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IS OUABAIN-SENSITIVE RUBIDIUM OR POTASSIUM UPTAKE A MEASURE OF SODIUM PUMP ACTIVITY IN ISOLATED CARDIAC MUSCLE?

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Summary

(1) The significance of the specific (ouabain-sensitive) $^{86}Rb^+$ or $^{42}K^+$ uptake by cardiac muscle preparations which are not 'sodium-loaded' was studied.

(2) In left atrial preparations of guinea-pig heart, resting $^{86}Rb^+$ uptake was relatively low. It was markedly increased by electrical stimulation. This stimulated uptake was further enhanced by isoproterenol and inhibited by verapamil.

(3) In rat atria, the resting $^{86}Rb^+$ uptake was somewhat higher than in guinea-pig atria, and the increase in uptake caused by electrical stimulation was smaller. In guinea-pig right ventricular papillary muscle, the resting uptake was highest among those tissues studied, and the response to electrical stimulation was smallest. In the latter tissue, verapamil produced only a minimal inhibition of the specific $^{86}Rb^+$ uptake.

(4) The effect of the frequency of electrical stimulation on $^{86}Rb^+$ uptake paralleled its influence on the force of contraction, suggesting the involvement of intracellular sodium in both events.

(5) In both left atrial and right papillary muscle preparations of guinea-pig heart, specific $^{42}K^+$ uptake observed with 5.8 mM K^+ was relatively high, and was increased only slightly by electrical stimulation. This electrical stimulation, however, increased ouabain-induced inhibition of $^{42}K^+$ uptake, suggesting that the stimulation increases the amount of Na^+ available to the sodium pump.

(6) When the K^+ concentration was 1 mM, the resting $^{42}K^+$ uptake was low, and could be enhanced by electrical stimulation.

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(7) Thus, in cardiac muscle preparations which are not sodium loaded, the specific $^{86}Rb^+$ or $^{42}K^+$ uptake can be used to estimate the rate of sodium influx, which is equivalent to the rate of sodium efflux under steady-state conditions, provided that neither Rb^+ nor K^+ is in excess compared to the Na^+ available to the pump. If Rb^+ or K^+ is in excess, its specific uptake may not reflect changes in transmembrane Na^+ movement.

Introduction

Ouabain-sensitive $^{86}Rb^+$ uptake has been widely used as an estimate of sodium pump activity in various tissue preparations [1]. Since K^+ (or Rb^+) is actively transported in exchange with Na^+ across the cell membrane by the sodium pump, and the stoichiometry of this reaction is believed to be three Na^+ pumped out per two K^+ or Rb^+ pumped in for each molecule of ATP hydrolyzed by $(Na^+ + K^+)$-ATPase [2—5], it is reasonable to assume that the ouabain-sensitive $^{86}Rb^+$ uptake is an accurate estimate of the capacity of the sodium pump in cardiac preparations preloaded with Na^+ [6—9].

Ouabain-sensitive $^{86}Rb^+$ uptake, however, does not appear to represent the capacity of the sodium pump in preparations which are not preloaded with Na^+. For example, in isolated cardiac muscle preparations beating under steady-state conditions, $^{86}Rb^+$ uptake represents the activity of the sodium pump which is apparently operating below its capacity [10]. In these studies, factors and agents which increase the rate of sodium influx increased the rate of the ouabain-sensitive $^{86}Rb^+$ uptake, thereby indicating that the sodium pump has a reserve capacity, and is capable of adjusting to the increased sodium influx by functioning at a higher activity. It follows that the ouabain-sensitive $^{86}Rb^+$ uptake assayed without Na^+ preloading may also represent the rate of sodium influx, since this uptake is coupled with sodium efflux, which in turn is equivalent to sodium influx under steady-state conditions.

This argument is based on the premise that the active exchange transport of Rb^+ and Na^+ is the major mechanism for the ouabain-sensitive $^{86}Rb^+$ uptake (in contrast to the Rb^+-K^+ exchange reaction, for example) and that the transport of Rb^+ and Na^+ is coupled at a fixed ratio. In cardiac muscle, however, this ratio must be flexible. For example, in isolated heart preparations, an increase in the frequency of stimulation is accompanied by a proportional increase in the rate of sodium influx but a minimal change in the rate of potassium efflux [11]. Despite apparent differences in the ratio of Na^+ and K^+ which may be exchanged at different stimulation frequencies, a steady-state tissue ion concentration is maintained, indicating that the sodium pump can adjust to different $Na^+ : K^+$ ratios. If the $Na^+ : K^+$ ratio is variable, the $Na^+ : Rb^+$ ratio is also likely to be variable.

Thus, there is some doubt as to whether the ouabain-sensitive $^{86}Rb^+$ or $^{42}K^+$ uptake in preparations which are not sodium loaded represents sodium pump activity. The present study was undertaken to examine the significance of the ouabain-sensitive $^{86}Rb^+$ or $^{42}K^+$ uptake by isolated cardiac muscle preparations.

Methods

Ouabain-sensitive $^{86}Rb^+$ uptake was assayed as described by Yamamoto et al. [10]. Hearts were obtained from guinea-pigs of either sex weighing 350—450 g or male Sprague-Dawley rats weighing 250—300 g, and were perfused (Langendorff preparations) for 5—10 min at 30°C with aerated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer of the following composition (mM): NaCl, 118.0; $NaHCO_3$, 27.2; KCl, 4.8; KH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 1.2 and glucose, 11.1. The pH value of the aerated solution was 7.4. After visible blood was removed from the tissue, the atrial or papillary muscle was excised. $^{86}Rb^+$ uptake was determined by incubating the tissue with or without drugs for 30 min at 36.5°C in a continuously aerated Krebs-Henseleit bicarbonate buffer in which KCl was replaced with 2 mM RbCl containing tracer amounts of $^{86}Rb^+$ (specific activity 0.15 Ci/mmol, New England Nuclear Corp., Boston, MA), and KH_2PO_4 was replaced with NaH_2PO_4. $^{86}Rb^+$ uptake studies were performed in both quiescent and electrically stimulated tissue preparations. In the latter preparations, tissues were stimulated at an indicated rate with square-wave pulses of 4 ms duration at a voltage 50% above threshold with platinum field-stimulation electrodes using a Grass S44 stimulator (Grass Instrument Co., Quincy, MA). After incubation in the presence of $^{86}Rb^+$, preparations were rinsed once in a solution of the same ionic composition, but without $^{86}Rb^+$. The preparations were blotted on filter paper. After weighing the tissue, the amount of radioactivity in the tissue was assayed using a gamma scintillation spectrometer. Ouabain-sensitive $^{86}Rb^+$ uptake is the difference in values observed in the absence and presence of 0.3 mM ouabain for guinea-pig heart preparations and 5 mM ouabain for rat heart preparations.

Ouabain-sensitive $^{42}K^+$ uptake studies were performed in a manner similar to the $^{86}Rb^+$ studies using $^{42}K^+$ (specific activity approx. 0.74 mCi/mmol). $^{42}K^+$ was produced from KOH by the Nuclear Reactor Laboratory of Michigan State University. The solution was neutralized with HCl before use.

The force of contraction and the transmembrane potential of isolated right ventricular papillary or left atrial muscle preparations were studied as previously reported by Temma et al. [12]. Tissue preparations were obtained as described above for $^{86}Rb^+$ uptake studies. After visible blood was removed from the Langendorff preparation, either the right ventricular papillary or the left atrial muscle was excised and suspended horizontally in the above solution. Muscle preparations were electrically stimulated at 36.5°C with square-wave pulses of 4 ms duration at a voltage 15% above threshold. Floating glass capillary microelectrodes, filled with 3 M KCl and possessing a tip resistance of approx. 30 MΩ, were used for intracellular potential recordings, and the force of contraction was recorded using a force-displacement transducer (Grass Instrument Co., model FT-03C). The transmembrane potential and isometric tension were simultaneously displayed on a storage oscilloscope and recorded with a Grass C4R kymograph camera. When the force of contraction was monitored without simultaneous recording of the transmembrane potential, preparations were mounted vertically between a pair of platinum field-stimulation electrodes. The isometric force of contraction was recorded with a polygraph recorder (Grass Instrument Co., model 7B). The resting tension was adjusted to

1.0 g. Tissue preparations were equilibrated for 60 min before the start of experiments. After the equilibration period, control preparations were stable during the experimental period.

Results were analyzed by Student's *t*-test for group comparison. All chemicals used were of reagent grade.

Results

$^{86}Rb^+$ uptake

We have previously reported [10] that electrical stimulation of left atrial preparations of guinea-pig heart enhances the ouabain-sensitive (specific) $^{86}Rb^+$ uptake by increasing the sodium influx associated with membrane depolarization when these preparations were not cold-incubated in the presence of sodium. Since a large fraction of sodium influx associated with the action potential occurs during the plateau phase rather than during the upstroke of action potential [11], the effect of verapamil, an agent which blocks slow sodium/calcium channels [13—15] was examined to see if it would reduce the effect of electrical stimulation on $^{86}Rb^+$ uptake.

In isolated left atrial preparations of guinea-pig heart, electrical stimulation caused a significant increase in the specific $^{86}Rb^+$ uptake (Table I) without affecting the nonspecific uptake (data not shown, but see Ref. 10). The increase in the specific $^{86}Rb^+$ uptake was roughly proportional to the frequency of electrical stimulation (Table I). Verapamil caused a marked inhibition of that portion of the specific $^{86}Rb^+$ uptake enhanced by electrical stimulation. The effect of verapamil was concentration-dependent between 10 and 100 μM. Isoproterenol, an agent which increases the action potential duration in these preparations (Fig. 1), caused a marked increase in specific $^{86}Rb^+$ uptake in preparations stimulated at 1.5 Hz (Table I). These results suggest that the enhancement of specific $^{86}Rb^+$ uptake caused by electrical stimulation of left atrial preparations of guinea-pig heart is mainly related to the sodium influx occurring during the plateau phase of the action potential.

In the rat heart, the action potential does not have a distinct plateau phase

TABLE I

$^{86}Rb^+$ UPTAKE BY ISOLATED ATRIAL PREPARATIONS OF GUINEA-PIG HEART

Specific $^{86}Rb^+$ uptake is the difference in values observed in the absence and presence of 0.3 mM ouabain. Nonspecific (ouabain-insensitive) $^{86}Rb^+$ uptake was 3.3 ± 0.4 nmol/mg tissue per 30 min (n = 5). Values are the mean ±S.E. n, number of experiments.

Electrical stimulation (Hz)	Drug	n	Specific $^{86}Rb^+$ uptake (nmol/mg protein per 30 min)
0	none	16	16.3 ± 1.0
0	verapamil (100 μM)	11	15.6 ± 1.5
1.5	none	22	32.6 ± 1.5
1.5	verapamil (10 μM)	7	23.2 ± 2.8
1.5	verapamil (30 μM)	13	19.0 ± 1.7
1.5	verapamil (100 μM)	15	15.4 ± 1.0
1.5	isoproterenol (40 nM)	12	50.3 ± 2.0
3.0	none	8	46.3 ± 3.2

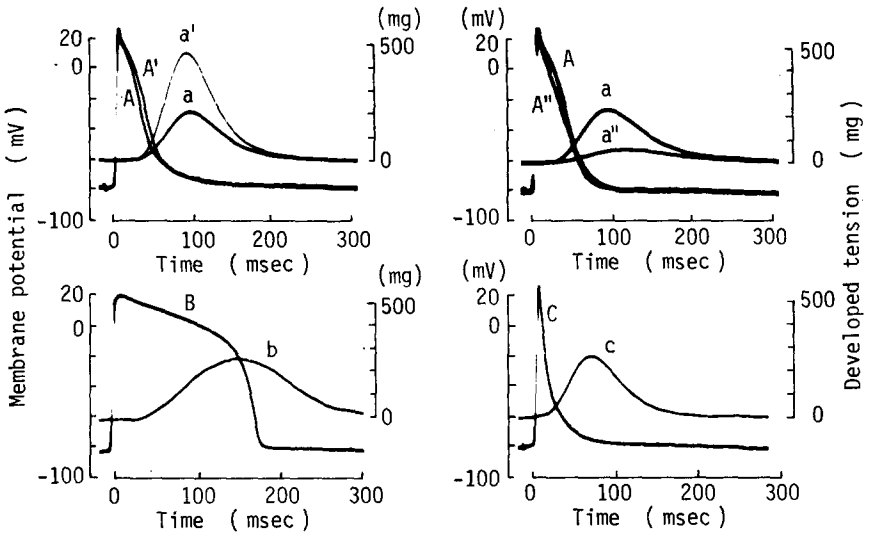

Fig. 1. Action potential configuration of cardiac muscle preparations and force of contraction. Left atrial muscle (A) or right ventricular papillary muscle preparation (B) of guinea-pig heart, or left atrial muscle of rat heart (C) was electrically stimulated at 1.5 Hz at 36.5°C. Transmembrane potentials were recorded with KCl-filled glass microelectrodes. In left atrial preparations of guinea-pig heart, recordings were obtained also in the presence of either 40 nM isoproterenol (A') or 10 μM verapamil (A''). a, a', a'', b and c represent corresponding recordings of the force of contraction.

(Fig. 1). Therefore, electrical stimulation is expected to cause a smaller increase in specific $^{86}Rb^+$ uptake in this tissue compared to guinea-pig heart preparations if the above hypothesis is correct. In quiescent left atrial preparations of rat heart, the specific $^{86}Rb^+$ uptake was slightly higher than the corresponding value observed in the guinea-pig (Table II). Electrical stimulation increased the specific $^{86}Rb^+$ uptake. In these preparations, 1.5 and 3 Hz stimulation caused a 28 and 54% increase in the specific $^{86}Rb^+$ uptake, respectively. These increases are significantly smaller than the corresponding values observed with guinea-pig heart preparations. In the latter, 1.5 and 3 Hz stimulation caused a 100 and 184% increase, respectively, above the value observed in quiescent preparations. These results establish the importance of the sodium influx associated with the

TABLE II

$^{86}Rb^+$ UPTAKE BY ISOLATED ATRIAL PREPARATIONS OF RAT HEART

Specific $^{86}Rb^+$ uptake is the difference in values observed in the absence and presence of 5.0 mM ouabain. Nonspecific (ouabain-insensitive) $^{86}Rb^+$ uptake was 7.8 ± 0.7 nmol/mg tissue per 30 min (n = 5). Values are the mean ±S.E. n, number of experiments.

Electrical stimulation (Hz)	n	Specific $^{86}Rb^+$ uptake (nmol/mg tissue per 30 min)
0	18	21.9 ± 1.1
1.5	6	28.1 ± 3.1
3.0	13	33.7 ± 2.6
4.0	4	39.3 ± 3.6
5.0	10	46.1 ± 3.5

TABLE III

$^{86}Rb^+$ UPTAKE BY ISOLATED PAPILLARY MUSCLE PREPARATIONS OF GUINEA-PIG HEART

Specific $^{86}Rb^+$ uptake is the difference in values observed in the absence and presence of 0.3 mM ouabain. Nonspecific (ouabain-insensitive) $^{86}Rb^+$ uptake was 3.1 ± 0.2 nmol/mg protein per 30 min (n = 5). Values are the mean ±S.E. n, number of experiments.

Electrical stimulation (Hz)	Drug	n	Specific $^{86}Rb^+$ uptake (nmol/mg tissue per 30 min)
0	none	34	30.8 ± 1.0
1.5	none	16	34.4 ± 1.5
3.0	none	22	37.9 ± 1.0
3.0	verapamil (100 μM)	7	34.6 ± 2.3

plateau phase of the action potential in the enhancement of the specific $^{86}Rb^+$ uptake by cardiac muscle preparations.

Among the three cardiac muscle preparations studied, papillary muscle of guinea-pig heart had the highest resting $^{86}Rb^+$ uptake (Table III). Papillary muscle preparations of guinea-pig heart have a long action potential duration (Fig. 1). Nevertheless, electrical stimulation of these preparations only minimally increased the specific $^{86}Rb^+$ uptake (Table III). Consistent with this observation, verapamil caused a modest inhibition of specific $^{86}Rb^+$ uptake in electrically stimulated guinea-pig papillary muscle preparations in a concentration (100 μM) which caused a marked inhibition of uptake in atrial preparations. These results indicate that the duration of the plateau phase of the action potential is not the sole determinant of the degree of enhancement of the $^{86}Rb^+$ uptake caused by electrical stimulation.

Force of contraction

An increase in the rate of sodium influx associated with membrane depolarization has been implicated in an enhancement of the force of contraction (positive staircase or Bowditch phenomenon) observed when cardiac muscle preparations were stimulated at higher frequencies [16,17]. Thus, the influence of the frequency of electrical stimulation on the force of contraction was compared in cardiac muscle preparations used in the above $^{86}Rb^+$ uptake studies.

In left atrial preparations of guinea-pig heart incubated in a Krebs-Henseleit bicarbonate buffer solution containing 5.8 mM K^+ and no Rb^+, increases in the frequency of electrical stimulation markedly enhanced the force of contraction (Fig. 2, panel A). When the frequency of stimulation was decreased, the force of contraction returned towards a lower level. In the presence of 10 μM verapamil, however, increases in the frequency of stimulation failed to augment the force of contraction (Fig. 2, panel B). When the frequency of stimulation was subsequently decreased, the developed tension returned towards a control level. In papillary muscle preparations of guinea-pig heart, increases in the frequency of electrical stimulation were accompanied by increases in the force of contraction (Fig. 2, panel C); however, the enhancement of the developed tension was markedly smaller than that observed with left atrial preparations of guinea-pig heart.

In left atrial preparations of rat heart, increases in the frequency of stimula-

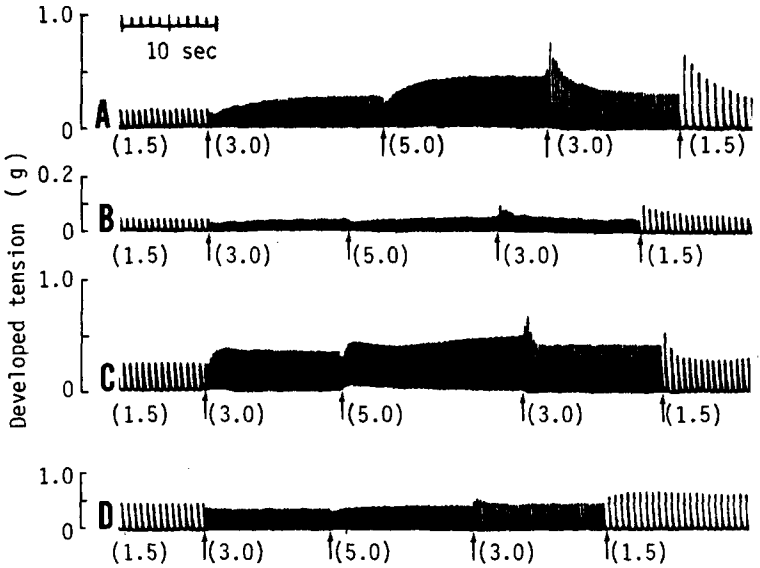

Fig. 2. Frequency of stimulation and developed tension. Isolated heart muscle preparations were incubated at 36.5°C in a Krebs-Henseleit bicarbonate buffer. Resting tension was adjusted to 1.0 g. After a 60 min equilibration period at 1.5 Hz stimulation, the frequency of stimulation was altered at the time indicated by the arrow. Numbers in parentheses indicate the frequency of stimulation (Hz). Typical tracings from several experiments in each group are shown. (A) Left atrial preparation of guinea-pig heart. (B) Left atrial preparation of guinea-pig heart in the presence of 10 μM verapamil. (C) Papillary muscle preparation obtained from guinea-pig right ventricle. (D) Left atrial preparation of rat heart.

tion were accompanied by decreases in the developed tension (Fig. 2, panel D). When the frequency of stimulation was decreased, the force of contraction returned towards a higher level. It is apparent that the extent of stimulus-induced increase in transmembrane sodium influx is highly dependent on the area of the heart used and the animal species from which preparations were obtained.

TABLE IV

$^{42}K^+$ UPTAKE BY ATRIAL AND PAPILLARY MUSCLE PREPARATIONS OF GUINEA-PIG HEART

Tissue preparations were incubated at 36.5°C for 30 min in a Krebs-Henseleit bicarbonate buffer in which KCl was replaced with the same concentration of ^{42}KCl (5.8 mM). Nonspecific (ouabain-insensitive) $^{42}K^+$ uptake was assayed in the presence of 0.3 mM ouabain. Specific (ouabain-sensitive) uptake is the difference in values observed in the absence and presence of ouabain. Values are the mean ±S.E. Numbers in parentheses indicate the numbers of experiments. Values of uptake are expressed as nmol/mg tissue per 30 min.

Preparations	Electrical stimulation (Hz)	Nonspecific $^{42}K^+$ uptake	Specific $^{42}K^+$ uptake
Left atria	0	21.5 ± 0.8 (6)	49.3 ± 2.4 (11)
	3.0	20.6 ± 1.0 (6)	54.7 ± 2.1 (10)
Papillary muscle	0	19.0 ± 0.9 (6)	61.0 ± 3.2 (12)
	3.0	20.8 ± 1.0 (6)	67.9 ± 3.0 (13)

$^{42}K^+$ uptake

In the first series of $^{42}K^+$ studies, the effect of electrical stimulation on $^{42}K^+$ uptake was examined in a medium which had a composition identical to that used in the force-frequency studies described above. In isolated non-stimulated right ventricular papillary or left atrial muscle preparations of guinea-pig heart, both nonspecific (ouabain-insensitive) and specific (ouabain-sensitive) $^{42}K^+$ uptake were higher (Table IV) than corresponding values obtained with $^{86}Rb^+$ uptake studies (Tables I and III). The higher uptake of K^+ is probably due to the presence of 5.8 mM K^+ as compared to 2.0 mM Rb^+ present in the medium in the earlier studies. Electrical stimulation did not change nonspecific $^{42}K^+$ uptake, and caused only a minimal increase in specific $^{42}K^+$ uptake in both atrial and papillary muscle preparations (Table IV).

In order to examine the possible cause of differences in results obtained with $^{86}Rb^+$ and $^{42}K^+$ uptake studies, the ouabain sensitivity of the specific $^{42}K^+$ uptake reaction was examined in quiescent and electrically stimulated preparations. Ouabain caused a concentration-dependent inhibition of the specific $^{42}K^+$ uptake in both right ventricular papillary and left atrial muscle preparations of guinea-pig heart (Fig. 3). Electrical stimulation significantly increased the degree of inhibition caused by ouabain. In quiescent preparations, the concentration of ouabain which produced a 50% inhibition of the specific $^{42}K^+$ uptake

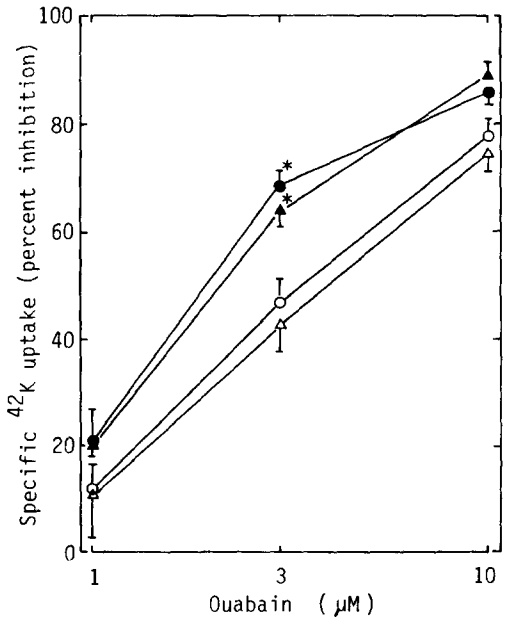

Fig. 3. Effect of electrical stimulation on ouabain-induced inhibition of specific $^{42}K^+$ uptake. Left atrial preparations (circles) or papillary muscle preparations (triangles) obtained from guinea-pig heart were incubated in the presence of an indicated concentration of ouabain at 36.5°C for 30 min in continuously aerated Krebs-Henseleit bicarbonate buffer in which K^+ was replaced with $^{42}K^+$. Open symbols: quiescent preparations. Filled symbols: preparations were electrically stimulated at 3 Hz. Values observed in the presence of 0.3 mM ouabain were subtracted to calculate the ouabain-sensitive (specific) $^{42}K^+$ uptake. Percent inhibition of the activity was calculated against the control value observed in the absence of ouabain (i.e., the difference in value observed in the absence and presence of 0.3 mM ouabain). For control values, see Table IV. Each point represents the mean of six to 12 experiments. Vertical lines indicate S.E.

TABLE V

$^{42}K^+$ UPTAKE BY ATRIAL MUSCLE PREPARATIONS OF GUINEA-PIG HEART IN A MEDIUM CONTAINING A LOW CONCENTRATION OF K^+

Tissue preparations were incubated at 36.5°C for 30 min in a Krebs-Henseleit bicarbonate buffer containing a tracer amount (approx. 0.003 mM) of ^{42}KCl with no carrier KCl. Final concentration of potassium was 1.0 mM (derived from KH_2PO_4). Nonspecific (ouabain-insensitive) $^{42}K^+$ uptake was 1.45 ± 0.08 nmol/mg tissue per 30 min (n = 5). Values are the mean ±S.E. n, number of experiments.

Electrical stimulation (Hz)	n	Specific $^{42}K^+$ uptake (nmol/mg tissue per 30 min)
0	5	5.76 ± 0.73
1.5	5	9.12 ± 0.95 *
3.0	9	8.34 ± 0.71 *

* Significantly different from the corresponding value observed with quiescent preparations.

was approx. 3.6 μM without electrical stimulation in both preparations, and 2 μM with 3 Hz electrical stimulation. These results indicate that the influence of electrical stimulation on the ouabain sensitivity of the specific $^{42}K^+$ uptake reaction was similar in the two types of muscle preparation obtained from guinea-pig heart.

It is reasonable to assume that the difference in results obtained with Rb^+ and K^+ is due to either the cation used, or to differences in their concentration. In order to choose between these possibilities, $^{42}K^+$ uptake studies were performed with a medium containing a low concentration (1 mM) of K^+. In this medium, both nonspecific and specific $^{42}K^+$ uptake by left atrial preparations of guinea-pig heart were markedly lower (Table V) than corresponding values observed in a medium containing 5.8 mM K^+ (Table IV). Electrical stimulation at 1.5 Hz markedly increased the specific $^{42}K^+$ uptake when the concentration of K^+ in the medium was 1 mM, although 3 Hz stimulation failed to cause a further increase (Table V). These results indicate that electrical stimulation can increase specific $^{42}K^+$ uptake under appropriate conditions. When the resting rate of cation uptake is high, it is relatively unaffected by electrical stimulation.

Discussion

Ouabain-sensitive $^{86}Rb^+$ or $^{42}K^+$ uptake has been used by many investigators as a means of estimating sodium pump activity. In earlier work [1,6—8], preparations were generally preincubated at low temperature or under anaerobic conditions in order to increase the intracellular Na^+ concentrations such that the sodium pump is maximally activated. Under these conditions, the capacity of the sodium pump is estimated.

Since the ouabain-sensitive $^{86}Rb^+$ accumulation by cardiac muscle preparations is linear over a 30—40 min period, whereas nonspecific (ouabain-insensitive) uptake does not significantly increase with time, a longer incubation time is preferable since it results in higher specific:nonspecific uptake ratios [10]. With an incubation time greater than 30 min, sodium preloading has a relatively minor effect on $^{86}Rb^+$ uptake [10]. Thus, recent studies [10,18] have been performed without sodium preloading. While this longer preincubation time with-

out sodium preloading offers the advantage of a high specific:nonspecific uptake ratio, it is unclear whether the value obtained measures the capacity of the sodium pump, the rate of sodium efflux, or is related to sodium influx rate. If the rate of sodium influx were the primary determinant of the ouabain-sensitive $^{86}Rb^+$ or $^{42}K^+$ uptake rate, then partial inhibition of the sodium pump produced by a positive inotropic concentration of the cardiac glycosides [6] might not be detected until the sodium pump inhibition reaches a point such that the capacity of the remaining pump is incapable of handling the prevailing sodium influx rate.

The present study indicates that the rate of ouabain-sensitive $^{86}Rb^+$ uptake in preparations which are not sodium loaded is substantially below the maximal rate (capacity), and therefore, the intracellular Na^+ available to the sodium pump is the determinant of the observed uptake value. This finding is consistent with our previous observation [7,10] that several agents or conditions which increase sodium influx also increase specific $^{86}Rb^+$ uptake. In left atrial preparations of guinea-pig heart, the rate of $^{86}Rb^+$ uptake was significantly enhanced by electrical stimulation. This enhanced $^{86}Rb^+$ uptake was reduced by high concentrations of verapamil, an agent which blocks the 'slow channels' for sodium and calcium influx and also the fast sodium channel at high concentrations [15]. These results are consistent with the observation that the force of contraction is markedly increased at higher stimulation frequencies and that this effect is eliminated by verapamil in guinea-pig atrial preparations. The increased force of contraction at higher stimulation rates has been proposed to result from an enhancement of sodium influx, which in turn indirectly increases intracellular Ca^{2+} concentrations [16,17].

In atrial preparations of rat heart, increases in frequency of stimulation had relatively minor effects on both ouabain-sensitive $^{86}Rb^+$ uptake and the force of contraction. The rat heart atrium lacks a clear plateau phase of the action potential. It is in this phase that a significant sodium influx may occur via slow channels. These results also support the concept that the rate of ouabain-sensitive $^{86}Rb^+$ uptake is primarily determined by the rate of sodium influx in preparations not preincubated for sodium loading.

In papillary muscle preparations of guinea-pig heart, electrical stimulation had statistically significant but minor effects on both specific $^{86}Rb^+$ uptake and force of contraction. In this tissue, verapamil had also a relatively small effect on $^{86}Rb^+$ uptake. Although these findings are consistent with the concept that influx determines both the rate of $^{86}Rb^+$ uptake and the developed tension, action potentials in this tissue do have a well characterized plateau phase. Moreover, electrical stimulation increased the potency of ouabain to inhibit $^{42}K^+$ uptake in papillary muscle as well as in atrial muscle preparations, indicating that electrical stimulation significantly increases the intracellular Na^+ available to the sodium pump. This is consistent with our previous observations on $^{86}Rb^+$ uptake [10]. It should be noted that the rate of $^{86}Rb^+$ uptake in quiescent preparations was higher in papillary muscle preparations of guinea-pig heart than in other preparations. In general, the extent of the increase in $^{86}Rb^+$ or $^{42}K^+$ uptake induced by electrical stimulation was smaller when the value observed with quiescent preparations was higher. For example, electrical stimulation failed to affect the rate of $^{42}K^+$ uptake by left atrial preparations of guinea-pig

heart in the presence of 5.8 mM K^+. Thus, it seems that when the resting uptake values are high, they are less responsive to an increased sodium influx.

These findings may be explained from the flexibility in the ratio of Na^+ and K^+ which may be exchanged by the sodium pump. In quiescent heart muscle, the rate of sodium influx and potassium efflux is approx. 0.3 and 0.7 mmol/kg per min, respectively [11]. This means that the Na^+-K^+ exchange rate of the sodium pump in quiescent preparations is approx. 0.4, assuming that other mechanisms for sodium efflux and potassium influx do not play a significant role. If a part of sodium efflux was mediated by a sodium-calcium exchange reaction, then the above ratio was even lower than 0.4. This value is significantly lower than the optimal ratio of 1.5. Thus, a slight increase in sodium influx may then result in more Na^+ to be transported per unit of potassium, and therefore may not increase the rate of potassium (or rubidium) influx/uptake to a significant degree. This concept is supported by the finding that when the concentration of potassium in guinea-pig atrial preparations was decreased from 5.8 to 1 mM, the rate of specific $^{42}K^+$ uptake became sensitive to an increased frequency of electrical stimulation.

Alternatively, the finding that the ouabain-sensitive $^{42}K^+$ uptake observed in the presence of 5.8 mM K^+ was only minimally increased by electrical stimulation and therefore was not markedly sensitive to an increase in the sodium influx rate may be explained if a K^+-K^+ exchange reaction is the primary mechanism by which $^{42}K^+$ is transported into the cell. This reaction is a partial reaction of (Na^+ + K^+)-ATPase, is ouabain sensitive [19—21], and is expected to be reduced when the concentration of intracellular Na^+ is relatively increased or that of extracellular K^+ lowered. The lowering of extracellular K^+, however, is most likely to decrease both the K^+-K^+ and Na^+-K^+ exchange reactions and therefore not to alter the relative predominance of each reaction. Since present results indicate that the Na^+-K^+ exchange reaction is predominant in the presence of reduced (1 mM) K^+, the latter explanation is somewhat less likely.

Therefore, ouabain-sensitive $^{86}Rb^+$ or $^{42}K^+$ uptake observed with isolated heart preparations, which are incubated under steady-state conditions and not preloaded with Na^+, is not indicative of the capacity of the sodium pump. When the extracellular K^+ concentration is in the physiological range, a change in sodium pump activity (sodium efflux rate) may even not be reflected by a corresponding change in the ouabain-sensitive $^{42}K^+$ uptake. Sodium preloading or a continuous enhancement of sodium influx by means of a high-frequency electrical stimulation or agents such as grayanotoxin [7,8,10] may provide conditions where the specific $^{86}Rb^+$ or $^{42}K^+$ uptake represents the capacity of the sodium pump.

When the extracellular Rb^+ or K^+ concentration is lowered, the specific uptake of these cations may be indicative of the rate of sodium efflux, which is equivalent to the rate of sodium influx in preparations which are not 'sodium-loaded'. Under these conditions, the Rb^+ or K^+ available to the sodium pump is not in excess compared to Na^+, and therefore the ratio between Na^+ and either Rb^+ or K^+ is near the optimal value. Thus, in the experiments in which the sodium pump activity is estimated from the rate of specific $^{86}Rb^+$ or $^{42}K^+$ uptake, the concentration of these cations in the medium should be reduced. Under these conditions, the sodium pump can turnover only slowly, and hence

a modest reduction in the capacity of the sodium pump resulting from a slight inhibition can be detected more easily.

In conclusion, the results obtained with $^{86}Rb^+$ or $^{42}K^+$ uptake studies in cardiac muscle should be carefully evaluated. These values may represent the activity or the capacity of the sodium pump under appropriate conditions. Under certain conditions, however, they are not indicative of the sodium efflux rate. Generally, lower extracellular concentrations of Rb^+ or K^+ are desirable for the assay.

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