EFFECTS OF CERTAIN ANTIBIOTICS ON ISOMETRIC CONTRACTIONS OF ISOLATED RAT HEART MUSCLE

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ANTIBIOTICS are among the most common drugs used clinically. Although their untoward effects in some areas are appreciated, their cardiac actions are less well known. Recently the cardiovascular effects of antibiotics have been studied *in vitro* and *in vivo* and depressant effects have been observed. Our previous study¹ and others²⁻⁶ have demonstrated that aminoglycoside antibiotics produce direct cardiac depression and potentiate the depression produced by halothane. However, no studies have shown concentration-dependent direct myocardial depression by antibiotics and the effects of kanamycin on Ca^{++} dependent contractions or the effects of Ca^{++} on depressed isometric contraction produced by kanamycin in an isolated heart muscle have not been recorded.

It is believed that excitation-contraction coupling in heart muscle is dependent upon a superficial membrane source of Ca^{++} , and in dependent depression of myocardial contractile tension that was reversible by adding Ca^{++} .⁷ Many studies have demonstrated that high K^+ concentration produced cardiac depression⁸ and even cardiac arrest.^{9,10} However, other studies¹¹ show that in both digitalized and non-digitalized dogs with the chest open potassium did not significantly alter right ventricular contractility and cardiac dynamics. Similar results are reported from *in vitro* studies of Green, *et al.*¹²

Since those findings differ from the results observed in our previous study, since antibiotics are sometimes given to patients by bolus intravenous administration and because we did not believe that these depressant effects are all-or-none phenomena, the present study was undertaken to determine concentration-dependent effects of antibiotics on cardiac contractility and the effects of K^+ and Ca^{++} on isometric contraction of rat heart muscle.

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MATERIALS AND METHODS

For the studies of the direct myocardial effects of potassium-penicillin-G (K-PNC-G), kanamycin (KM), streptomycin (SM), and chloramphenicol (CM), the isometric contractions of isolated rat heart muscle were examined in peak developed tension (Tpd), maximum rate of tension development and relaxation ($\pm \text{dP/dt max}$), resting tension (Tr), time to peak tension (TPT) and relaxation time (RT).

Sixty experiments were done with seven rat heart muscle preparations to determine the direct effect of K-PNC-G, KM, SM and CM on isometric contractions. The techniques used in this study are essentially similar to those previously reported.¹

After sacrificing the rats, isolated columns of left ventricular trabeculae carneae muscle were prepared for measurement of isometric contractions. Each muscle preparation was placed in an oxygenated (O_2 95 per cent and CO_2 5 per cent premixed) 80 ml capacity muscle bath containing modified Krebs-bicarbonate solution. The composition of the bathing solution in millimols per litre was: Na^+ 146, K^+ 5.0, Ca^{++} 2.25, Mg^{++} 1.25, Cl^- 130, HCO_3^- 25, H_2PO_4 1.4, SO_4^{--} 1.25, and glucose 11.1. The pH was 7.40 (H^+ 40 nmol/l). Temperature of the bath solution was held constant at 34° C by a thermoregulator (Precision Scientific Co.). One end of the muscle was fixed and the other end was connected to the Statham tension transducer (G10B-0.15-350) and its output recorded on one channel, while another output was connected to the differentiator (Optical Electronic Inc. Model 9009) and recorded on another channel of the recorder (Brush Recorder 440). This differentiator was checked at the factory using automatic test equipment and calibrated with rat heart muscle twitches in isometric contractions. Each muscle was stimulated with 2×8 mm platinum electrodes placed about 1 cm apart, parallel to the muscle, at a frequency of 15 per minute by a square wave pulse 10 per cent above threshold in volts and 6.0 msec in duration. In that condition, no significant amounts of catecholamine are released from muscle.¹³

The length-tension curve of each preparation was determined after 30 minutes of isometric contractions. The average rat weight was $154.29 \text{ g} \pm 15.10$ (mean $\pm$ standard deviation) and maximum muscle length (the length at the peak of the length-tension curve) was $4.02 \text{ mm} \pm 0.53$. Changes in muscle length, measured with a micrometer transformer, could be detected to 0.01 mm.

The muscle length was then held at the peak of the length-tension curve over the next 30 minutes for equilibration, and then peak development tension (Tpd), resting tension (Tr), maximum rate of tension development and relaxation ($\pm \text{dP/dt max}$), time to peak tension (TPT) and relaxation time (RT) were measured from the twitch curve recorded at paper speed of 50 mm per second. After control twitches were obtained the antibiotic was added directly into the bath solution. When equilibration was achieved, which took about two minutes after administration of the antibiotic, twitches were recorded at the same paper speed. The bath solution was then drained, muscles were washed twice with fresh modified Krebs-bicarbonate solution and the bath was refilled. We selected K-PNC-G (Pfizerpen-G, Pfizer) from the penicillin group of antimicrobial agent, KM (Kantrex, Bristol) and SM, (Streptomycin, Pfizer) from the aminoglycosides group, CM (Chloromycetin, Parke-Davis & Co.) from others and sterile water for injection as diluent. We studied the effects of the antibiotics on the myocardium with final concentrations of 0.962 mmol of K-PNC-G, 0.207 mmol KM, 0.215 mmol SM, 0.235 mmol CM, and concentrations three and five times higher for concentration-response studies. An 80 ml bath solution was used and the different concentrations were studied in random order. We selected potassium chloride injection, USP (Elkins-Sinn, Inc.) which contains 2 mmol (2 mEq) of K^+ per ml and 10 per cent calcium gluconate 10 ml ampule (Elkins-Sinns, Inc.) for studies of K^+ and Ca^{++} on isometric contractions. Percentage changes from control produced by antibiotics were calculated and compared. Analyses for statistical significance were made employing the paired *t*-tests; P values less than 0.05 were considered significant.

RESULTS

A. Potassium-Penicillin-G

There were increases in Tpd, $+\text{dP/dt max}$ and $-\text{dP/dt max}$ as a result of administration of

K-PNC-G in concentrations of 0.962, 0.962×3 , 0.962×5 mmol to the bath while no changes in TPT and RT were noted, but no significant changes were noted among different concentration groups (Table I). We observed that twitches in rat heart muscle were augmented by adding K^+ in amounts of $0.5 (4 \times 10^{-2})$, $1 (8 \times 10^{-2})$ and $5 (4 \times 10^{-1})$ mmol (mEq) as KCl but larger amounts of K^+ , 30 mmol, produced an initial increase followed by depression and complete standstill of heart muscle in the bath (Figure 1).

B. Kanamycin

There were reductions in Tpd, $+\text{dP/dt max}$ and $-\text{dP/dt max}$ in concentrations of 0.207, 0.207×3 , 0.207×5 mmol of KM, while no significant changes were seen in TPT and RT in any of the three groups. Not only were all these reductions significantly different from control but significant changes were noted among these three different groups (Table I), showing concentration-dependent depression (Figure 2). Addition of Ca^{++} reversed the twitch depression induced by KM (Figure 3A). In Ca^{++} pre-treated muscle isometric contraction could be depressed by KM but not as much as in muscle which was not so pre-treated (Figure 3B). Much less initial depression followed by recovery were seen when KM and Ca^{++} were added together to the bath solution (Figure 3C).

C. Streptomycin

As seen in KM studies, SM significantly reduced Tpd, $+\text{dP/dt max}$ and $-\text{dP/dt max}$ at all the concentrations tested (Table I).

Although there were no statistically significant changes between two close concentrations, that is 0.215 and 0.215×3 or 0.215×3 and 0.215×5 mmol it was noted that there were significant changes between the extremes (0.215 and 0.215×5 mmol) in Tpd and $+\text{dP/dt max}$ without any changes in TPT and RT (Figure 2).

These results may suggest that SM is a less potent depressant on rat heart muscle than KM.

D. Chloramphenicol

The three different concentrations of CM studied show much less depression in Tpd, $+\text{dP/dt max}$ and $-\text{dP/dt max}$ than the other drugs, and no concentration-dependency was noted. There were no significant changes in TPT and RT (Table I).

Resting Tension

Resting tension was reduced and showed no

TABLE 1
EFFECTS OF POTASSIUM PENICILLIN-G, KANAMYCIN, STREPTOMYCIN AND CHLORAMPHENICOL ON ISOMETRIC CONTRACTIONS (MEANS $\pm$ SD)

	mmol	Tpd	+dP/dt max	-dP/dt max	Tr	TPT	RT
K-PNC-G 0.962	$\times 1$	$+12.64 \pm 5.03$	$+13.17 \pm 4.15$	$+14.46 \pm 3.46$	7.61 ± 3.48	0	$+1.43 \pm 3.19$
	$\times 3$	$+19.27 \pm 11.27$	$+23.41 \pm 17.73$	$+25.07 \pm 19.43$	8.80 ± 6.15	0	0
	$\times 5$	$+14.74 \pm 13.21$	$+17.07 \pm 12.62$	$+20.40 \pm 13.44$	6.52 ± 3.17	$+0.08 \pm 1.34$	2.22 ± 3.05
KM 0.207	$\times 1$	$21.76 \pm 5.69^*$	$19.84 \pm 5.67^*$	$18.65 \pm 4.17^*$	1.98 ± 2.04	0	3.40 ± 5.01
	$\times 3$	$31.80 \pm 3.52^*$	$29.84 \pm 4.55^*$	$30.72 \pm 4.30^*$	7.48 ± 3.82	2.22 ± 4.97	1.18 ± 2.63
	$\times 5$	$52.26 \pm 13.37^\dagger$	$51.18 \pm 14.63^\dagger$	$51.54 \pm 14.14^\dagger$	3.74 ± 4.60	7.22 ± 11.04	5.75 ± 5.72
SM 0.215	$\times 1$	13.78 ± 6.44	13.12 ± 6.11	13.04 ± 7.23	$2.59 \pm 1.77^*$	0	0
	$\times 3$	20.91 ± 7.21	$18.38 \pm 7.22^*$	18.37 ± 9.35	5.38 ± 1.31	2.0 ± 4.47	1.00 ± 2.24
	$\times 5$	$20.47 \pm 4.88^\dagger$	$21.71 \pm 5.54^\dagger$	20.89 ± 7.07	3.89 ± 2.94	0	1.05 ± 2.35
CM 0.235	$\times 1$	5.62 ± 2.95	4.22 ± 2.40	4.62 ± 3.19	1.21 ± 1.24	0	0
	$\times 3$	3.86 ± 2.95	3.28 ± 3.41	4.36 ± 5.60	2.11 ± 1.45	2.22 ± 4.97	0
	$\times 5$	5.05 ± 3.21	4.10 ± 5.43	2.91 ± 6.46	4.66 ± 3.80	0	0

Per cent depressions from control: K-PNC-G – potassium penicillin-G; KM – kanamycin; SM – streptomycin; CM – chloramphenicol; Tpd – peak developed tension; $\pm$ dP/dt max – maximum rate of tension development and relaxation; Tr – resting tension; TPT – time to peak tension; RT – relaxation time; mmol – millimols.

*P < 0.05, between two near dose groups of same antibiotic.

† P < 0.05, between two far dose groups of same antibiotic.

FIGURE 1 These experiments show the changes in isometric contraction produced by K^+ . Arrows ($\uparrow$) indicate the time when K^+ was administered. The isometric twitches could be increased by adding small amount of K^+ (0.5, 1, or 5 mmol as KCl), while large enough doses of K^+ (30 mmol) produced an initial increase in a few seconds and then depression and complete standstill.

FIGURE 2 Per cent depression of peak developed tension (Tpd), maximum rate of tension development ($+dP/dt$ max) and relaxation ($-dP/dt$ max) as a result of administration of $0.207 \times 1, \times 3$ and $\times 5$ mmol kanamycin and $0.215 \times 1, \times 3$ and $\times 5$ mmol streptomycin.

KM: kanamycin experiments. SM: streptomycin experiments.

* $p < 0.05$ between two near groups ($\times 1$ and $\times 3$, $\times 3$ and $\times 5$).

$p < 0.05$ between two far groups ($\times 1$ and $\times 5$).

concentration dependency with all antibiotics studied.

DISCUSSION

Some doses studied are greater than recommended clinical doses but were chosen because transient cardiac concentrations of an antibiotic immediately after a bolus intravenous administration could be much higher than steady state therapeutic concentrations of the antibiotic in blood. K-PNC-G increased isometric contractions of rat heart muscle. Those increases in twitch were not significantly different between different concentrations. Twiches in rat heart muscle were augmented by adding a small amount of K^+ as KCl, but a large enough amount K^+ produced an initial increase and then depression and complete standstill of the muscle. The possible mechanism of standstill produced by large doses of K^+ is the change in resting cell membrane potential produced by changing extracellular K^+ concentration.⁹ The amounts of K-PNC-G which we used contain approximately 0.85 (0.07), 2.55 (0.2) and 4.25 (0.34) mmol (mEq) of K^+ . Therefore the increases in isometric contraction of isolated rat heart muscle produced by K-PNC-G seem to be due to small amounts of K^+ in K-PNC-G but not to penicillin itself. This interpretation is supported from studies by Swain³ who found that high doses of K-PNC-G produced cardiac decompensation in the dog heart-lung preparation. The addition of K^+ as KCl produced changes similar to those seen with K-PNC-G but no changes were seen with the sodium salt of penicillin-G. Comparable results were observed by Cohen, *et al.*²

In contrast, KM and SM, both aminoglycoside antibiotics, produced depression of isometric contractions of rat heart muscle that were concentration-dependent. It has been previously reported^{1-6,14,15} that the aminoglycoside antibiotics produce negative inotropic effects in cardiac muscle. In our previous studies¹ KM and SM not only produced depression by themselves but potentiated the depression produced by halothane. Although the mechanisms of antibiotic-induced depression of cardiac contractility are uncertain, recent studies^{1,2,6,16} have provided evidence that these depressions may not be metabolic in origin and are rather due to alteration of Ca^{++} availability in the contractile unit of heart muscle. The changes of cardiac contractility occur within seconds after administration of these agents, which is well before any metabolic

FIGURE 3 These experiments demonstrate that Ca^{++} has an antagonist effect against the depressant effects of kanamycin on isometric contractions.

A. Depression produced by kanamycin is antagonized by Ca^{++} .

B. Kanamycin cannot depress the Ca^{++} pretreated myocardial contractility.

C. Much less depression followed by recovery can be seen in this experiment when kanamycin and Ca^{++} are added together.

KM: kanamycin, Ca^{++} , calcium.

mM: mmol: millimol.

$\pm dP/dt$ max: maximum rate of tension development and relaxation.

changes may occur and isometric twitches return to normal after the bath solution is drained and refilled with fresh solution. In the Ca^{++} and kanamycin studies, we demonstrated that kanamycin could not depress the Ca^{++} pretreated myocardium as much as the non-treated myocardium, that much less initial depression followed by recovery was seen when KM and Ca^{++} were added to the bath at the same time, and that Ca^{++} restored the kanamycin-depressed twitch to normal level. It seems likely that Ca^{++} has an antagonistic effect against the negative inotropic action of KM. Although CM depressed isometric contraction, the degree of depression was small and CM did not show any concentration-dependency. Those findings are somewhat different from those of Cohen.² It may be that the concentrations of CM which we studied were not sufficient to produce marked depression or to produce concentration-dependent changes.

SUMMARY

The direct effects of potassium-penicillin-G, kanamycin, streptomycin and chloramphenicol on isometric contraction of isolated rat heart muscle were examined. Potassium-penicillin-G did not depress myocardial contractility but

rather increased it. Those increases are not due to penicillin itself but due to a small amount of K^+ in potassium-penicillin-G.

Kanamycin and streptomycin did show not only direct myocardial depressant effects but concentration-dependent depressions. The depression produced by kanamycin could be restored to normal by adding Ca^{++} to the bath solution.

Chloramphenicol did not show any significant concentration-dependent depression in our studies.

We conclude that it is important to be aware of the potential depression of cardiac function by antibiotics, particularly in patients who have diminished cardiac reserve and who are undergoing surgical procedures under anesthesia which may also depress cardiac function.

RÉSUMÉ

Les effets de la pénicilline G potassique, de la kanamycine, de la streptomycine et du chloramphénicol sur la contraction isométrique du muscle cardiaque isolé ont été étudiés chez le rat.

Les changements produits par la pénicilline G potassique sont causés par le potassium et non

par l'antibiotique lui-même. Quant à la kanamycine et à la streptomycine, leurs effets inotropes négatifs sont directs et proportionnels à la concentration de la solution employée. Le calcium antagonise l'action inotrope négative de la kanamycine sur le muscle cardiaque isolé. Le chloramphénicol déprime moins la contraction isométrique du muscle cardiaque isolé que les autres antibiotiques qui ont fait l'objet de cette étude. Les auteurs concluent qu'il faut être conscient que certains antibiotiques peuvent être la cause de dépression myocardique et que cette dépression peut aggraver l'état de patients dont la réserve cardiaque est limitée par la maladie ou les anesthésiques lors d'interventions chirurgicales.

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