POLYMYXIN B AND HEART MUSCLE

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SUMMARY

The effects of the polypeptide antibiotic, polymyxin B, on myocardial contractility were studied in the isolated rat heart muscle. Five different doses of polymyxin B were tested. There were no changes in contractility with doses ranging from the clinical therapeutic value to three times greater. There was an initial increase and then depression with a dose six times greater than the therapeutic dose. There was no direct competitive interaction between polymyxin B and halothane or Ca$^{2+}$. This suggests that polymyxin B does not depress the myocardium in clinical doses and does not interfere with Ca$^{2+}$ influx at the myocardial cell membrane.

It is well known that certain antibiotics can produce direct depression of the heart (Swain, Kiplinger and Brody, 1956; Cohen et al., 1970; Adams, 1976; Sohn and Katz, 1978) and induce neuromuscular paralysis (Pittinger and Adamson, 1972; Singh, Harvey and Marshall, 1978). There is some correlation between cardiac and neuromuscular activity. For example, penicillin neither depresses the heart nor induces neuromuscular paralysis. On the other hand, aminoglycoside antibiotics are direct cardiac depressants and produce neuromuscular block in clinical doses. Polymyxin B, a polypeptide, has been shown by experiment and clinical observation (Van Nyhuis, Miller and Fogdall, 1976) to have a strong neuromuscular blocking effect. Investigations in our department (Lee, Ricker and Katz, 1979) have shown that polymyxin B produced severe cardiovascular depression, but the mechanisms of action are not clear yet. We have evaluated the direct effect of polymyxin B on myocardial contractility.

METHOD

The isometric contractions of isolated rat heart muscle were examined. Isolated columns of left ventricular trabeculae carneae muscle were prepared. Each preparation was placed in an oxygenated (oxygen 95% and carbon dioxide 5%), 80-ml capacity muscle bath containing modified Krebs-bicarbonate solution, as described previously (Sohn and Katz, 1978). The temperature of the bath solution was held constant at 32 °C by a thermostat (Precision Scientific Co.). One end of the muscle was fixed and the other was connected to a Statham tension transducer (G 10 B-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 also at our laboratory.

Each muscle was stimulated with 2x8-mm platinum plate electrodes (placed about 10 mm apart, parallel to the muscle) at a frequency of 0.25 Hz with square wave pulse of 6.0 ms duration. In this preparation, no significant amount of catecholamine is released from the muscle (Koch-Weser, 1965). The length–tension curve of each preparation was determined after 30 min of isometric contractions. The average rat weight was 142.33 ± 10.97 g (mean ± SD) and maximum muscle length (the length at the peak of the length–tension curve) was 4.74 ± 0.64 mm.

Changes in muscle length, measured with a micrometer transformer, could be detected to 0.01 mm. The muscle length was held at the peak of the length–tension curve for the next 30 min for stabilization and then peak developed tension ($T_{pd}$), maximum rate of tension development (+dP/dt max) and relaxation (−dP/dt max) were recorded at a paper speed of 5 mm min$^{-1}$.

Polymyxin B studies

Polymyxin B (Pfizer) was prepared in a solution of 1 mg ml$^{-1}$ in sterile water (Invenex). Doses of 0.64 mg (therapeutic dose in 80-ml bath) and 0.5, 2, 3 and 6 times this dose were chosen for dose–response studies. The final concentrations in the 80-ml bath were 5.708 μmol (A) and 0.5 (B), 2 (C), 3 (D) and 6 times (E) respectively. One selected dose was added directly to the muscle bath solution and changes in...
isometric contractions were recorded. After completion of the recording, the bath solution was drained, the muscle was washed twice with fresh modified Krebs–bicarbonate solution and the bath was refilled.

**Interaction between polymyxin B and halothane**

The muscles in the bath were exposed to 1.4% of halothane before (one group) and after (another group) concentration E of polymyxin B was added to the bath. $T_{pd}$ and $\pm dP/dt_{\text{max}}$ were recorded continuously at the same paper speed, 5 mm min$^{-1}$.

Gases were collected just below-bath. Halothane concentrations were determined with a Perkin–Elmer 900 gas chromatograph with a standard halothane concentration supplied by the company.

**Interaction between polymyxin B and calcium**

Concentrations D and E of polymyxin B were tested with 0.05, 0.1 and 0.2 ml of 10% calcium chloride (Elkins–Sinn, Inc.) which contain 0.034, 0.068 and 0.136 mmol litre$^{-1}$ of Ca$^{2+}$ respectively.

Two different recordings were made at the same paper speed of 5 mm min$^{-1}$. One was a continuous recording with Ca$^{2+}$ added first and then polymyxin B. The other was with polymyxin B first and then Ca$^{2+}$.

**Sterile water for injection USP**

This was added in volumes (0.64 ml, $\times 0.5$, $\times 2$, $\times 3$ and $\times 6$) equal to those in the test groups, to evaluate the effect of the solvent itself.

**RESULTS**

Polymyxin B did not depress cardiac muscle contraction in clinical concentration or in higher concentrations up to three times greater (table I, fig. 1). A small initial increase in twitch was observed, but this was equal to that seen when the same volume of solvent was tested. In studies with the greatest concentration, E, of polymyxin B, initial increases and then slow progressive depressions were seen. About 30% depressions occurred at 30 min after administration of polymyxin B in concentration E (table I, figs 1 and 2). These depressions were progressive and

<table>
<thead>
<tr>
<th>B (µmol)</th>
<th>A</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tbody>
<tr>
<td>Experiments performed</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
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<tr>
<td>% Changes (mean ± SD)</td>
<td></td>
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<tr>
<td>$T_{pd}$</td>
<td>$-1.4 \pm 4.4$</td>
<td>$+0.2 \pm 7.9$</td>
<td>$+2.8 \pm 13.4$</td>
<td>$+2.4 \pm 5.2$</td>
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<td>$+dP/dt_{\text{max}}$</td>
<td>$-0.6 \pm 6.7$</td>
<td>$+3.4 \pm 9.7$</td>
<td>$-0.9 \pm 10.7$</td>
<td>$+0.9 \pm 4.1$</td>
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<tr>
<td>$-dP/dt_{\text{max}}$</td>
<td>$0 \pm 3.7$</td>
<td>$+2.3 \pm 11.1$</td>
<td>$-0.7 \pm 12.3$</td>
<td>$+1.5 \pm 4.5$</td>
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more than 80% depression occurred at 90 min after administration. These initial increases were produced by the same amount of solvent itself.

In the studies of concentration D of polymyxin B and 1.4% halothane, an interaction on isometric contraction was not seen. Polymyxin B in concentration D did not alter the direct effects of 1.4% halothane on myocardial contractility (fig. 3). In addition, 1.4% halothane depressed myocardium pretreated with polymyxin B to the same extent as halothane alone.

In the studies of interaction between polymyxin B and Ca$^{2+}$, concentration D or E of polymyxin B did not alter the magnitude of positive inotropic action of Ca$^{2+}$ on heart muscle. Ca$^{2+}$ caused the same degree of positive inotropy with concentration D.
DISCUSSION

Polymyxin B is among the very few membrane-active polypeptide antibiotics which show sufficient selective membrane toxicity and produce irreversible damage in cell membranes containing phosphatidylethanolamine as the dominant phospholipid (Hsu Chen and Feingold, 1973). Tissue phospholipid is located mainly in the cell membrane. One of its major functions is to contribute to the structure and function of cell membranes (Ansell, Hawthorne and Dawson, 1973). All biological membranes throughout nature are generally similar (Hsu Chen and Feingold, 1973) and all mammalian tissues contain, qualitatively, the same phospholipids but, quantitatively, different ones (Ansell, Hawthorne and Dawson, 1973). Thus one would expect polymyxin B to be clinically effective by affecting various host cell membranes.

Although the phosphatide content of the brain and the nervous system has been found to vary with age and development, nervous tissue possesses the largest phosphatide content of any of the organs and contains about three times as much as heart muscle (Witcoff, 1951; Rouer, Nelson and Fleischer, 1968). Thus one would anticipate a neuromuscular blocking action of polymyxin B, particularly under anaesthesia involving a neuromuscular blocker, as reported in a recent article (Van Nyhuis, Miller and Fogdall, 1976). The early investigation of the phosphatide content of muscle showed that the heart contains the greatest percentage of phosphatides (Witcoff, 1951). Since profound cardiovascular depression produced by polymyxin B was observed by other investigators (Lee, Ricker and Katz, 1979) and the mechanisms of action are not clear yet, we would postulate that polymyxin B may depress the cardiovascular system by means of (1) direct myocardial cell membrane damage similar to that seen in nervous tissue, (2) a direct ganglion blocking effect, (3) histamine release or (4) any combination of the above. So far, it has been reported that polymyxin B has a ganglion blocking effect and releases histamines (Naiman and Martin, 1967; Lee, Ricker, and Katz, 1979).

For studies of the direct effect of polymyxin B on heart muscle, the dose tested was chosen to reflect a blood concentration which can be achieved when polymyxin B is used to treat infection in man (up to 0.034 0.068 0.136 mmol litre⁻¹).
8 µg ml⁻¹). In an 80-ml muscle bath, the dose would be 640 µg (0.64 mg) which is equivalent to 5.708 µmol concentration. When polymyxin B is administered i.v., transient cardiac concentrations may be much greater than the clinical therapeutic concentration. Thus we performed these studies with clinical concentrations of polymyxin B in blood, 0.5, 2, 3 and 6 times greater than steady-state values. There were no depressant effects in clinical and greater concentrations (up to three times). Although initial increases in tension were seen, similar increases could be produced with the same amount of solvent alone. In studies with very large doses (6 × clinical dose) progressive depression could be observed. Based on the work of Lee, Ricker and Katz (1979) and the present findings, we conclude that the causes of cardiovascular depression produced by clinical doses of polymyxin B are not a result of direct myocardial depression by polymyxin B but may be produced by direct sympathetic ganglion blocking effects of polymyxin B and histamine release.

Our results from heart muscle preparations are different from those of Van Nyhuys, Miller and Fogdall (1976), who showed a dose-dependent depression of the hemidiaphragm–phrenic nerve preparation. ED₅₀ of polymyxin B (23 µg ml⁻¹) observed with skeletal muscle did not depress cardiac muscle contractility in our experiments.

In the studies by Van Nyhuys, Miller and Fogdall (1976) with therapeutic doses (5 µg ml⁻¹) of polymyxin B, the twitch tension was not depressed. These findings support the conclusion of Hsu Chen and Feingold (1973) that the polymyxin B susceptibility of biological membranes requires not only the presence of target sites in membrane such as phosphatidylethanolamine and a threshold density of these sites on the membrane surface, but also certain concentrations of polymyxin B. Myocardial depression produced by our large dose (6 × clinical dose) of polymyxin B is likely to be a result of toxicity from massive overdose. Ca²⁺ exerted a positive inotropic effect in heart muscle with or without pretreatment with polymyxin B. Similar results were observed in the halothane and polymyxin B interaction studies.

In previous studies, the direct myocardial depression and neuromuscular blocking action produced by aminoglycoside antibiotics were antagonized readily by Ca²⁺ (Corrado, 1963; Sohn and Katz, 1978), but lack of antagonism of polymyxin B–induced paralysis by Ca²⁺ (Adamson, Marshall and Long, 1960; Naiman and Martin, 1967) probably indicates a different mechanism of action. Although a clinical dose of polymyxin B does not interfere with Ca²⁺ influx, depressions produced in cardiac muscle by large doses of polymyxin B can be restored to normal by Ca²⁺. It appears that small fractions of phosphatidylethanolamine in myocardial cell membrane are damaged by polymyxin B, but other fractions of phospholipid in membrane continue to possess a high affinity for Ca²⁺ (Ansell, Hawthorne and Dawson, 1973).

In conclusion, clinical therapeutic doses of polymyxin B did not depress myocardial contractility, but massive overdoses did so. Clinical therapeutic doses do not produce any clinical side-effects but may produce undesirable effects in the presence of anaesthetic agents capable of producing depression.

ACKNOWLEDGEMENTS

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REFERENCES


LA POLYMYXINE B ET LE MUSCLE DU COEUR

RESUME
On a procedé à une étude sur les effets qu’a l’antibiotique polypeptide: polymyxine B, sur la contractilité du myocarde, sur le muscle isolé du cœur d’un rat. On a essayé cinq doses différentes de polymyxine B. Il n’y a eu aucun changement dans la contractilité avec les doses allant de la plage des niveaux thérapeutiques cliniques aux doses trois fois supérieures. Il y a eu une augmentation initiale, suivie d’une dépression, avec une dose six fois plus forte que la dose thérapeutique. Il n’y a eu aucune interaction concurrente directe entre la polymyxine B et l’halothane. Ceci laisse penser que la polymyxine B ne déprime pas le myocarde lorsqu’on l’administre à des doses cliniques et ne gêne pas l’ influx de Ca²⁺ à la membrane de la cellule myocardiale.

POLIMIXINA B Y EL MUSCULO CARDIACO

SUMARIO
Se estudiaron los efectos del antibiótico polipéptico, polimixina B, sobre la contractilidad miocardial en el músculo cardíaco aislado de un ratón. Se hicieron pruebas con cinco dosis distintas de polimixina B. No hubo cambio en la contractilidad con dosis fluctuando del nivel terapéutico clínico hasta tres veces mayor. Hubo un aumento inicial seguido de una depresión con una dosis seis veces mayor de la dosis terapéutica. No hubo ninguna interacción competitiva directa entre la polimixina B y el halotano o el Ca²⁺. Esto hace pensar que la polimixina B no deprime el miocardio en dosis clínicas y no interfiere con el influxo Ca²⁺ en la membrana celular miocardial.

POLYMYXIN B UND DER HERZMUSKEL

ZUSAMMENFASSUNG

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