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The influence of pH on the cardiac depressant action of tobramycin

C. F. NAME*, O. VETTORE, A. ANTONIO, *Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto-U.S.P., 14049 Ribeirão Preto, S.P., Brazil*

Abstract—Tobramycin, like other aminoglycoside antibiotics, is a basic compound which causes a reduction of cardiac contractile force and inhibits the inotropic action of calcium. Its antagonistic action towards calcium had been analysed at different pH values of the solution bathing the rat left atrium. The isometric tension of the electrically driven atrium (4 Hz, 2 ms, supramaximal voltage) in response to cumulative increase in the bath calcium concentration (2·0 to 32·0 mM) was measured in the absence and in the presence of $2{\cdot}0 \times 10^{-5}$ M tobramycin. The increase in pH from 6·6 to 8·2, which decreased the ionization of tobramycin, reduced the cardiac depression. Similar experiments were carried with other cardiac depressants. Thus, the effect of methoxyverapamil ($3{\cdot}5 \times 10^{-7}$ M), also a basic compound, was increased at higher values of pH; the acidic pentobarbitone (4×10^{-5} M) was less effective at more alkaline pH; the cardiac depression of the non-electrolyte ethanol (2×10^{-4} M) was practically the same over the pH range studied. The time for equilibrium of the antagonism was small for tobramycin, moreover, its antagonism could only be fully reversed by an increase in extracellular calcium. These results suggest a competitive type of antagonism, between the ionized fraction of tobramycin and calcium, for superficial sites of the sarcolemma. The effect of methoxyverapamil, pentobarbitone and ethanol seems to occur at a more internal site of the cardiac cell since the unionized form of the drug is the most active.

Several groups of chemically unrelated drugs have in common the ability to depress the cardiac contractility, an effect which can be overcome by increasing the extracellular calcium concentration. These chemicals are called calcium antagonists or calcium channel blockers since they are believed to act by interfering with the influx of extracellular calcium into cells (Fleckestein 1977; Rahwan et al 1979; Needleman et al 1985). Some are more specific and currently used in therapeutics, but most of the other drugs exhibit the calcium blocking action as an additional or side effect to its main use. This is the case, for example, of the aminoglycoside antibiotics which are known to depress the cardiac contractility (Adams 1975). They are polybasic, highly hydrophilic compounds that do not penetrate the cell membranes and their action is, therefore, assumed to be at superficial sites of the sarcolemma (Adams & Durrett 1978; Tulkens & Trouet 1978; Lüllmann & Schwarz 1985). Most of the other drugs, including the therapeutically used calcium antagonists have, on the other hand, a lipophilic moiety which is suggestive of an internal site of action (Church & Zsóter 1980; Spedding 1985).

The present experiments were designed to test whether changes in the ionization of the aminoglycoside tobramycin, induced by different values of pH of the nutrient solution, could affect the magnitude of its negative inotropic action. The cardiac depressant action of tobramycin was compared with that of the basic compound methoxyverapamil, the acidic pentobarbital and with ethanol which does not ionize.

Materials and methods

Wistar rats of either sex, 350–450 g, were killed by stunning and exsanguination. The heart was quickly removed and placed in a 100 mL dish containing a modified Ringer-Locke solution aerated with pure oxygen. The left atrium was carefully dissected and suspended in a 7·0 mL organ bath containing the oxygenated nutrient solution and kept at a temperature of 30°C. One end of the atrium was fixed between two stimulation platinum electrodes and the other end was tied to a Stathan UC3 force displacement transducer. A resting tension of 1 g was maintained throughout each experiment. The preparation was stimulated by square-wave impulses of 2 ms duration and supramaximal voltage at a frequency of 4 Hz. The resulting contractions were continuously recorded on a Beckman Dynograph. To analyse the influence of pH on the action of the test drugs at increasing values of extracellular calcium concentrations, we used a modified Ringer-Locke solution of the following composition, in mM: NaCl 154·0, KCl 5·6, $CaCl_2$ 2·0, THAM 5·0, glucose 5·5. The use of THAM (tris-hydroxymethyl-aminomethane) instead of bicarbonate as the buffer, although decreasing the basal contractility slightly (Durrett & Adams 1979), was necessary to avoid precipitation of the higher concentrations of calcium in the presence of bicarbonate and phosphate (see Thyrum 1974). With this THAM-buffered medium we were able to study the influence of pH over the range from 6·6 to 8·2 on the tension developed by the electrically driven atrium. Below 6·6 the tension developed was small and irregular, and above 8·2 the preparation did not last for more than 30–40 min. The adjustments of the pH of the nutrient solution were achieved immediately before starting the experiments by addition of 1·0 M HCl or NaOH.

After an equilibration period of 50 min, the calcium antagonistic action of the test drugs were determined by comparing two cumulative dose-response curves for calcium obtained before the addition of the inhibitor and 30 min after. The cumulative dose-response curves were obtained by adding each concentration of calcium (in a volume of 0·05 mL) directly to the muscle chamber only after the inotropic effect of the previous concentration had reached a plateau. In most experiments the maximal tension developed by the left atria was in the range of 16–32 mM $[Ca]_o$. The preparation was then thoroughly washed and 30 min later a second dose-response curve constructed in the absence or presence of the antagonist added directly to the organ bath.

Only one concentration of each antagonist producing an approximately 50–60% reduction of the basal tension (at pH 7·4 and 2·0 mM Ca) was used; in the case of tobramycin the concentration used was higher because its effect was easier to reverse by calcium.

The time for equilibrium of the antagonist with the preparation was shorter for ethanol (less than 1 min) and tobramycin (2–3 min) than for pentobarbitone (12–15 min) or methoxyverapamil (18–20 min).

Drugs. The drugs used were ethanol (Merck), methoxyverapamil (compound D600, Knoll), sodium pentobarbitone (Abbott), tobramycin sulphate (Lilly), tris-hydroxymethylaminomethane (THAM, Sigma).

Results

In the electrically driven left antria increases in tension occurred with increasing pH over the range from 6·6 to 8·2. This was the same for extracellular calium concentrations of

*Present address: Departamento de Ciências Fisiológicas, Universidade Estadual de Londrina, 86100-Londrina, PR, Brazil.
Correspondence to: A. Antonio, Departmento de Farmacologia, Faculdade de Medicina de Ribeirão Preto-U.S.P., 14049 Ribeirão Preto, S.P., Brazil.

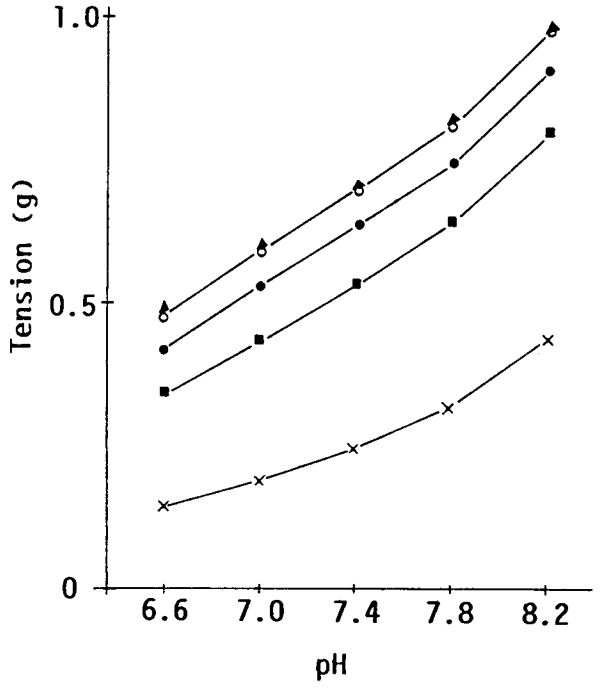

FIG. 1. The influence of pH on the tension developed by the electrically driven left atrium at increasing values of nutrient solution calcium, in mM: 2·0 (X), 5·3 (■), 9·0 (●), 16·0 (○), 32·0 (▲). The individual points represent the mean of 20–24 experiments. The maximal tension developed at each value of pH is already attained with 16·0 mM calcium.

2·0 up to 16·0 mM (Fig. 1); no further increase in tension was obtained when the bath calcium concentration was doubled.

Tobramycin, 2×10^{-5} M, inhibited the increase in tension of the atria elicited by calcium; the inhibitory action was most marked at pH values below 7·4 and was fully reversed by increasing the extracellular calcium concentration (Fig. 2C). The same Figure shows the effects of methoxyverapamil ($3{\cdot}5 \times 10^{-7}$ M), pentobarbitone (4×10^{-5} M) and ethanol (2×10^{-4} M). Methoxyverapamil was more potent than tobramycin as an inhibitor of the responses to calcium, and was oppositely affected by the changes in pH; that is, the greater inhibition was obtained at pH values above 7·4 (Fig. 2A).

The influence of pH on the inhibitory action of pentobarbitone was similar to that observed with tobramycin; that is, the greater inhibition was obtained at pH values below 7·4 (Fig. 2B). The effects of ethanol were not markedly influenced by variations of the pH in the range 6·6 to 8·2 (Fig. 2D).

From the concentrations selected and comparison of the inhibitory effect of the four compounds at pH 7·4 and at the 2·0 mM calcium concentration, it can be seen that methoxyverapamil was about 50–100 times more potent than tobramycin or pentobarbitone while ethanol was about 5–10 times less potent than tobramycin or pentobarbitone as a cardiac depressant. It can also be seen that the antagonistic action of methoxyverapamil, pentobarbitone or ethanol, contrary to that of tobramycin, was not fully reversed by the increase in the extracellular calcium concentration.

Discussion

It is well known that the tension developed by cardiac muscle is increased as the pH of the bathing medium increases. This is probably due to an antagonism between the hydrogen ions and calcium (Vaughan Williams 1955; Mattiazzi et al 1979) and explains the increased baseline tension of the electrically driven left atria at higher values of pH.

Apart from the change in tension, the modification of the pH of the nutrient solution bathing the atrium may influence the ionization and probably the pharmacological action of drugs acting on the cardiac muscle. Our experiments, therefore, were designed to test whether ionization may effect the cardiac depression of tobramycin, a basic compound ($pKa \simeq 8{\cdot}5$) which shows a negative inotropic response similar to other aminoglycoside antibiotics (Adams 1975; Adams & Durrett 1978). The inhibitory effect of tobramycin was compared with that of the barbituric acid derivative, pentobarbitone ($pKa \simeq 7{\cdot}5$), with methoxyverapamil which is also a basic compound ($pKa \simeq 8{\cdot}5$) and with ethanol, which does not ionize.

We observed that tobramycin was more potent at lower values

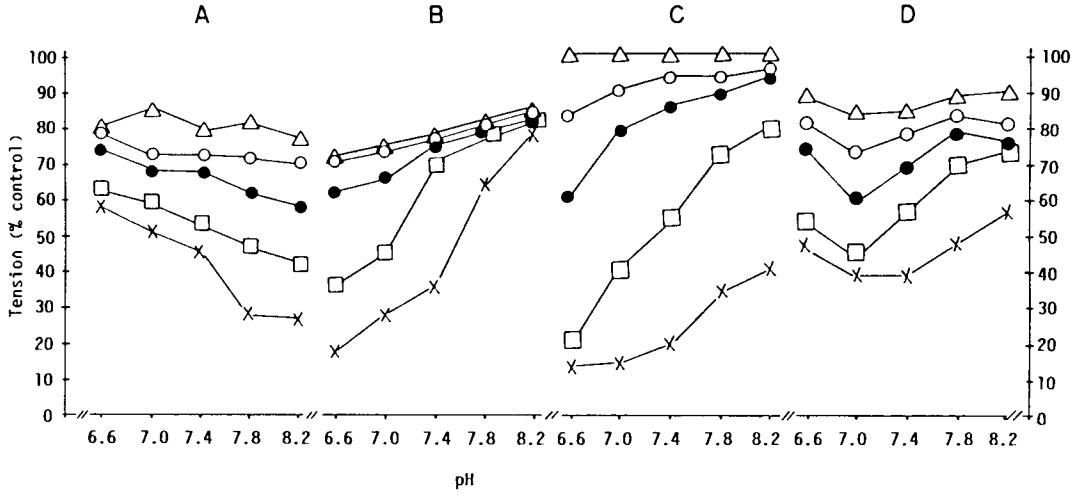

FIG. 2. The influence of pH on the negative inotropic action of $3{\cdot}5 \times 10^{-7}$ M methoxyverapamil (A), 4×10^{-5} M pentobarbitone (B), 2×10^{-5} M tobramycin (C) and 2×10^{-4} M ethanol (D) at increasing values of the nutrient solution calcium concentration, in mM: 2·0 (X), 5·3 (□), 9·0 (●), 16·0 (○), 32·0 (△). In abscissa the values of pH and in ordinates the tension of the electrically driven left atrium as percent of the control. Each value represent the mean of 5–6 experiments. Only the effect of tobramycin (C) is fully reversed by the increase in calcium concentration.

of pH; in other words, the cardiac depressant action and calcium-blocking effect of the antibiotic increase with the ionization of the drug. This is suggestive of a superficial site in the sarcolemma for the calcium-antagonistic action of tobramycin and reinforces a similar proposal for the action of other aminoglycoside antibiotics (Tulkens & Trouet 1978; Lüllmann & Vollmer 1982; Lüllman & Schwarz 1985). The rapid equilibration of the antagonism, in about 2–3 min, is another indication of a superficial site for the action of tobramycin.

Contrary to tobramycin, the cardiac depressant action of methoxyverapamil, which is also a basic compound, was more intense at higher values of pH; i.e. the inhibitory action of this calcium-channel blocking agent decreased with the ionization of the drug. This suggests that the unionized form of methoxyverapamil, which is lipid-soluble, penetrates the cell membrane of the cardiac cell and blocks the calcium channel at an internal site. Our results are in agreement with previous studies indicating a more profound locus for the action of verapamil and its derivatives (Church & Zsóter 1980). The longer time required for the equilibrium of methoxyverapamil with the atria, of about 20 min, reinforces the above conclusion of a more internal site for its inhibitory action.

The negative inotropism of pentobarbitone, a weak acid, was more intense at the lower values of pH; in other words, the calcium-antagonistic action of pentobarbitone increases with the increase in the unionized form of the compound. This finding and the relatively long time for equilibration of the antagonism, about 15 min, are in agreement with other studies indicating an internal site for the action of the barbiturate (Daniel et al 1962; Nayler & Szeto 1972; Khan 1980).

As should be expected, the cardiac depressant action of the non-electrolyte ethanol was not substantially influenced by the pH, a property already observed in other tissues (Antonio et al 1970).

These results suggest that only tobramycin is more active when the ionized form predominates. The effect of methoxyverapamil, pentobarbitone and ethanol may be obtained after the inhibitor has been dissolved in the lipid material of the cell membrane as the unionized form of the drug is the most active.

The antagonism studied in the present work is reversed by increasing the bath calcium concentration; however, only the cardiac depressant action of tobramycin was completely reversed.

When taken together, these observations suggest a competitive type of antagonism between the ionized form of tobramycin and calcium for superficial sites of the sarcolemma. The calcium-antagonistic action of the three other compounds is only obtained after the inhibitor has been dissolved in the lipid material of the cell membrane.

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