Biochimica et Biophysica Acta 861 (1986) 251–258
Elsevier

BBA 73279

Unsuitability of the $^{86}Rb^+$ uptake method for estimation of $(Na^+ + K^+)$-ATPase activity in innervated tissues

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(Received 20 March 1986)

Key words: $^{86}Rb^+$ uptake; $(Na^+ + K^+)$-ATPase; Cardenolide; (Canine saphenous vein)

$(Na^+ + K^+)$-ATPase activity was estimated by $^{86}Rb^+$ uptake in dog saphenous vein to determine the validity of the technique in tissues that have a sympathetic innervation. When saphenous vein rings were incubated at 37°C in Krebs' solution containing $^{86}Rb^+$, the cardenolide acetylstrophanthidin caused a concentration-dependent inhibition of Rb^+ uptake. The threshold for inhibition was approx. 10 nM acetylstrophanthidin and the maximum effect was obtained at 9 μM. In the upper part of this concentration range (> 1 μM) acetylstrophanthidin released noradrenaline from the sympathetic nerve terminals associated with the tissue. In this upper part of the acetylstrophanthidin concentration range the α-adrenoceptor antagonist phentolamine (8 μM) reduced, by up to 25%, the degree of $^{86}Rb^+$ uptake inhibition caused by the cardenolide. In other experiments, saphenous vein strips were loaded with $^{86}Rb^+$ and perifused with Krebs' solution containing acetylstrophanthidin. At concentrations which release noradrenaline, acetylstrophanthidin increased the efflux of $^{86}Rb^+$. Phentolamine (8 μM) prevented the acetylstrophanthidin-evoked efflux of the isotope as did prior in vitro denervation of $^{86}Rb^+$ loaded strips with 6-hydroxydopamine. Exogenous noradrenaline (1–100 μM) added to the perifusing fluid also caused an efflux of $^{86}Rb^+$ that was attenuated by phentolamine. The data indicate for dog saphenous vein that with low concentrations of acetylstrophanthidin the extent of $^{86}Rb^+$ accumulation might accurately reflect prevailing $(Na^+ + K^+)$-ATPase activity. At higher concentrations of acetylstrophanthidin, however, noradrenaline is released from the nerve endings and causes $^{86}Rb^+$ efflux from the smooth muscle cells consequent upon α-adrenoceptor activation. Since this efflux reduces the extent of Rb^+ accumulation, measurement of the latter does not adequately reflect uptake mediated by the activity of $(Na^+ + K^+)$-ATPase. This is significant because in most applications of the $^{86}Rb^+$ uptake method it is the estimate of Rb^+ accumulation made in the presence of a high concentration of cardenolide that forms the basis of all subsequent calculations with respect to $(Na^+ + K^+)$-ATPase activity.

Introduction

Because rubidium is considered to be handled by the cell membrane in a manner comparable to that of potassium [1–3] and because the half-life of $^{86}Rb^+$ (18.7 days) is substantially longer than that for ^{42}K (12 h), this nuclide has been used widely as a tracer for K. Specifically, $^{86}Rb^+$ uptake by a tissue has been used as an index for $(Na^+ + K^+)$-ATPase activity (see, for example, Refs. 4–9). The method is based on the principle that the enzyme pumps Rb^+ as if it were K^+ to the interior of the cell.

In practice some Rb^+ is accumulated by mech-

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anisms independent of $(Na^+ + K^+)$-ATPase. Therefore in all methods $^{86}Rb^+$ uptake measured during an experimental manoeuvre is always related to that measured in a control tissue exposed to a sufficient concentration of a cardenolide, like acetylstrophanthidin or ouabain, to abolish the $(Na^+ + K^+)$-ATPase activity. Accordingly it is the cardenolide-sensitive accumulation of Rb^+ by the tissue during the experimental period that is said to reflect the $(Na^+ + K^+)$-ATPase activity.

The current experiments were undertaken to investigate the validity of the method for use in innervated tissues. The lateral saphenous vein of the dog was used; this tissue comprises predominantly smooth muscle but contains also a profuse sympathetic noradrenergic innervation.

Specifically the acceptibility of using the value for Rb^+ accumulation obtained in the presence of high concentrations of cardenolide as the basis for all subsequent calculations is questioned.

A preliminary account of some of the findings reported here has been presented elsewhere [10].

Methods

Experiments were performed with rings (10–20 mg) or helical strips (width 1.5–2 mm, length approx. 30 mm, weight approx. 50 mg) cut from the lateral saphenous vein removed from dogs post mortem (sodium pentobarbital, 150 mg/kg).

Three types of experiments were performed. Initially all preparations were incubated for two 90-min periods at 37°C in gassed ($95\%O_2/5\%CO_2$), modified Krebs' solution containing (mM): NaCl (118.3), KCl (4.7), $CaCl_2$ (1.8), KH_2PO_4, $MgSO_4$ (1.2), $NaHCO_3$ (25.0) and glucose (11.0). Depending upon the type of experiment intended the incubation solution in each 90 min period contained also:

(I) No additions.

(II) (±)-[7-^{3}H]noradrenaline (1.5 μM; spec. act. 12–18 Ci/mmol, New England Nuclear) and ascorbic acid (0.5 mg/ml) to load the sympathetic nerve terminals with labelled neurotransmitter. In some experiments, prior to loading with [^{3}H] noradrenaline, in vitro chemical denervation of the saphenous veins was accomplished with 6-hydroxydopamine (Sigma, St. Louis, MO, U.S.A.) using the method of Aprigliano and Hermsmeyer [11] as modified by Tan and Powis [12].

(III) ^{86}RbCl (7.5–20 μCi/ml; spec. act. 9–11 mCi/mg; New England Nuclear) to load the tissues with a marker for K^+.

I. Measurement of $^{86}Rb^+$ uptake

Vein rings were further incubated for 30 min in modified Krebs' solution (KCl replaced with RbCl, 2.0 mM; KH_2PO_4 replaced with NaH_2PO_4 1.2 mM). Each ring was then transferred to an incubation vessel containing 2 μCi $^{86}Rb^+$ in 4.5 ml modified Krebs' solution. In each experiment two rings served as controls, their incubation vessels contained no further additives. In order to ensure total inhibition of $(Na^+ + K^+)$-ATPase activity two other rings were placed in vessels containing 500 μM acetylstrophanthidin or ouabain. The other rings were exposed to a range of concentrations (1 nM–100 μM) of acetylstrophanthidin to determine the effects of the cardenolide on $^{86}Rb^+$ uptake. In some experiments phentolamine (8 μM) was present throughout preincubation and incubation periods.

After 75 min incubation, duplicate 0.1 ml aliquots of the incubation fluid were taken, 10 ml scintillant was added to each and the samples were stored overnight. The vein rings were washed (three times) in 10 ml cold, modified Krebs' solution, blotted dry and weighed. The rings were solubilized (NCS, Amersham) at 50°C overnight, cooled, acidified with 0.1 ml glacial acetic acid and 10 ml scintillant was added. The content of radioactivity in the incubation fluids and solubilized tissues was measured by β-scintillation spectrometry on the same day to eliminate errors resulting from isotope decay. Efficiency of counting was greater than 90%.

II. Measurement of ^3H efflux

After loading the saphenous vein rings with [^{3}H]noradrenaline each was rinsed three times with Krebs' solution to remove excess radioactivity from the extracellular spaces and transferred to preincubation vessels containing continuously gassed ($95\%O_2/5\%CO_2$) Krebs' solution (10 ml) at 37°C where they were equilibrated for 15–30 min. The rings were transferred to incubation vessels containing 10 ml of the same basic solution ± acetylstrophanthidin (10 nM–9 μM) where they

were incubated for 75 min. At this time 1 ml aliquots of the incubation media were taken for estimation of total radioactivity. The rings were removed, blotted dry, weighed and the radioactivity remaining in the tissue was measured by the method described by Tan and Powis [12]. From these determinations change in ^{3}H efflux in response to acetylstrophanthidin was expressed as a percentage of total tissue tritium content.

III. Measurement of $^{86}Rb^{+}$ efflux

After incubation to load the tissues with $^{86}Rb^{+}$, the helical strips were rinsed three times, each time with 10 ml Krebs' solution to remove excess radioactivity and mounted in a perifusion chamber of the same form and dimensions as that described by Bolton and Clark [13]. Isometric tension was recorded (Grass FT03 force transducer and Grass Polygraph recorder); basal tension was set at 1 g. Perifusion at a rate of 2.2 ml/min with gassed ($95\%O_2/5\%CO_2$), warm (37°C) Krebs' solution was commenced and a period of 30 min was allowed before effluent from the chamber was collected for analysis. Successive samples were then collected at 2-min intervals. The amount of radioactivity in 1 ml aliquots of each 2-min sample was estimated by Cerenkov counting in a β-scintillation spectrometer. (10 ml Krebs' solution was added to each aliquot to make these samples comparable with tissue samples treated as below). The rate constant for $^{86}Rb^{+}$ efflux was calculated for each sample as described by Bolton and Clapp [2]. Briefly the number of counts in each sample was divided by the number of counts calculated to be present in the tissue at that time. This latter value was obtained from the residual $^{86}Rb^{+}$ content of the tissue at the end of the experiment (Cerenkov counting of tissue immersed in 10 ml Krebs' solution in counting vial) to which had been added, in reverse order, the total $^{86}Rb^{+}$ content of each sample collected. (Computer programme and data plotting routine developed with Lotus 123 software).

The effects of noradrenaline and acetylstrophanthidin upon Rb^{+} efflux rate were measured as follows: Noradrenaline was added to the perifusing Krebs' solution for 2-min periods at 15–20-min intervals. When required phentolamine was added to the Krebs' solution 20 min before noradrenaline. Acetylstrophanthidin ± phentolamine was added as required to the reservoir of perifusing fluid to give the appropriate concentration at the tissue.

Analysis of data

All data are expressed as mean ± S.E. and the number of samples contributing to the mean value is one from each experiment performed. Each experimental value, however, might itself have been calculated from individual data obtained from several vein rings or helical strips. Where this is relevant the information is given in the text. Statistical analysis, where relevant, was performed with Student's *t*-test; *P* values less than 0.05 were considered to be significant.

Drugs

Acetylstrophanthidin was synthesised from strophanthidin (Sigma S6626) by the method of Koechlin and Reichstein [14]; composition and purity of the preparation was confirmed by the Australian Microanalytical Service (Amdel, Port Melbourne, Victoria). Ouabain octahydrate, noradrenaline bitartrate and 6-hydroxydopamine were obtained from Sigma; phentolamine mesylate from Ciba-Geigy. All drugs were dissolved directly into Krebs' solution as required.

Results

I. $^{86}Rb^{+}$ uptake into the tissues of the saphenous vein

Saphenous vein rings were incubated at 37°C for 75 min in Krebs' solution containing $^{86}Rb^{+}$ to determine a control value for uptake of the nuclide. In twenty experiments involving 41 rings (1–4 rings per experiment) uptake of Rb^{+} was 54.5 ± 2.93 pmol/mg tissue per min. In the same 20 experiments cardenolide inhibitable uptake was determined in 30 rings (one or two rings per experiment) in the presence of either ouabain or acetylstrophanthidin (500 μM). Uptake of Rb^{+} in the presence of cardenolide was 13.0 ± 0.77 pmol/mg per min. (The nature of the cardenolide was immaterial, the values obtained in the presence of ouabain were no different from those with acetylstrophanthidin.) From these data cardenolide-sensitive Rb^{+} uptake accounted for 76% of

total uptake in innervated rings.

Other rings were exposed to acetylstrophanthidin at a range of concentrations between 1 nM and 100 μM; Rb^+ uptake at each concentration was related to the maximal cardenolide-sensitive uptake. The relationship is shown in Fig. 1. The curve indicates a threshold for acetylstrophanthidin inhibition of uptake around 10 nM. The maximum effect occurred at around 10 μM; at this concentration of acetylstrophanthidin $^{86}Rb^+$ uptake was no different from that measured in other rings in the same experiments exposed to 500 μM ouabain. A notable feature of the curve shown in Fig. 1, and one which is accentuated by the larger number of data points in the μM concentration range, is the distinct kink in the uptake inhibition curve which appears to divide $^{86}Rb^+$ uptake inhibition into a two-phase phenomenon.

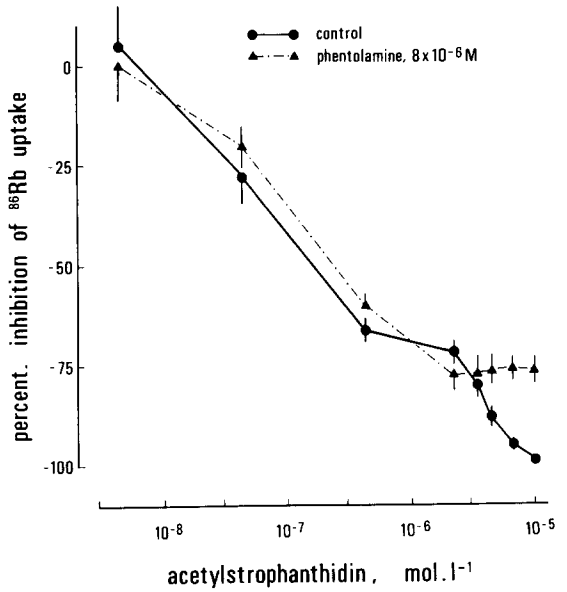

Fig. 1. Effect of acetylstrophanthidin upon cardenolide-sensitive Rb^+ uptake in dog saphenous vein. Illustrated is the concentration-response relationship for acetylstrophanthidin upon Rb^+ uptake into saphenous vein rings both in the absence (●———●) and presence (▲···▲) of 8 μM phentolamine. The ordinate is scaled from 0 to 100% which represents that range of Rb^+ uptake sensitive to inhibition by cardenolides (see Methods). Absolute values for control and cardenolide-sensitive Rb^+ uptake are given either in the text or in Table I. Each point is the mean ± S.E. of 7–15 determinations.

Effects of phentolamine upon acetylstrophanthidin sensitive $^{86}Rb^+$ uptake by saphenous vein rings. One physiological consequence of $(Na^+ + K^+)$-ATPase inhibition in saphenous vein rings is noradrenaline release from the sympathetic nerve terminals [12]. Experiments were conducted to determine whether noradrenaline released by acetylstrophanthidin could account for the biphasic character of the Rb^+ uptake curve shown in Fig. 1.

Firstly, experiments were performed to investigate the effects of acetylstrophanthidin upon Rb^+ uptake in the presence of phentolamine (8 μM); a concentration expected to attenuate dramatically the effect of released noradrenaline acting upon α-adrenoceptors in the tissues of the saphenous vein. Phentolamine itself at this concentration had no significant effect upon control $^{86}Rb^+$ uptake. In 20 experiments (30 rings, one or two rings per experiment) Rb^+ uptake was 49.1 ±

TABLE I

EFFECT OF PHENTOLAMINE UPON THE INHIBITION BY ACETYLSTROPHANTHIDIN OF Rb^+ UPTAKE INTO DOG SAPHENOUS VEIN

Pairs of rings of saphenous vein were separately incubated in modified Kreb's solution (see Methods) containing 2 μCi $^{86}RbCl$ plus either acetylstrophanthidin alone or acetylstrophanthidin and phentolamine (8 μM). After 75 min the rings were removed and Rb^+ uptake was estimated by measurement of isotope accumulation. Values are the mean ± S.E. of n experiments. Also shown is the statistical significance ($2P$) of the difference between the mean values for Rb^+ uptake in the absence and presence of phentolamine. n.s., not significant.

Acetylstrophanthidin (μM)	Rb^+ uptake (pmol/mg tissue/min)		n	$2P$
	− phentolamine	+ phentolamine (8 μM)		
0	54.5 ± 2.93	49.1 ± 2.16	20	n.s.
0.044	41.9 ± 2.88	45.0 ± 1.80	11	n.s.
0.44	30.1 ± 3.38	31.0 ± 2.66	7	n.s.
2.2	24.6 ± 2.07	22.1 ± 1.94	10	n.s.
3.5	22.8 ± 3.02	23.0 ± 2.34	12	n.s.
4.4	18.7 ± 1.31	23.1 ± 2.30	12	n.s.
6.7	15.8 ± 1.17	22.8 ± 1.76	15	< 0.005
8.8	15.0 ± 0.86	25.4 ± 2.57	7	< 0.005

2.16 pmol/mg per min (Table I). Likewise phentolamine had no effect upon $^{86}Rb^+$ uptake inhibition produced by acetylstrophanthidin at sub-micromolar concentrations, but at higher concentrations of the cardenolide there was an increasing disparity between Rb^+ uptake measured in the presence and absence of phentolamine (Fig. 1, Table I). It is clear that phentolamine prevents the expression of the second phase of inhibition of $^{86}Rb^+$ uptake caused by acetylstrophanthidin but has no significant effect upon the first phase.

II. The effects of acetylstrophanthidin upon [^{3}H]noradrenaline efflux from saphenous vein

Experiments were performed to investigate further the implication that the second phase of the Rb^+ uptake inhibition curve is due to noradrenaline release caused by acetylstrophanthidin. Firstly it was found that over the 75 min incubation period, acetylstrophanthidin caused release of ^{3}H from saphenous vein rings loaded with [^{3}H]noradrenaline. (Fig. 2). Previous experiments of this type have shown that efflux of ^{3}H evoked by cardenolides reflects qualitatively the evoked efflux of noradrenaline [12,15]. Fig. 2 contains also data from Fig. 1 and show clearly that the second phase of $^{86}Rb^+$ uptake and of [^{3}H]noradrenaline release coincide with respect to their relationship to acetylstrophanthidin concentration.

In another series of experiments ($n = 5$) it was determined that in saphenous veins previously denervated with 6-hydroxydopamine the effects of acetylstrophanthidin (1 nM–9 μM) on $^{86}Rb^+$ uptake were described by a curve that was not altered by phentolamine (8 μM; data not shown).

III. The effects of exogenous noradrenaline and of acetylstrophanthidin upon $^{86}Rb^+$ efflux from loaded helical strips of saphenous vein

In twelve innervated strips the basal rate con-

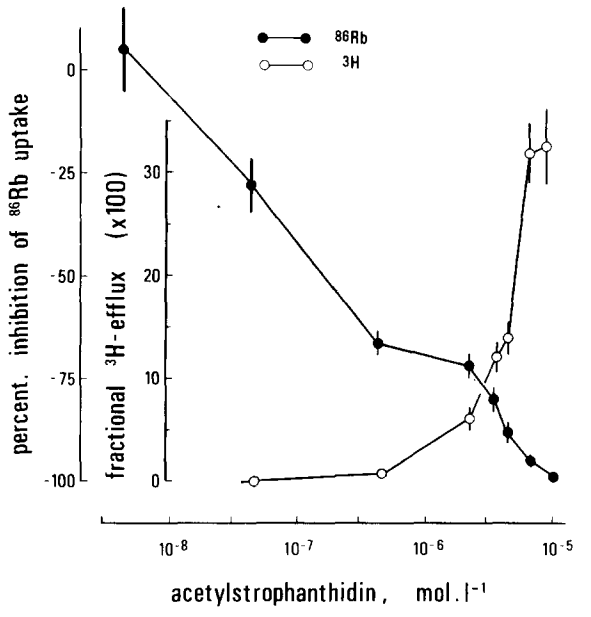

Fig. 2. Effect of acetylstrophanthidin upon ^{3}H efflux and cardenolide sensitive Rb^+ uptake in dog saphenous vein. Illustrated is the superimposition of data from Fig. 1. (●——●) upon the relationship between acetylstrophanthidin concentration and ^{3}H efflux (○——○) from saphenous vein rings. With respect to ^{3}H efflux, each point reflects the increase in fractional efflux of ^{3}H compounds over a 75 min period and is the mean ± S.E. of 6–18 determinations.

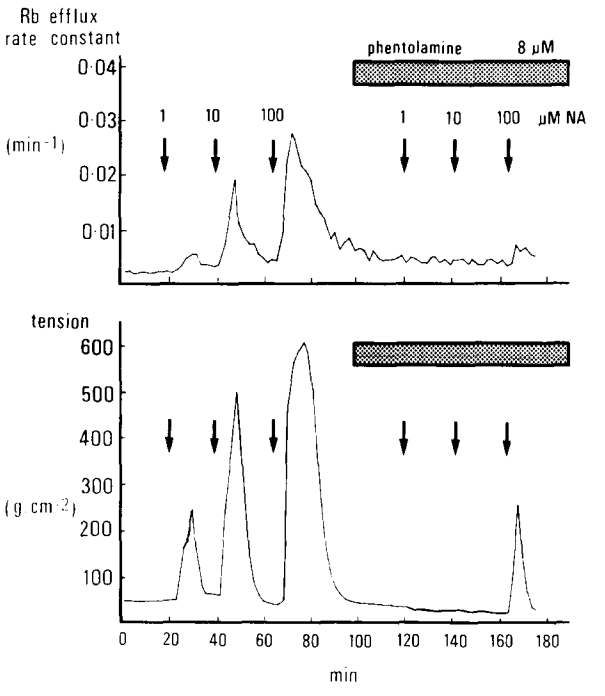

Fig. 3. Effect of exogenous noradrenaline on $^{86}Rb^+$ efflux rate and tension in dog saphenous vein. Illustrated are the data from a single experiment in which $^{86}Rb^+$ efflux and tension were recorded simultaneously in a saphenous vein strip. Noradrenaline (1–100 μM) for 2 min caused an increase in efflux and tension both of which were attenuated by phentolamine (8 μM) present in the perifusing fluid over the period shown by shaded bar.

stant for $^{86}Rb^+$ efflux measured over a 10 min period at the start of each experiment was 0.0029 $\pm$ 0.00019 per min; a value that is of the same order as that given by Bolton and Clapp [2] for strips of rabbit aorta (0.0048 $\pm$ 0.0004 per min). In these twelve strips, mean basal tension over the same 10-min, period was 26.4 $\pm$ 3.60 g/cm^2.

In seven helical strips preloaded with $^{86}RbCl$, noradrenaline, in a dose dependant fashion (1–100 μM) caused an increase in isometric tension and also an efflux of $^{86}Rb^+$. Furthermore in three strips it was shown that such efflux, like the increase in tension, is attenuated by phentolamine. Fig. 3 shows the result obtained in one representative experiment.

In three other helical strips loaded with $^{86}Rb^+$ it was established that acetylstrophanthidin, like noradrenaline, is able to evoke both an increase in isometric tension and an increase in Rb^+ efflux. Fig. 4 shows that exposure of a saphenous vein strip to acetylstrophanthidin at a concentration

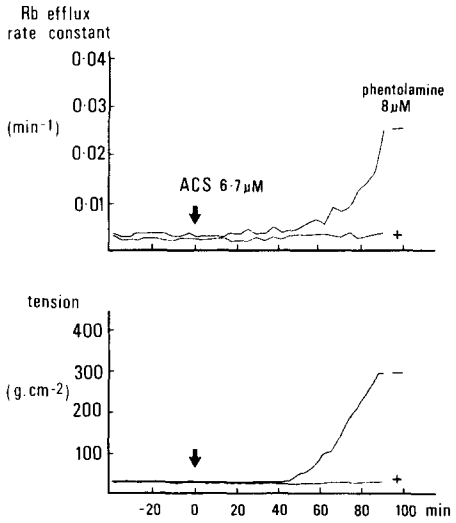

Fig. 4. Effect of acetylstrophanthidin (ACS) upon $^{86}Rb^+$ efflux rate and tension in dog saphenous vein. Illustrated are the data from a single experiment with a pair of saphenous vein strips. Acetylstrophanthidin (6.7 μM) caused an increase in $^{86}Rb^+$ efflux and tension in that strip not exposed to phentolamine. Phentolamine (8 μM) added to the perifusing fluid of the other strip 20 min before introduction of acetylstrophanthidin prevented the increase in efflux. Following their addition the drugs were present at the concentration indicated for the duration of the experiment.

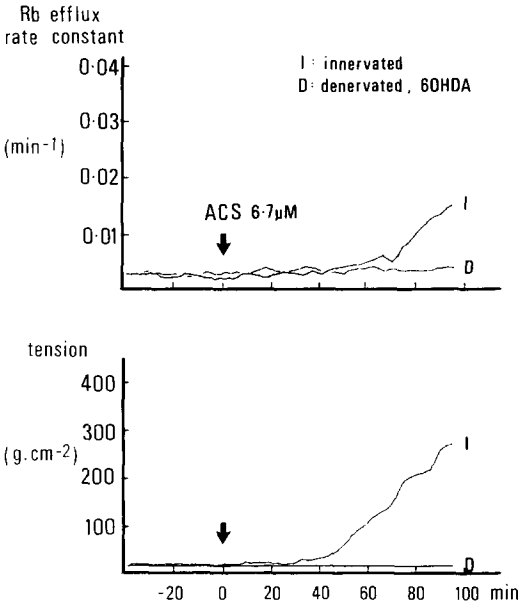

Fig. 5. Effect of acetylstrophanthidin upon $^{86}Rb^+$ efflux rate and tension in innervated and denervated dog saphenous vein. Illustrated are the data from a single experiment with a pair of saphenous vein strips. Acetylstrophanthidin (6.7 μM) caused an increase in $^{86}Rb^+$ efflux and in tension only in the innervated strip. In a strip previously acutely denervated with 6-hydroxydopamine (6OHDA), acetylstrophanthidin (ACS) was without effect.

shown above to cause noradrenaline release (6.7 μM) likewise caused $^{86}Rb^+$ efflux. The kinetics of $^{86}Rb^+$ efflux caused by acetylstrophanthidin parallel the efflux of ^{3}H-noradrenaline (see Refs. 12 and 15). At lower concentrations (e.g. 0.44 μM), acetylstrophanthidin over this timecourse had no effect upon either isometric tension or on $^{86}Rb^+$ efflux (data not shown).

In two experiments it was confirmed that the acetylstrophanthidin (6.7 μM) evoked efflux of $^{86}Rb^+$ is prevented by phentolamine (8 μM; Fig. 4) and did not occur in a strip loaded with $^{86}Rb^+$ after previous acute denervation by 6-hydroxydopamine treatment (Fig. 5).

Discussion

The $^{86}Rb^+$ uptake method for estimation of $(Na^+ + K^+)$-ATPase activity has been widely used. In brief the method is based on the principle that $(Na^+ + K^+)$-ATPase pumps Rb^+ as if it were K^+

to the interior of the cell where its concentration at the end of the experiment is used to indicate net $(Na^+ + K^+)$-ATPase activity. In practice cellular Rb^+ is accumulated by mechanisms independent of the enzyme, hence, all methods must incorporate a control containing a sufficient concentration of ouabain, or other cardenolide, to abolish $(Na^+ + K^+)$-ATPase activity to allow estimation of non-specific Rb^+ accumulation. It is the difference between total Rb^+ uptake and that measured in the presence of high concentration of cardenolide that defines the range of $(Na^+ + K^+)$-ATPase activity. The present experiments indicate that this methodology is not appropriate for tissues innervated by sympathetic, noradrenergic nerves. Fig. 6 is based on data obtained in the present experiments with dog saphenous vein. The figure shows that inhibitors of $(Na^+ + K^+)$-ATPase not only inhibit Rb^+ uptake but also cause Rb^+ efflux as a consequence of α-adrenoceptor activation by noradrenaline released from the nerve terminals. The noradrenaline release itself is a parallel consequence of $(Na^+ + K^+)$-ATPase inhibition [12].

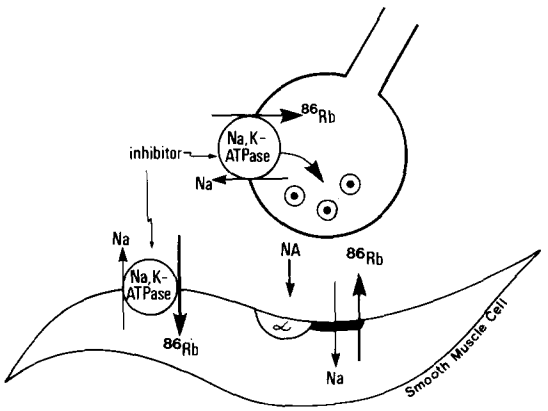

Fig. 6. The probable effects of $(Na^+ + K^+)$-ATPase inhibition upon Rb^+ distribution in dog saphenous vein. The diagram shows that for an innervated tissue like saphenous vein, inhibitors of $(Na^+ + K^+)$-ATPase affect not only $^{86}Rb^+$ uptake into nerve and muscle cell but also, by virtue of their ability to release noradrenaline would cause $^{86}Rb^+$ efflux. Measurement of $^{86}Rb^+$ accumulation at the end of an experiment would reflect the balance of uptake and efflux which would not be an accurate index of $(Na^+ + K^+)$-ATPase activity. (See text for further detail.)

Accordingly, in the presence of high concentrations of cardenolide the amount of $^{86}Rb^+$ present in the tissue at the end of the experiment does not reflect the Rb^+ influx mediated by $(Na^+ + K^+)$-ATPase activity but rather the net balance between this and Rb^+ efflux evoked by noradrenaline. It follows that the experimental estimate of the range of uptake inhibition and, by implication the apparent range of $(Na^+ + K^+)$-ATPase activity, would be falsely increased by the Rb^+ efflux. Fig. 1 indicates that under the present experimental conditions the over-estimate of uptake inhibition is around 25%.

In view of the findings reported above, it is quite probable that many investigators using the method with tissues containing stored neurotransmitter would have obtained such a spurious value for cardenolide-sensitive Rb^+ accumulation. Use of this value would undoubtedly have had an influence, perhaps significant, upon their numerical estimates of $(Na^+ + K^+)$-ATPase activity. Indeed it is quite possible that, based on this estimate, the relationship between apparent $(Na^+ + K^+)$-ATPase activity to the concentration of inhibitor of the enzyme would be sufficiently disparate from the relationship between inhibitor concentration and the physiological effect under study that a link between $(Na^+ + K^+)$-ATPase and physiological effect was deemed unlikely (Ref. 19; note also Ref. 7, p. 1472 and Ref. 8, pp. 789–790).

In the present experiments $^{86}Rb^+$ uptake was measured over a 75 min period, the time at which acetylstrophanthidin-evoked noradrenaline release is at a maximum in dog saphenous vein [15], but a time which is longer than usual for $^{86}Rb^+$ uptake determinations. Since most determinations of this type are performed over 10–30 min, it might thus be argued that there would usually be insufficient cardenolide-evoked neurotransmitter release occurring to confound $^{86}Rb^+$ uptake measurements. This argument cannot be sustained. In many experimental tissues in which the $^{86}Rb^+$ uptake method has been employed, maximum cardenolide-evoked neurotransmitter release occurs within seconds or very few minutes [16].

It is concluded that the $^{86}Rb^+$ uptake method for estimation of $(Na^+ + K^+)$-ATPase activity is not appropriate in tissues innervated by nor-

adrenergic fibres unless steps, such as those described above, have been taken to eliminate, or at least, evaluate the effects of concomitantly released neurotransmitter upon $^{86}Rb^{+}$ movement.

References

1 Pocock, G. (1983) Molec. Pharmac. 23, 681–697
2 Bolton, T.B. and Clapp, L.H. (1984) J. Physiol., Lond. 355, 43–63
3 Bartschat, D.K. and Blaustein, M.P. (1985) J. Physiol., Lond. 361, 419–440
4 Overbeck, H.W., Pamnani, M.B., Akera, T., Brody T.M. and Haddy, F.J. (1976) Circ. Res. 38, II-48-II-52
5 Erdmann, E., Phillipp, G. and Scholz, H. (1980) Biochem. Pharmac. 29, 3219–3229
6 Meyer, E.M. and Cooper, J.R. (1981) J. Neurochem. 36, 467–475
7 Vyas, S. and Marchbanks, R.M. (1981) J. Neurochem. 37, 1467–1474
8 Akera, T., Yamamoto, S., Temma, K., Kim, D-H. and Brody, T.M. (1981) Biochim. Biophys. Acta 640, 779–790
9 Lechat, P., Malloy, C.R. and Smith, T.W. (1983) Circ. Res. 52, 411–422
10 Powis, D.A. (1985) Proc. Aust. Physiol. Pharmac. Soc. 16, 103P
11 Aprigliano, O. and Hermsmeyer, K. (1976) J. Pharmac. exp. Ther. 198, 568–577
12 Tan, C.M. and Powis, D.A. (1985) Naunyn-Schmiedeberg's Arch. Pharmac. 329, 1–8
13 Bolton, T.B. and Clark, J.P. (1981) Br. J. Pharmac. 72, 319–334
14 Koechlin, H. and Reichstein, T. (1947) Helvetica Chim. Acta 30, 1673–1676
15 Lorenz, R.R., Powis, D.A., Vanhoutte, P.M. and Sepherd, J.T. (1980) Circ. Res. 47, 845–854
16 Powis, D.A. (1983) J. Autonomic Pharmac. 3, 127–154