half maximal inhibition and n_H, the Hill number or slope of the relationship. There were no significant differences in n_H between drugs ($P > 0.05$, one-way ANOVA). IC_{50} for streptomycin was significantly higher than that for gentamicin and neomycin in all cases ($P < 0.001$, one-way ANOVA; $P < 0.05$, Tukey). IC_{50} for gentamicin and neomycin were not significantly different from each other ($P > 0.05$, Tukey). Parameters for the effect of a given drug on cell shortening in the presence and absence of fura-2 were not significantly different from each other with the exception of the IC_{50} for the gentamicin and neomycin ($P < 0.01$, unpaired *t*-test).

mycin (Fig. 2C). It can be seen that the antibiotics reversibly reduced the $[Ca^{2+}]_i$ transients. In addition to the fall in systolic shortening and $[Ca^{2+}]_i$, it can be seen that there was a consistent fall in diastolic $[Ca^{2+}]_i$ and a corresponding increase in diastolic cell length.

Fig. 3 gives the dose–inhibition curves for $[Ca^{2+}]_i$ (Fig. 3A) and cell shortening (Fig 3B) in fura-2 loaded cells. The changes in $[Ca^{2+}]_i$ in response to antibiotics were similar to those for cell shortening, that is, the effect of gentamicin and neomycin were similar, but the curves for streptomycin are shifted to the right.

Table 1 compares the characteristics of the data for each antibiotic. There were no differences between the slopes (n_H) for the three antibiotics ($P > 0.05$, one-way ANOVA). However, the IC_{50} for streptomycin was significantly greater ($P < 0.001$, one-way ANOVA; $P < 0.05$, Tukey), for both shortening and $[Ca^{2+}]_i$, than the IC_{50} for gentamicin and neomycin (which were not significantly different from each other, $P > 0.05$, Tukey). Fura-2 loading shifted the IC_{50} for shortening to the left and reduced the n_H, however these differences were only statistically significant for the IC_{50} of gentamicin and neomycin ($P < 0.01$, unpaired *t*-test).

The time-course of the shortening and $[Ca^{2+}]_i$ transients was investigated. The time to peak response from stimulation in the absence of drug was 211 ± 5 ms ($n = 37$ cells) and 69 ± 5 ms ($n = 26$ cells), respectively. The time taken for cell shortening and $[Ca^{2+}]_i$ to fall from peak response to half peak levels in the absence of drug was 317 ± 12 ms and 426 ± 35 ms, respectively. None of these parameters were significantly altered by exposure to antibiotics ($P > 0.05$, one-way ANOVA, on changes in values following exposure to drug concentration closest to IC_{50} that was tested).

Aminoglycosidic antibiotics are cationic in nature and may interfere with the access of Ca^{2+} to Ca^{2+} influx pathways. It has been reported that the block of gentamicin of I_{Ca} (Hino et al., 1982) and contraction (Adams, 1975) in multicellular preparations is relieved by elevating $[Ca^{2+}]_o$. Fig. 4 shows the effect of increasing extracellular Ca^{2+} from 1 to 5 mM on the negative inotropic response of 1 mM streptomycin in single cardiac cells. It can be seen that as $[Ca^{2+}]_o$ was elevated the effect of streptomycin was reduced.

The effect of gentamicin (0.125 and 0.25 mM) and streptomycin (0.4 and 1 mM) on action potential configuration was tested (Fig. 5). Lower traces in each panel show the simultaneous measurement of cell shortening, confirming the negative inotropic effect described in the absence of a microelectrode (e.g. Fig. 1). The effects of the different drug doses, on action potential parameters, were not significantly different from each other, so data was pooled for each antibiotic for brevity.

The response of the action potential duration to the antibiotics was varied, in most cells the action potential duration shortened (16 out of 22 tested, Fig. 5) but in others it lengthened (6 out of 22). Effects in individual cells were reversible on wash. The action potential duration at 20% repolarisation, of cells exposed to gentamicin was shortened from 12 ± 11 ms by 3.6 ± 6.3% and the

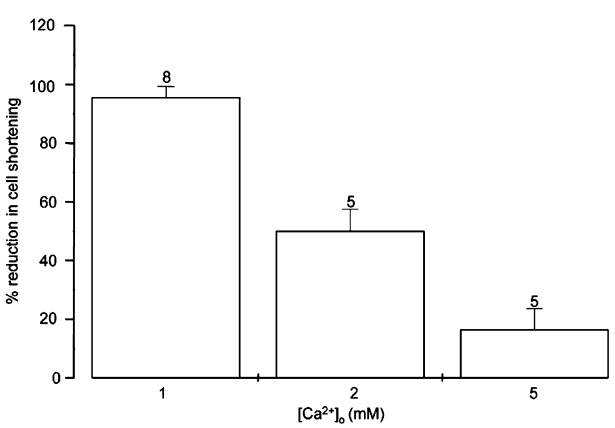

Fig. 4. Effect of $[Ca^{2+}]_o$ on the negative inotropic effect of 1 mM streptomycin. Mean ± S.E.M., values for number of cells given in figure. The negative inotropic effect was decreased by increasing $[Ca^{2+}]_o$ ($P < 0.05$, one-way ANOVA).

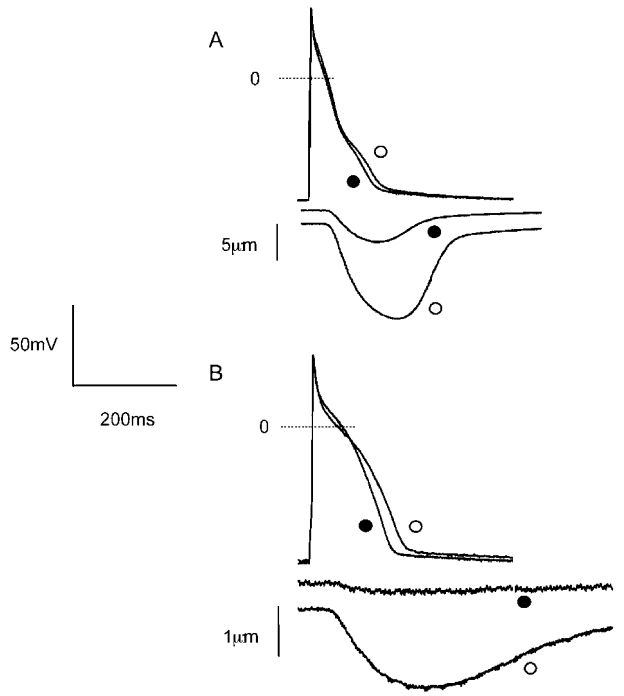

Fig. 5. Representative traces of action potentials (upper traces) and simultaneously recorded contraction (lower traces) before (○), and during (●) exposure to (A) 0.125 mM gentamicin (B) 0.4 mM streptomycin. Exposure to antibiotics decreased cell shortening in all cells and abbreviated the action potential duration most cells.

action potential duration at 90% repolarisation from 117 ± 18 ms by 6.5 ± 8.0% ($n = 14$ cells). In cells exposed to streptomycin, action potential duration at 20% repolarisation, shortened from 19 ± 5 ms by 11.5 ± 15.8% and action potential duration at 90% repolarisation from 131 ± 26 ms by 1.2 ± 7.8% ($n = 8$ cells). None of the changes in action potential duration at 20% repolarisation were significant ($P > 0.05$, paired *t*-test). There were no significant effects upon resting membrane potential (74 ± 1 mV; $n = 22$) nor on action potential amplitude (110 ± 4 mV; $n = 22$).

A decrease in the $[Ca^{2+}]_i$ transient (Fig. 3) is often reported to shorten the action potential duration, however when $[Ca^{2+}]_o$ is lowered action potential duration is reported to increase (see Section 4). The antibiotics we tested are routinely manufactured in sulphate form $(SO_4^{2-})_5$ for gentamycin and $(SO_4^{2-})_3$ for neomycin and streptomycin. Sulphate ions can bind to Ca^{2+} in solution, form the sparingly soluble $CaSO_4$ and reduce the level of free Ca^{2+} in solution. Such a response might contribute to the negative inotropic effect and the lengthening of the action potential duration seen in some cells. In order to test this possibility cells were superfused with Tyrode solution containing 5 or 10 mM Na_2SO_4. There was no significant effect of elevating sulphate concentrations on the cell shortening of rat ventricular myocytes ($n = 6$ cells at each concentration).

4. Discussion

4.1. Comparative effects of antibiotics on cell shortening and $[Ca^{2+}]_i$

Our observations, that antibiotics reduce cell shortening in single cardiac cells (e.g. Fig. 1) and that this effect is modulated by $[Ca^{2+}]_o$ (Fig. 4) are in agreement with previous studies in multi-cellular preparations (e.g. Adams, 1975, gentamicin). However, our study gives the first direct comparison of the inotropic actions of the three agents under investigation. In addition this is the first demonstration that the negative inotropic effect of antibiotics is associated with a decrease in the $[Ca^{2+}]_i$ transient and resting $[Ca^{2+}]_i$ levels (Figs. 2 and 3). The effects of neomycin and gentamicin were similar but streptomycin appears to be a less potent negative inotropic agent in cardiac tissue. These observations contrast somewhat with observations made in skeletal muscle by Haws et al. (1996) where the K_d for I_{Ca} was 11.1 mM for gentamicin compared to 2.1 mM for streptomycin and 0.8 mM for neomycin. In cardiac muscle, it seems likely that a decrease in Ca^{2+} influx via I_{Ca} (Hino et al., 1982) leads to decreased Ca^{2+} loading of sarcoplasmic reticulum and/or trigger for sarcoplasmic reticulum Ca^{2+} release. This leads to a reduced $[Ca^{2+}]_i$ transient and as a result, a reduced contractility (see Bers, 1991). A fall in free Ca^{2+} due to the formation of the sparingly soluble $CaSO_4$, does not appear important.

4.2. Effects of antibiotics on action potentials

In most cells, exposure to antibiotics shortened the action potential duration but in some cells it lengthened. We therefore conclude that several mechanisms that affect action potential duration were modulated. Action potential shortening could arise from decreased inward currents, likely to be associated with the fall in the $[Ca^{2+}]_i$ transient, e.g. I_{Ca} and the Na^+:Ca^{2+} exchanger operating in Ca^{2+} extrusion mode. Alternatively action potential duration can lengthen if $[Ca^{2+}]_o$ is reduced (Schouten and Ter Keurs, 1985; Leitch and Brown, 1996; see Janvier and Boyett, 1996 for review). Positively charged antibiotics might reduce the 'effective' $[Ca^{2+}]_o$ close to the membrane by interacting with surface negative charges on the cell (surface charge screening, Green and Andersen, 1991). Indeed, the charges on the molecules (neomycin +4.4; gentamicin +3.5; streptomycin +2) do resemble their relative potencies in reducing contractility and $[Ca^{2+}]_i$.

4.3. Use of antibiotics as blockers of stretch-dependent mechanisms

There is an absence of data relating to the effects of neomycin and gentamicin on stretch-activated events in the

heart, however data relating to streptomycin does exist. In whole hearts, Nazir et al. (1995) reported a reduction in stretch-activated arrhythmias by 80 μM streptomycin. Salmon et al. (1997) reported similar effects with 200 μM (but not 50 μM). In unstretched hearts Slinker and Tobias (1996) reported a decrease in force at 500 μM (but not 100 μM). In single cells, we observed the threshold for the inotropic effects of streptomycin to be lower (about 50 μM). This observation is consistent with the lack of effect of 40 μM streptomycin on I_{Ca} in guinea pig myocytes, a dose which did reverse stretch-activated increases in $[Ca^{2+}]_i$ (Gannier et al., 1994).

Thus, in single cells, here is evidence to suggest some separation between concentrations of streptomycin that are effective upon stretch-dependent mechanisms (< 50 μM) and on contractility and $[Ca^{2+}]_i$ (> 50 μM). This compares favourably with the most widely used stretch-activated channel blocker Gd^{3+}. At 10 μM, the dose frequently used to block stretch effects (Hamill and McBride, 1996; Pascarel et al., 1997a), Gd^{3+} irreversibly abolishes cell shortening and $[Ca^{2+}]_i$ transients in rat ventricular myocytes (Ward and White, 1994). Thus, the use of streptomycin to investigate stretch dependent mechanisms seems preferable to Gd^{3+}.

Acknowledgements

The authors thank Profs. M.R. Boyett, J-Y. Le Guennec and Dr. S.C. Calaghan for helpful discussions. This work was supported by the British Heart Foundation.

References

Adams, H.R., 1975. Direct myocardial depressant effects of gentamicin. Eur. J. Pharmacol. 30, 272–279.

Belus, A., White, E., 1999. Effects of aminoglycosidic antibiotics on the contraction and intracellular calcium transients of isolated rat ventricular myocytes. J. Physiol. 521P, 29P (abstract).

Bers, D.M., 1991. Excitation–Contraction Coupling and Cardiac Contractile Force. Kluwer Academic Publishing, Dordrecht, Netherlands.

Frampton, J.E., Orchard, C.H., Boyett, M.R., 1991. Diastolic, systolic and sarcoplasmic reticulum $[Ca^{2+}]$ during inotropic interventions in isolated rat myocytes. J. Physiol. 437, 351–375.

Gannier, F., White, E., Lacampagne, A., Garnier, D., Le Guennec, J-Y., 1994. Streptomycin reverses a large stretch-induced increase in $[Ca^{2+}]_i$ in isolated guinea-pig ventricular myocytes. Cardiovasc. Res. 28, 1193–1198.

Green, W.N., Andersen, O.S., 1991. Surface charges and ion channel function. Ann. Rev. Physiol. 53, 341–359.

Hamill, O.P., McBride, D.W., 1996. The pharmacology of mechanogated membrane ion channels. Pharmacol. Rev. 48, 231–252.

Haws, C.M., Winegar, B.D., Lansman, J.B., 1996. Block of single L-type Ca^{2+} channels in skeletal fibres by aminoglycoside antibiotics. J. Gen. Physiol. 107, 421–432.

Hino, N., Ochi, R., Yanagisawa, T., 1982. Inhibition of the slow inward current and time-dependent outward current of mammalian ventricular muscle by gentamycin. Pfluegers Arch. 394, 243–249.

Hongo, K., Pascarel, C., Cazorla, O., Gannier, F., Le Guennec, J-Y., White, E., 1997. Gadolinium blocks the delayed rectifier potassium current in isolated guinea pig ventricular myocytes. Exp. Physiol. 82, 647–656.

Janvier, N.C., Boyett, M.R., 1996. The role of Na–Ca exchange current in the cardiac action potential. Cardiovasc. Res. 32, 69–84.

Leitch, S.P., Brown, H.F., 1996. Effect of raised extracellular calcium on characteristics of the guinea-pig ventricular action potential. J. Mol. Cell. Cardiol. 28, 541–551.

Miller, A.L., Langton, P.D., 1998. Streptomycin inhibition of myogenic tone, K^+-induced force and block of L-type calcium current in rat cerebral arteries. J. Physiol. 508, 793–800.

Nazir S.A., Dick, D.J., Coen, B.A., Lab, M.J., 1995. Anti-arrhythmic action of streptomycin in the ventricle of the isolated, Langendorff perfused guinea-pig heart. J. Physiol. 489.P, 166P (abstract).

Pascarel, C., Cazorla, O., Brette, F., Le Guennec, J-Y., 1997a. Can a specific blocker of mechano-sensitive channels be found? Curr. Top. Pharmacol. 3, 229–238.

Pascarel, C., Cazorla, O., Le Guennec, J-Y., Orchard, C.H., White, E., 1997b. The effect of the venom of a Chilean tarantula *Phrixotrichus spatulatus* on isolated guinea-pig ventricular myocytes. Toxicol. Appl. Pharmacol. 147, 363–371.

Salmon, A.H.J., Mays, J.L., Dalton, G.R., Jones, J.V., Levi, A.J., 1997. Effect of streptomycin on wall stress induced arrhythmias in the working rat heart. Cardiovasc. Res. 34, 493–503.

Schouten, T.J., Ter Keurs, H.E.J.D., 1985. The slow repolarization phase of the action potential in rat heart. J. Physiol. 360, 13–25.

Slinker, B.K., Tobias, A.H., 1996. Previous-beat contraction history is not influenced by mechanosensitive ion channel blockade. Cardiovasc. Res. 32, 131–137.

Ward, H., White, E., 1994. Reduction in the contraction and intracellular calcium transient of single rat ventricular myocytes by gadolinium and the attenuation of these effects by extracellular NaH_2PO_4. Exp. Physiol. 79, 107–110.

White, E., Boyett, M.R., Orchard, C.H., 1995. The effects of mechanical loading and changes of length on single guinea-pig ventricular myocytes. J. Physiol. 482, 93–107.

Winegar, B.D., Haws, C.M., Lansman, J.B., 1996. Subconductance block of single mechanosensitive ion channels in skeletal muscle fibres by aminoglycoside antibiotics. J. Gen. Physiol. 107, 433–443.