Effects of amiloride in guinea-pig and rat left atrial contraction as affected by frequency of stimulation and $[Ca^{2+}]_0$ – $[Na^+]_0$ ratio: role of Na^+/Ca^{2+} exchange

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- 1 The effect of amiloride (0.5 mM) on guinea-pig and rat left atria driven at various rates of stimulation and different $[Ca^{2+}]_0$ – $[Na^+]_0$ ratios has been studied.
- 2 Amiloride elicited a positive inotropic response in guinea-pig left atria driven at 0.1 Hz, 0.5 Hz and 1 Hz when $[Ca^{2+}]_0$ was 3.6 mM, 1.8 mM and 0.9 mM respectively but not when $[Ca^{2+}]_0$ was 2.7 mM at 0.1 Hz, 0.9 mM at 0.5 Hz and 0.45 mM at 1 Hz.
- 3 A positive inotropic response was obtained in guinea-pig left atria driven at 0.1 Hz and 1 Hz when $[Ca^{2+}]_0$ – $[Na^+]_0^2$ was increased respectively from 8×10^{-5} to 16×10^{-5} and from 2×10^{-5} to 8×10^{-5} . The positive inotropic effect was evident only when the ratio was increased by increasing $[Ca^{2+}]_0$ and not by decreasing $[Na^+]_0$.
- 4 In the presence of amiloride, the force of contraction of guinea-pig left atria decreased instead of increasing, when the rate of stimulation was lowered from 1 Hz to 0.01 Hz. Amiloride inhibited the post-rest potentiation.
- 5 In rat left atria amiloride was devoid of any effect in all the above-mentioned experimental conditions.
- 6 It is suggested that the pattern of cardiac actions of amiloride can be explained by the inhibition of the Na^+/Ca^{2+} exchange system.

Introduction

Amiloride is a potassium-sparing diuretic that has been proposed as a useful pharmacological tool for elucidating the molecular basis and physiological role of Na transport systems in tissues and cells (Benos, 1982). In various epithelial tissues amiloride was shown to inhibit Na transport through two pathways, namely a conductive pathway and a Na^+/H^+ exchange system (Cuthbert & Fanelli, 1978; Frelin *et al.*, 1984). Recently, it has been demonstrated that amiloride inhibits Na^+/Ca^{2+} exchange in cardiac sarcolemmal vesicles (Floreani & Luciani, 1984; Siegl *et al.*, 1984; Debetto *et al.*, 1987) and prevents vascular smooth muscle contraction most probably through inhibition of Na^+/Ca^{2+} exchange (Bova *et al.*, 1988). *In vivo* and *in vitro* studies have shown that amiloride affected the inotropic and toxic responses of the cardiac muscle to digitalis glycosides (Seller *et al.*, 1975a,b; Waldorff *et al.*, 1981; Floreani & Luciani, 1984; Kennedy *et al.*,

1985). In electrophysiological studies amiloride induced lengthening of the action potential duration and reduction of the amplitude and the maximum rate of rise in ventricular muscle, sino-atrial node and Purkinje fibres (Marchese *et al.*, 1985; Satoh & Hashimoto, 1986). Furthermore, it has been shown that amiloride induced positive inotropic and negative chronotropic effects in isolated atrial muscle (Pousti & Khoyi, 1979). These effects have been confirmed (Floreani & Luciani, 1984; Kennedy *et al.*, 1986; Barret & Kau, 1986) and the hypothesis that the positive inotropic effect of amiloride is due to the inhibition of Na^+/Ca^{2+} exchange has been put forward (Floreani & Luciani, 1984; Luciani & Floreani, 1985; Kennedy *et al.*, 1986). A negative inotropic effect in cultured chick heart cells has been reported for amiloride (Kim & Smith, 1986) and a negative (Siegl *et al.*, 1984) or positive inotropic response (Floreani *et al.*, 1987) in guinea-pig papillary muscle has been observed for the amiloride derivative, 3'-4'-dichlorobenzamil.

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Figure 1 Record showing the time course of the positive inotropic effect of amiloride (A) in guinea-pig left atria driven at 1 Hz in normal $[Ca^{2+}]_o$ (1.8 mM) and $[Na^+]_o$ (149.2 mM). The mean values ($\pm$ s.e. mean) of control contraction and of the contraction in the presence of amiloride were 90 ± 27 mg and 650 ± 140 mg respectively (5 preparations).

Since it is known that the Na^+/Ca^{2+} exchange system can move calcium in either direction across the cardiac sarcolemma (Sheu & Blaustein, 1986), the discrepancies in the effects of amiloride on cardiac contractility may be explained by postulating that the direction of Ca^{2+} movements through the Na^+/Ca^{2+} exchange may have different relevance in cardiac muscle of different species and under different experimental conditions.

In addition to the inhibition of the Na^+/Ca^{2+} exchange, several mechanisms have been proposed to account for the cardiac effects of amiloride, namely changes in ionic currents through membrane channels, inhibition of Na^+/K^+ ATPase and of Na^+/H^+ exchange (Frelin *et al.*, 1984; Marchese *et al.*, 1984; Kennedy *et al.*, 1985; Kim & Smith, 1986; Satoh & Hashimoto, 1986).

It has been hypothesized that Na^+/Ca^{2+} exchange may play a role in the influence exerted on cardiac contractility by the pattern of stimulation and by the $[Ca^{2+}]_o/[Na^+]_o$ ratio, which is believed to determine the amount of intracellular calcium available for binding to the contractile proteins (Orkand, 1968; Allen *et al.*, 1976; Lee *et al.*, 1985; Sutko *et al.*, 1986; Gibbons, 1986).

The effect of amiloride in guinea-pig and rat atrial myocardium driven at various frequencies of stimulation and in ionic conditions that alter the transmembrane distribution of either sodium or calcium has been investigated. Results show that the positive inotropic effect of amiloride is species-dependent (the drug is not active in rat atria) and is strictly related to the frequency of stimulation and to the $[Ca^{2+}]_o/[Na^+]_o$ ratio.

Methods

The experiments were performed on guinea-pig (250–350 g) and rat (200–300 g) left atria. The tissues were attached to two platinum hooks mounted on one

end of a plexiglas holder, and placed vertically in water-jacketed 16 ml organ baths containing physiological salt solution continuously bubbled with 95% O_2 : 5% CO_2 at 35°C and pH 7.36–7.40. The composition of the medium was (mM): NaCl 137, KCl 5.4, $CaCl_2 \cdot 2H_2O$ 1.8, $MgCl_2 \cdot 6H_2O$ 1, $NaH_2PO_4 \cdot 2H_2O$ 0.4, $NaHCO_3$ 12 and glucose 5.4.

The preparations were stimulated through the platinum hooks, which were connected to a GRASS S44 stimulator, by square wave pulses at a strength of twice the threshold voltage and a duration of 0.8–1.2 ms. Force generation was monitored by connecting one end of the tissue to an isometric force transducer (Battaglia Rangoni KV 200). A rough length-tension relationship was mapped out and the tissues were kept at the length at which submaximal tension had developed. An equilibration time of 1–1.5 h was allowed before the start of the experiments. The low- Na^+ solution was made by equiosmolar substitution of sucrose for NaCl.

In the trials designed to explore the force-frequency relationship, the frequencies ranged from 1 Hz to 0.01 Hz or from 1 Hz to 3 Hz and the steady force at each stimulation rate was considered. The amplitude-interval relationship of the first contraction elicited after different times of rest was determined by driving the atria under steady-state conditions at 1 Hz. The amplitude measured was that of the first contraction elicited 1 min, 2 min, 4 min, 8 min, 16 min after the end of the periods of steady-state condition. Since the effect of amiloride was very slow to reach equilibrium, in all the experiments in which the effect of the drug was observed in relation to the changes of the parameters of stimulation, we used one atrium as a control, driving it at 1 Hz for the same time as the atrium exposed to the drug.

In this study only one concentration of amiloride was used (0.5 mM), a concentration which is very close to the EC_{50} of the inotropic effect (Florenzi & Luciani, 1984).

Results

Effect of frequency of stimulation and $[Ca^{2+}]_o$ on inotropic response to amiloride

Amiloride (0.5 mM) elicited a very slowly developing positive inotropic response (peak effect within about 4 h, increase from the control $659 \pm 129\%$) in guinea-pig left atria bathed in normal physiological solution and stimulated at 1 Hz (Figure 1). Rat left atria in the same experimental conditions did not respond to amiloride.

When amiloride was applied to guinea-pig left atria driven at 0.1 Hz, the positive inotropic response did not occur (Figure 2a) unless Ca^{2+} was increased to 3.6 mM (Figure 2b). Since a direct relation has been found between intracellular Ca^{2+} activity and extracellular $[Ca^{2+}]$ or frequency of stimulation (Chapman, 1983), the positive inotropic effect of amiloride appeared to be strictly related to intracellular $[Ca^{2+}]$. To analyse this effect, we exposed guinea-pig left atria to amiloride which were driven at different frequencies of stimulation in different extracellular $[Ca^{2+}]$. As shown in Figure 3, a positive inotropic effect was not observed at 0.1 Hz,

Figure 3 In guinea-pig left atria driven at 0.1 Hz, 0.5 Hz and 1 Hz two critical extracellular Ca^{2+} concentrations for each frequency of stimulation were determined: the lowest concentration (●) at which amiloride elicited positive inotropic response and the highest concentration (○) at which amiloride had no positive inotropic effect. The observations were made on separate atria, and at least four atria were used for each combination of $[Ca^{2+}]_o$ and frequency.

Figure 2 Guinea-pig left atria driven at 0.1 Hz. (a) Amiloride (A) had no positive inotropic effect in the atria bathed in normal Ca^{2+} ($CaCl_2$ 1.8 mM). The mean value ($\pm$ s.e. mean) of contraction was 110 ± 35 mg. Five preparations were used. (b) Amiloride elicited positive inotropic response in left atria bathed in high Ca^{2+} ($CaCl_2$ 3.6 mM). The mean values ($\pm$ s.e. mean) of control contraction and of contraction in the presence of amiloride were 519 ± 76 mg and 1170 ± 136 mg respectively. $[Na^+]_o$ was 149.2 mM in (a) and (b).

Figure 4 Record showing the positive inotropic effect of amiloride (A) in guinea-pig left atria driven at 0.1 Hz previously exposed to Bay K 8644 ($2 \mu\text{M}$) (CaCl_2 1.8 mM). The mean value ($\pm$ s.e. mean) of the contraction in the presence of Bay K 8644 was $333 \text{ mg} \pm 45$; after the addition of amiloride the contraction was $593 \text{ mg} \pm 75$. Three preparations were used.

0.5 Hz or 1 Hz in extracellular $[\text{Ca}^{2+}]$ below 2.7 mM, 0.9 mM or 0.45 mM respectively. The positive inotropic response was elicited by amiloride at 0.1 Hz in $[\text{Ca}^{2+}]_o$ 3.6 mM, at 0.5 Hz in $[\text{Ca}^{2+}]_o$ 1.8 mM and at 1 Hz in $[\text{Ca}^{2+}]_o$ 0.9 mM.

Therefore for each rate of stimulation a certain extracellular Ca^{2+} concentration existed below which amiloride was not able to induce the positive inotropic response. Further support for the relationship between intracellular $[\text{Ca}^{2+}]$ and the effect of amiloride was given by the experiment illustrated in Figure 4 in which positive inotropic response to the drug was observed in guinea-pig left atria driven at 0.1 Hz in $[\text{Ca}^{2+}]_o$ 1.8 mM provided that the tissue was previously exposed to Bay K 8644, which is known to enhance Ca^{2+} entry into cardiac cells (Brown *et al.*, 1984). An initial negative inotropic effect was observed, the duration and magnitude of

which varied in different experimental conditions (Figures 1, 2, 4). Although no clear explanation of this effect can be given at present, we suggest that the transient negative inotropic response is due either to the known inhibitory effect of amiloride on Na^+/H^+ exchange (Frelin *et al.*, 1984) or to a blockade of T-type Ca^{2+} channels (Tang *et al.*, 1988).

Guinea-pig left atria driven at 1 Hz or 0.1 Hz in different $[\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2$

Contractility of isolated heart preparations is strongly influenced not only by extracellular $[\text{Ca}^{2+}]$ but also by extracellular $[\text{Na}^+]$ (Blaustein, 1974). It has been found that intracellular Ca^{2+} and force of contraction are approximately related to the ratio $[\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2$ (Chapman, 1983). The pattern of the cardiac action of amiloride with respect to the

Figure 5 Influence of $[\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2$ ratio at 0.1 Hz and 1 Hz on the ability of amiloride to induce positive inotropic responses in guinea-pig left atria. The effect of amiloride was tested at two different $[\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2$ ratios for each frequency of stimulation. The $[\text{Ca}^{2+}]_o/[\text{Na}^+]_o^2$ was changed either by increasing $[\text{Ca}^{2+}]_o$ or by decreasing $[\text{Na}^+]_o$. In each condition at least four preparations were used.

$[Ca^{2+}]_o/[Na^+]_o^2$ ratio respectively at 1 Hz and 0.1 Hz was therefore examined. A positive inotropic response was obtained at 0.1 Hz when the $[Ca^{2+}]_o/[Na^+]_o^2$ ratio was increased from 8×10^{-5} to 16×10^{-5} and at 1 Hz from 2×10^{-5} to 8×10^{-5} . However, as shown in Figure 5, a positive inotropic effect was elicited only when the ratio was increased by increasing $[Ca^{2+}]_o$ and not by decreasing $[Na^+]_o$.

Thus the drug did or did not induce positive inotropic response at equivalent $[Ca^{2+}]_o/[Na^+]_o^2$ ratios, according to how the value was reached.

Force-frequency relationship and post-rest contractions of guinea-pig and rat left atria

The force-frequency relationship and post-rest inotropy have been used as an indirect approach to study the mechanisms which determine at any time the amount of Ca^{2+} available for the contractile proteins (Blinks & Koch-Weser, 1961; Kruta, 1964; Lipsius *et al.*, 1982).

Amiloride did not modify the force-frequency relationship of guinea-pig left atria in the range of high frequencies, from 1 Hz to 3 Hz but strongly influenced the behaviour of the force of contraction in response to a decrease in the rate of stimulation, from 1 Hz to 0.01 Hz. In the presence of the drug, the contractility of guinea-pig left atria decreased, instead of increasing, when the frequency was lowered from 1 Hz to 0.01 Hz (Figure 6). Moreover, amiloride inhibited the potentiation of the first beat observed when the preparations driven at 1 Hz were left quiescent for 1 min, 2 min, 4 min, 8 min and 16 min (Figure 7). Amiloride did not influence rat left atria exposed to the same changes of the modalities of stimulation.

Discussion

The results show that the positive inotropic effect of amiloride in guinea-pig left atria was markedly affected by frequency of stimulation and extracellular calcium and sodium concentrations, in that it was elicited only at certain combinations of values of these parameters. Rat left atria did not respond to amiloride.

The dependence of contractile behaviour on external $[Ca^{2+}]$ and stimulation frequency of isolated cardiac muscle preparations from most mammalian species is a well known phenomenon (Blinks & Koch-Weser, 1961; Gibbons, 1986). In ventricular muscle a relationship has been established between the strength of the twitch, intracellular calcium concentration and frequency of driving (Bers, 1983; Chapman, 1983; Allen *et al.*, 1984). The relation

Figure 6 Force-frequency relationship of guinea-pig left atria as affected by amiloride. In the range of low frequencies, the contraction increases with decreasing frequency (○); in the presence of amiloride (●) a direct relation between force and frequency was observed. Since the effect of amiloride was very slow to reach equilibrium, two sets of four atria were used, one set serving as a control. The atria were driven at 1 Hz for at least 210 min and then the frequency was decreased. Each point represents the mean value of four observations, vertical bars showing s.e.mean.

between the force of contraction and intracellular calcium, calculated from aequorin signals, was found to be unchanged when the contraction was increased either by raising the calcium concentration of the bathing solution or by increasing the frequency of stimulation (Allen & Kurihara, 1980). Measurement of intracellular Ca^{2+} activity with Ca^{2+} -sensitive microelectrodes has demonstrated that intracellular calcium increases with stimulation rate (Lado *et al.*, 1982) or with increasing extracellular calcium (Sheu & Fozzard, 1982).

Amiloride elicited positive inotropic responses in guinea-pig atria driven at 1 Hz and 0.5 Hz when bathed in external $[Ca^{2+}]$ 1.8 mM but did not when the external $[Ca^{2+}]$ was lowered to 0.45 mM and to 0.9 mM respectively. At 0.1 Hz, amiloride did not exert a positive inotropic effect unless $[Ca^{2+}]_o$ was increased from 1.8 mM to 3.6 mM or the atria were previously exposed to the calcium agonist Bay K 8644. This pattern of action suggests that intracellular $[Ca^{2+}]$ critically influenced the effect of amiloride on the force of contraction, since the appearance of the inotropic effect seemed to be closely linked to experimental conditions in which intracellular calcium was raised above a certain level by increasing the frequency, the bathing $[Ca^{2+}]$ or by adding Bay K 8644.

Figure 7 Inhibition of guinea-pig left atria post-rest potentiation by amiloride (●). The atria were subjected to rest intervals of 1, 2, 4, 8 and 16 min separated by 15 min periods of uninterrupted 1 Hz stimulation. The force of contraction before and of the first twitch after the rest periods were considered respectively as the steady-state (C) and the post-rest (T) response. To avoid bias due to the long time required for reaching the equilibrium effect of amiloride, two sets of 4 atria were used, one serving as a control (○). Each point is the mean of four observations, vertical bars showing s.e.mean.

However, two points have to be considered. (1) At the temperature used in our experiments, the frequency-strength relation of guinea-pig atria revealed that the steady level of the strength at 0.1 Hz and at 1 Hz were quite similar. Although intracellular $[Ca^{2+}]$ was not measured, assuming that the magnitude of developed tension gives an estimate of the Ca^{2+} involved in excitation-contraction coupling (Sheu & Fozzard, 1982; Chapman, 1983; Blinks, 1986), it is likely that the amount of Ca^{2+} available for the contraction was almost the same at 0.1 Hz and at 1 Hz. Moreover, in the range of frequencies we explored, the lowest contraction was observed at 0.5 Hz, a frequency at which amiloride was effective in eliciting positive inotropic response in normal external $[Ca^{2+}]$. (2) It has been established that the contractility of isolated heart preparations depends on the ratio of $[Ca^{2+}]$ to $[Na^+]$ in the bath (Sheu & Blaustein, 1986; Gibbons, 1986). Therefore intracellular $[Ca^{2+}]$ and developed tension can be increased not only by raising external $[Ca^{2+}]$ but also by lowering external $[Na^+]^2$ (Sheu & Fozzard, 1982; Allen *et al.*, 1983; Chapman, 1983). When $[Ca^{2+}]_0$ was too low to allow the inotropic effect of amiloride, raising external $[Ca^{2+}]$ or lowering external $[Na^+]$ should have increased the availability of intracellular Ca^{2+} to a similar extent, since these changes were designed to increase the value of the $[Ca^{2+}]_0/[Na^+]_0^2$ ratio by the same magnitude at each rate of stimulation. If

the positive inotropic response to amiloride was determined by a certain level of intracellular $[Ca^{2+}]$, irrespective of the mechanism by which this level was achieved, the drug should have had an inotropic effect when intracellular $[Ca^{2+}]$ was increased, but this was not the case. Thus, the inotropic ability of amiloride appears to be critically correlated not only with intracellular $[Ca^{2+}]$ but also with the concentration of Na^+ in the medium.

In isolated vesicle studies, amiloride has been shown to inhibit the sarcolemmal Na^+/Ca^{2+} exchange (Floresani & Luciani, 1984; Siegl *et al.*, 1984; Debetto *et al.*, 1987). This exchange system and the ATP-driven Ca^{2+} pump are the two major mechanisms for Ca^{2+} efflux from cardiac cells (Sheu & Blaustein, 1986). Although the relative contribution of these two systems in extruding Ca^{2+} is not well determined, it has been established that the pump has high affinity and low capacity for internal Ca^{2+} , whereas the Na^+/Ca^{2+} exchange is a low affinity, high capacity system (Blaustein, 1977; Trumble *et al.*, 1980; Caroni & Carafoli, 1981; Wakabayashi & Goshima, 1981; Di Polo, 1983; Barry & Smith, 1984; Philipson, 1985). The direction and level of operation of Na^+/Ca^{2+} exchange are determined by the electrochemical gradient for Na^+ and the membrane potential (Mullins, 1979; Reeves & Hale, 1984; Eisner & Lederer, 1985; Philipson, 1985). It has been proposed that a decrease of external $[Na^+]$, lowering the $[Na^+]_0/[Na^+]_i$ ratio and

hence the Na^+ gradient across the sarcolemma, depresses the Ca^{2+} extrusion through the Na^+/Ca^{2+} exchange (Sheu & Fozzard, 1982; Lee *et al.*, 1985). Therefore in low external $[Na^+]$ the operational level of the exchange is expected to be low. This may be consistent with the lack of inotropic effect of amiloride in low external $[Na^+]$, since the inhibition of an already depressed exchange may have no functional consequences because of the contribution of the system in Ca^{2+} efflux is actually too low. If we hypothesize that the relative contribution of Na^+/Ca^{2+} exchange and ATP-driven Ca^{2+} pump in extruding Ca^{2+} is dependent on external $[Ca^{2+}]$ and rate of stimulation, we can also explain the dependence of the positive inotropic effect of amiloride on different $[Ca^{2+}]$ at different rates of stimulation. Amiloride had no inotropic effect on guinea-pig left atria driven at 1 Hz, 0.5 Hz, 0.1 Hz in external $[Ca^{2+}]$ of respectively 0.45 mM, 0.9 mM, 2.7 mM. At each of these external $[Ca^{2+}]$ and rate levels, the ATP-driven Ca^{2+} pump may have a more important role than Na^+/Ca^{2+} exchange in extruding Ca^{2+} , as the intervals between stimuli in relation to external $[Ca^{2+}]$ (and hence to $[Ca^{2+}]_i$) are long enough to enable Ca^{2+} efflux through a system with high affinity for Ca^{2+} such as the ATP-driven Ca^{2+} pump.

It has been suggested that differences in the level of operation of Na^+/Ca^{2+} exchange in removing Ca^{2+} from the cells are responsible for the differences in contractile behaviour, with respect to the parameters of stimulation, existing between both atrial and ventricular myocardium and cardiac muscle from rat and other mammalian species (Sutko *et al.*, 1986). Rat cardiac muscle has some anomalous features (Noble & Powell, 1985); its contractility is less dependent on external $[Ca^{2+}]$ than that of other mammalian species (Willerson *et al.*, 1978; Sutko & Willerson, 1980; Mitchell *et al.*, 1984); its force of contraction does not demonstrate positive staircase in the range of high rates, but increases as the rate of stimulation is decreased (Langer, 1983; Orchard & Lakatta, 1985; Stemmer & Akera, 1986). At the low-frequency end of the frequency-strength curve, guinea-pig left atria exhibits similar behaviour (Blinks & Koch-Weser, 1961; Gibbons, 1986). Although most of the Ca^{2+} directly involved in activating contraction comes from intracellular stores (mainly sarcoplasmic reticulum (SR)), the magnitude of the tension depends on the relative contribution of the sarcolemmal mechanisms which regulate the Ca^{2+} fluxes in and out the cells, since these mechanisms determine the filling of the SR with Ca^{2+} and its release (Gibbons & Fozzard, 1975; Lipsius *et al.*, 1982; Chapman, 1983; Fabiato, 1983; Winegrad, 1986). Since the amount of Ca^{2+} entering the cells during each

cardiac cycle does not change appreciably with rate changes (Beeler & Reuter, 1970; New & Trautwein, 1972), it is likely that the progressive increase of contraction induced by the progressive slowing of stimulation rate may be correlated with the progressive decline of operation of a Ca^{2+} extruding system, caused by the fact that more time is allowed to SR for Ca^{2+} loading. A time-dependent recycling of Ca^{2+} by the SR has been proposed by Orchard & Lakatta (1985) as a mechanism for the inverse relationship between strength and frequency in rat heart muscle. Moreover it has been postulated that Ca^{2+} efflux via Na^+/Ca^{2+} exchange may be less favoured in rat cardiac muscle (Sutko *et al.*, 1986). In the low frequency range, a time-dependent recycling of Ca^{2+} may be responsible for the increase of contraction as the frequency is decreased in guinea-pig atria as well. If this is so, the lack of positive inotropic effect of amiloride both on guinea-pig atria driven at very low frequencies and normal external $[Ca^{2+}]$ and on rat atria driven at 1 Hz can be accounted for by the fact that the Na^+/Ca^+ exchange is not relevant in removing Ca^{2+} from the cells: in rat atria as a species feature, in guinea-pig atria as a consequence of the lengthening of intervals between the stimuli.

Amiloride induced profound changes in guinea-pig (but not in rat) atria force-frequency relationship in the range of low frequencies, since in the presence of the drug the guinea-pig atria responded to the decrease of frequency with a decrease rather than increase of developed tension. Moreover, amiloride abolished the post-rest potentiation of the first contraction elicited after different times of rest in guinea-pig but not in rat atria.

The Na^+/Ca^{2+} exchange system has been implicated not only as a Ca^{2+} efflux but also as a Ca^{2+} influx mechanism in cardiac cells (Philipson, 1985; Sheu & Blaustein, 1986). It has been proposed that the direction of the carrier operation changes in each cardiac cycle (Gibbons, 1986), Ca^{2+} influx occurring on depolarization and Ca^{2+} efflux on repolarization (Mullins, 1979; Eisner & Lederer, 1985; Kimura *et al.*, 1986; Mechmann & Pott, 1986). Although the relative magnitude of the contribution of the Na^+/Ca^{2+} exchange in determining respectively Ca^{2+} entrance and loss is not known, evidence has been presented indicating a significant contribution to Ca^{2+} entry during depolarization by Na^+/Ca^{2+} exchange (see Gibbons, 1986). The contractile behaviour of guinea-pig atria, in which the force of contraction increases after rest (very low frequencies of stimulation or first contraction after rest) suggests a progressive Ca^{2+} gain, most probably due to a decreased activity of Ca^{2+} extruding systems between one beat and the following (the time-dependent recycling of Ca^{2+} by SR mentioned

above). If we assume that in atrial tissue at each contraction after rest the contribution of the $\text{Na}^+/\text{Ca}^{2+}$ exchange is mainly in Ca^{2+} entry and not in Ca^{2+} efflux, we can explain the response to amiloride: the net effect of the inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange by the drug is a decrease of Ca^{2+} influx. Moreover, the lack of effect of amiloride on the contractile response of rat atria to low frequencies and to rest can be ascribed to the peculiarity of the cardiac tissue of this species in which the sarcolemmal mechanisms of

Ca^{2+} flux are probably less important in the control of contractility than in other mammalian species.

In conclusion, we propose that the pattern of cardiac action of amiloride may be correlated with the relative level and the prevailing direction of operation of $\text{Na}^+/\text{Ca}^{2+}$ exchange.

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