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Chronotropic Effect of Alkali Metals on Spontaneously Beating Right Atria

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ROBERTS, L A *Chronotropic effect of alkali metals on spontaneously beating right atria* PHARMACOL BIOCHEM BEHAV **21**: Suppl 1, 81–85, 1984 —The alkali metal ions lithium, potassium, rubidium and cesium depress the rate of spontaneous beating of isolated rabbit right atria At low concentrations (2 to 4 mM) the negative chronotropic effect was in the order Cs > Rb > K or Li, at a higher concentration (12 mM) it was Rb or K > Cs or Li Force of contraction was also depressed by potassium and cesium at all levels, but was stimulated by lithium and by low levels of rubidium (2 mM) Lithium had little chronotropic effect up to relatively high concentrations, decreasing spontaneous beating rate to 80% of control at 100 mM LiCl The significant positive inotropic effect at 2 mM LiCl (to 120% of control) increased (to 180% of control) at 40 mM LiCl Rate of beating was significantly depressed by 0.2 mM CsCl The chronotropic effect of cesium was biphasic, the decrease in SBR at 2 and 4 mM cesium was greater than the negative chronotropic effect at 10 mM CsCl The effect of rubidium (above 4 mM) closely resembled that of increased potassium in decreasing spontaneous beating rate and contractile force

Alkali metals Atria Chronotropic response Contractile force Cs Heart rate Inotropic response K Li Rb Sinoatrial node

THE alkali metal ions sodium (Na) and potassium (K) are the dominant cations in blood and cytoplasm, respectively Their high concentration, maintained by active transport, acts as a source of potential energy for cellular function. Flux of these ions across the cell membrane, down their concentration gradients, is determined by the properties of membrane channels [9] and is coupled with other ion movements controlling functional responses, e.g., myofibrillar contraction [19]. Other alkali metal ions, lithium (Li), rubidium (Rb), and cesium (Cs), because of their similarity to Na and K, modify the ionic flux across membranes either by substituting for Na [2] or K [6,17] or by blocking specific membrane channels [1, 5, 7, 14] The alkali metal ions have different affinities for the charged pockets which define membrane channels and pumps [4], and therefore changes in alkali ion composition of the cellular "milieu" lead to modified ionic currents and altered levels of the coupled response Contractile and pacemaker cell activity in the heart is highly dependent on ionic flux; both contractile force (CF) and spontaneous rate of beating (SBR) are influenced by the alkali metal ions in the extracellular environment.

The present study relates primarily to modification of the pacing function of the sinoatrial node It compares the effect of alkali metal ions added to a normal Tyrode bathing medium in modifying SBR, and the accompanying CF, of isolated rabbit right atria

METHOD

Spontaneously beating rabbit right atria from New Zealand White rabbits were used in the study. The rabbits were killed with a cervical blow, the chest was opened along the midline and the entire heart was removed and placed in a tissue bath containing Tyrode medium, aerated with 95% O_2 + 5% CO_2. The ventricular muscle and all other extraneous tissue was removed from the two atria (atrial cap), except for some attached vascular tissue (the entering superior and inferior *vena cavae*). The left atrium was then severed from the right leaving a spontaneously beating right atrium with an intact sinoatrial node region.

The right atrium was suspended in a tissue bath with the base of the atrium tied to a glass hook in the bottom of the bath, and with the tip of the atrial appendage attached by a silk thread to the microscale accessory arm of a Statham tension transducer. The transducer provided signals to a Beckman R611 dynograph recorder indicating tension changes in the beating preparation. Rate of spontaneous beating was determined by counting the contractions per minute on the dynograph recording taken at a paper speed of 5 mm/sec

A second method of monitoring SBR was used in the high K experiments, when tension fell to zero while electrical activity of the atrium continued. Two silver/silver-chloride electrodes, spaced vertically 6 mm apart at the tip, were mounted together, insulated, in a glass tube so as to make a "bipolar electrode" to monitor spontaneous electrical activity of the atrium. The signal from the bipolar electrode was fed into a voltage/pulse coupler and recorded at the same speed as for tension.

The chloride salts of the alkali metal ions were added to a normal Tyrode medium which contained (in mM): Na^+, 145; K^+, 5.5; Ca^{2+}, 1 8, Cl^-, 133; HCO_3^-, 25; and glucose, 10. All salts were added above the normal Na and K concentrations of the bathing medium so that replacement of K or Na of the

medium was not involved. Osmolality of the control Tyrode medium was 297±4, as measured by freezing point depression using an Advanced osmometer. No attempt was made to correct for changes in osmolality of experimental media since their effect could be compared to that of hyperosmolar sucrose and NaCl solutions studied previously [15]

The atria were equilibrated for one hour at 36°C The bathing solution was exchanged (by suctioning out the Tyrode medium and replacing it with Tyrode solution at bath temperature) after 2, 5, and 10 min and at 15 min intervals thereafter throughout the equilibration period Exchanging the bathing medium did not alter SBR even though the atrium was exposed to air for a few seconds. After equilibration, the tyrode bathing medium was replaced with Tyrode solution containing a given concentration of the alkali metal salt. Spontaneous rate of beating and CF were measured at each concentration, allowing approximately 20 min for the response to stabilize.

Significance of the changes in SBR and CF for each alkali metal was estimated by a specific paired t-test at the concentration where there appeared to be a response. The differences in effect on SBR and CF among alkali metal ions was tested at specific levels by a one-way analysis of variance using the Statistical Analysis System (SAS)—General Linear Models procedure—on an IBM 4341 computer Responses to the alkali metal ions are plotted as mean±standard error (SE)

RESULTS

Addition of alkali metal ions to the normal Tyrode bathing medium caused a depression of SBR with a time course and degree of response which varied among the alkali metal ions Contractile force was stimulated by Li and by low levels of Rb, but was depressed at high levels of Rb and by Cs or increased K Control atria which remained active in normal Tyrode medium decreased their SBR at a rate of approximately 2 beats/min each hour for over 3 hours This small change in activity did not contribute significantly to the depression of SBR observed in this study The responses to the alkali metal ions were as follows

Potassium

Increasing bathing medium K above the normal 5.4 mM level (Fig. 1) caused a decrease in SBR and contractile force. Contractile force decreased to 50% of control in 11 mM KCl (5.6 mM above normal) and contractile activity ceased in 13.7 mM KCl. Spontaneous electrical activity was more resistant to the depressant action of high KCl When monitored with bipolar silver/silver-chloride electrodes, spontaneous rate remained at an average 70% of control in Tyrode containing 15 mM KCl, dropping to zero after 1 to 15 min in 17.5 mM KCl.

Lithium

Very low levels of LiCl (1 to 4 mM) caused a decrease in SBR in 3 of 4 atria (Fig 2), although the small effect (on only three atria) was not significantly different from control As the Li concentration was increased, the level of spontaneous beating remained near control up to relatively high concentrations of Li (above 60 mM) At very high concentrations (150 mM LiCl), Li not only depressed SBR but caused strong arrhythmias; activity stopped in Tyrode containing 200 mM LiCl. The negative chronotropic effect of 100 mM lithium

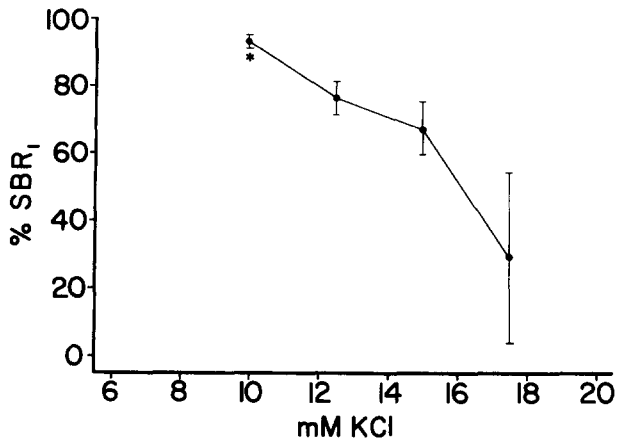

FIG 1 Chronotropic response, as percent of initial SBR of spontaneously beating rabbit right atria to increased levels of KCl in Tyrode medium The response at 10 mM KCl was significantly different (by paired t-test of SBR) from the control in normal 5 4 mM KCl *$p<0$ 01 (n=6)

was opposite to the positive chronotropic effects previously observed with hyperosmotic sucrose or sodium chloride at this level [15] Contractility was increased by Li even at low concentrations. The maximum increase in CF (to 180% of control) occurred in 40 mM Li and the response remained significantly above control at 100 mM LiCl. The response at 40 and 100 mM Li was greater than the hyperosmolar induced positive inotropic effects previously observed for sucrose or sodium chloride at this concentration [15] Contractile force was maintained near or above control levels up to concentrations at which beating became irregular

Cesium

Cesium had a biphasic effect on SBR Spontaneous rate of beating was significantly depressed by low concentrations of Cs (0.2 mM), decreasing to 65% of control at 2 mM CsCl and to 62% at 4 mM CsCl (Fig 3). However, at 10 mM CsCl, all 3 atria showed a stimulation above this level, i.e , an increase of 3 to 20 beats/min above the rate in 4 mM CsCl The SBR remained at greater than 60% of control in Tyrode medium containing 15 mM CsCl Cesium depressed contractile force even at the 0.2 and 1 mM levels where force fell to 86 and 80% of control respectively. After 20 min at 15 mM CsCl, force averaged 70% of control, but force decreased to zero within one minute at 20 mM CsCl

Rubidium

The effect of rubidium chloride on SBR was intermediate between that of potassium and cesium (Fig 4) at levels of 4 mM and below. At 10 mM RbCl, contractile force fell to zero, and SBR was strongly depressed.

Comparison

The responses to the four alkali metal ions are compared in Fig 4 (for SBR) and in Table 1 (for CF) Lithium was unique in causing only slight depression of SBR up to very high levels (100 mM) and in stimulating contractility over a wide range of concentrations. Cesium caused the greatest depression of SBR at low concentrations (at levels of 4 mM and below) but was more resistant than KCl or RbCl to con-

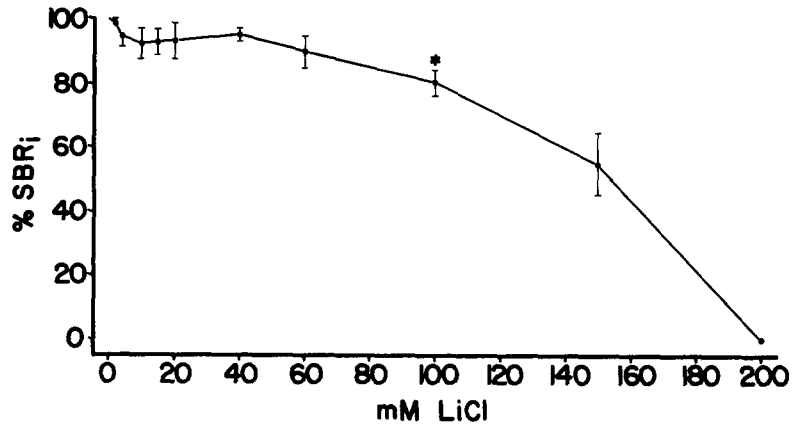

FIG. 2 Chronotropic response of spontaneously beating rabbit right atria to increasing levels of LiCl added to the Tyrode bathing medium The response at 100 mM LiCl was significantly different from control by paired t-test of SBR. *$p<0.05$ (n=3)

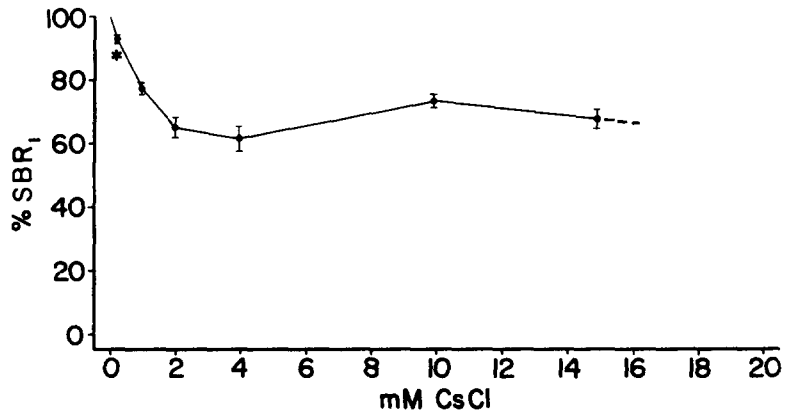

FIG. 3. Chronotropic response of spontaneously beating rabbit right atria to increasing levels of CsCl in Tyrode bathing medium The response at 0 2 mm CsCl was significantly different from control by paired t-test of SBR *$p<0$ 01 (n=3)

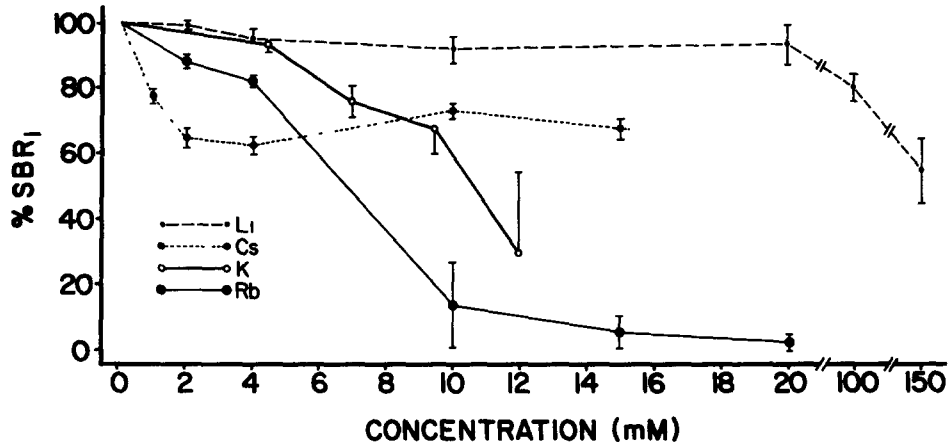

FIG 4 Comparison of the chronotropic respones to Li, Cs, K, and Rb added as chloride salts to Tyrode bathing medium The concentration of KCl for each response is that above the normal Tyrode ionic concentration, i.e , 0=5 4 mM KCl The responses at the 4 and 12 mM levels were tested by one-way analysis of variance There was a significant difference in the responses among agents at 4 and 12 mM (see text)

TABLE 1

EFFECT OF ALKALI METAL IONS ON FORCE OF CONTRACTION OF SPONTANEOUSLY BEATING ATRIA

Concentration (mM)	Alkali Metal Ions			
	Cs	K	Li	Rb
2	79 ± 2 0	87 ± 8 0	120 ± 6 6	145 ± 31
4	75 ± 4 0	84 ± 17	116 ± 9 5	81 ± 12
12	67 ± 19	0	133 ± 14	2

Percent of initial force of contraction (mean ± SE) of spontaneously beating atria in response to alkali metals added to normal tyrode bathing medium Initial CF averaged 224 mg One way analysis of variance at each dose level indicated that the differences in response among ions was only significant at the 12 mM level $F(3,11)=43\ 4$, $p<0\ 001$, Rb or K > Cs > Li

centrations of 10 to 15 mM Although Rb stimulated contractility at 2 mM, its pattern of effect on both rate and force resembled that of increased K more closely than did Cs or Li

One-way analysis of variance of the chronotropic response at the 4 and 12 mM levels indicated that the responses to the alkali ions were significantly different· $F(3,11)=19\ 9$, $p<0.001$, and $F(3,11)=15\ 9$, $p<0.001$, respectively. At the 4 mM concentration, the negative chronotropic response to the alkali metal ions was in the order Cs > Rb > Li or K (Duncan's multiple range test) At a higher concentration (12 mM) this sequence changed to Rb or K > Cs or Li.

The inotropic responses were more variable than the chronotropic responses and were not significantly different among agents at the 2 or 4 mM level At 12 mM, there was an obvious significant difference among agents, since contractility disappeared in 12 mM K or Rb· $F(3,11)=43\ 4$, $p<0\ 001$ The sequence of effect in depressing contractile force was K or Rb > Cs > Li at 10 to 12 mM concentrations of the chloride salts. Li still exhibited a stimulatory effect on force at this level, to 140% of control.

DISCUSSION

Comparison of the effects of alkali metals on heart rate using the past literature is difficult because the studies have been done on different species and in different laboratories, however, it is well known that some alkali metal ions will decrease SBR and CF of spontaneously beating cardiac preparations. High potassium, for example has been shown to lead to complete cessation of spontaneous activity of guinea pig atria at levels of 12.6 mM [13] and in rabbit atria at 16 2 mM [18]. Rubidium and Cs initiated but failed to sustain activity of atria made quiescent by zero K [6]. And both rubidium and cesium at 2, 4, and 8 mM were found to depress SBR of guinea pig atria [12,13] None of these studies, however, compared the chronotropic response of isolated rabbit atria to the alkali metals added to normal bathing medium over the range in which activity persists. In the present study the alkali metals, Li, Rb, K, and Cs, all caused a depression of SBR. Potassium and Cs added to normal tyrode solution, depressed atrial contractility as well. Lithium, even up to 100 mM, caused an increase in contractility while Rb increased force only at low concentrations (2 mM) and at higher levels caused a negative inotropic effect.

Concentrations of K above 10 mM (an increase of 4 mM above normal Tyrode ionic strength) caused a nearly linear decrease in SBR and CF. Electrical activity was more resistant to the depressant effect of high K than was CF since electrical discharge continued up to 17 mM KCl while atria ceased to contract at levels of 10 to 12 mM KCl This resistance of electrical discharge of the rabbit sinoatrial node to increased KCl was reported by others [3,18] The effects of Rb on SBR and CF were generally similar to that of high K Rubidium has previously been shown to mimic K in stimulating the electrogenic Na pump in rabbit sinoatrial node, and in maintaining spontaneous action potentials, i e , in restoring electrical activity after a period in K-free medium [6]

Cesium, in these experiments, had a biphasic effect on SBR which has not been reported previously Although Cs depressed SBR and CF at all concentrations used, its effect was not linear Cesium was more effective than Rb, K, or Li in depressing SBR at low concentrations, however, its depressent chronotropic effect was less at 10 mM than at 2 or 4 mM concentrations The biphasic nature of the response to Cs may indicate a dual effect on activity of true pacemaker cells in the sinoatrial node or may be due to a difference in the action of Cs on true and subsidiary pacemaker cells, possibly related to the difference in sensitivity of these cells to K [8] The depressant effect of Cs may involve changes in an electrogenic Na/K pump or a blocking of K channels, each of which may contribute to the membrane potential in sinoatrial node cells [10,11] Cesium was less effective than Rb in stimulating the Na/K pump in sinoatrial node or in sustaining electrical activity in the absence of K [6] Cesium has been shown to block K channels in cardiac muscle [7], and so might be expected to cause a response different from the effect of increased K In papillary muscle, Cs exerted a biphasic effect on CF, e g , by depressing contractility at low levels, but stimulating contractility at higher levels [12] In our study, such an inotropic effect might have been masked by the depression of SBR, due to the secondary effect of SBR on CF

Lithium had only a slight depressant effect on SBR up to very high levels (above 60 mM) although over this range it stimulated CF The positive inotropic effect of Li was greater than the effect of increased osmolality [15] and may in part be a hyperosmolar effect The stimulation of force by Li at very low concentrations (2 to 4 mM), however, is not a hyperosmolar effect and must be due to some other action of Li on the sinoatrial node The action of low levels of Li does not predict any arrhythmogenic effect on the sinoatrial node as is occasionally found in patients treated with Li [16] Be-

cause of secondary changes in intracellular ionic levels after modification of membrane ionic flux [2], the chronic effect of the alkali metal ions may be significantly different from their short term dose-response effects.

Information about the pattern of changes in SBR and CF due to increased levels of alkali metal ions, and eventually of the specific currents which are altered, should be important in clarifying the role of specific membrane channels in cardiac function. This is particularly true in sinoatrial node where there is still some uncertainty as to the specific currents underlying spontaneous activity [11]. Knowledge of the action of alkali metal ions on SBR and CF should also be useful in predicting possible side effects of drug treatments which may lead to appreciable levels of alkali metal ions in blood.

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