Br. J. Pharmac. (1971), **41**, 65–75.

Effects of Na^+ and K^+ on the uptake of metaraminol by rabbit ventricular slices

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Summary

1. The ionic dependence of [^{3}H]-metaraminol transport by rabbit ventricular slices was studied.

2. Transport was Na^+ dependent. Choline, Li^+, K^+, Rb^+ or Cs^+ could not be substituted for Na^+.

3. Transport was K^+ dependent. Rb^+ and Cs^+, but not Li, could be substituted for K^+, their relative potencies being K^+=or $>Rb^+>Cs^+>>Li^+$. Higher concentrations of K^+, Rb^+, Cs^+ and Li inhibited [^{3}H]-metaraminol transport.

4. The inhibitory effects on transport of either a marked reduction in the concentration of Na^+ or of omission of K^+ were rapidly reversible on exposure of slices to Krebs solution.

5. The inhibitory effect of ouabain on [^{3}H]-metaraminol transport was markedly time dependent, being significantly increased by preincubation with ouabain. The inhibitory action of ouabain was not affected, however, by the Na^+ concentration present during preincubation.

6. An inwardly directed Na^+ gradient did not increase [^{3}H]-metaraminol transport in slices in which Na^+ pumping had been prevented by 10^{-5}M ouabain, 4° C, omission of K^+ or by metabolic inhibition.

7. These findings provide additional evidence that the Na^+ and K^+ activated adenosine triphosphatase may participate in the transport of metaraminol and related amines. In the absence of Na^+ pumping, induced Na^+ gradients were unable to produce transport of amine as would be predicted by the co-transport model proposed by Bogdanski & Brodie (1969).

Introduction

Tissues accumulate noradrenaline and structurally related compounds such as metaraminol, by a process which is temperature dependent, requires energy, is Na^+ and K^+ dependent and is inhibited by ouabain (Dengler, Michaelson, Spiegel & Titus, 1962; Bogdanski & Brodie, 1969; Giachetti & Shore, 1966; Iversen & Kravitz, 1966; Gillis & Paton, 1967; Colburn, Goodwin, Murphy, Bunney & Davis, 1968; Paton, 1968; Wakade & Furchgott, 1968). These findings suggest that the Na^+ and K^+ activated adenosine triphosphatase (membrane ATPase) is involved in the transport of noradrenaline. Bogdanski & Brodie (1969) have postulated that the transport of noradrenaline occurs as the result of the operation of a process similar to that proposed by Crane (1965) for the Na^+ dependent transport of sugars by intestine. According to this model noradrenaline transport results from an

interaction with a membrane carrier whose affinity for the amine is Na^+ dependent; the carrier transports both Na^+ and amine intracellularly where the carrier affinity for amine falls, releasing noradrenaline which is then stored in intraneuronal granules; the inward transport of amine continues since the inward Na^+ gradient is maintained by the extrusion of Na^+ from the cell due to the activity of the membrane ATPase (Bogdanski & Brodie, 1969).

The aim of the present study was to characterize further the relationship of Na^+ and K^+ to amine transport and in particular to attempt to test the model proposed by Bogdanski & Brodie (1969). For this purpose the transport of [^{3}H]-metaraminol by rabbit ventricular slices was studied. Metaraminol is accumulated by rabbit heart by the same process as noradrenaline, but, unlike noradrenaline, is not a substrate for either monoamine oxidase or catechol-O-methyl transferase and thus is accumulated *in vitro* following reserpine pretreatment (Giachetti & Shore, 1966). Preliminary accounts of a portion of this work have been presented elsewhere (Paton, 1970).

Methods

Technique for incubation of slices

Male New Zealand white rabbits, weighing 2–3 kg, were killed by a blow to the neck, after which their hearts were excised rapidly. The hearts were then rinsed in cold Krebs solution and the atria, fat and surrounding tissues removed. Ventricular tissue slices each about 30 mg in weight were prepared using a Stadie-Riggs tissue slicer.

Slices were placed in 10 ml of the medium to be studied in 25 ml Erlenmeyer flasks, which were then set in a Dubnoff metabolic shaking incubator usually at 37° C and gassed with either 95% oxygen (O_2) and 5% carbon dioxide (CO_2) or 100% O_2. After varying periods of preincubation, 2×10^{-8}M [^{3}H]-metaraminol was added to the flasks and the incubation was continued for up to 30 minutes.

Measurement of [^{3}H]-metaraminol uptake

At the end of the incubation period, slices were removed, blotted and weighed. Slices were then dissolved in NCS solubilizer (Amersham Searle) in scintillation vials using 0·4 ml per vial at 37° C for 12–18 hours. Ten millilitres of Bray's phosphor (Bray, 1960) were then added to each vial. Duplicate 0·2 ml portions of the media were added to 10 ml Bray's phosphor in scintillation vials. Total [^{3}H] in tissues and media were then measured in a Picker nuclear liquid scintillation spectrometer that had a practical counting efficiency for [^{3}H] of more than 50%. In all cases, samples were corrected for quenching using the channels ratio method.

Retention of [^{3}H]-metaraminol was expressed as a distribution ratio (R) calculated by dividing the [^{3}H] (disintegrations/min)/g of heart slice by the [^{3}H] (disintegrations/min)/ml of medium.

Chromatographically pure ±metaraminol-7-(^{3}H)-hydrochloride (in 20% ethanol) with a specific activity of 6·5 Ci/mmol was obtained from the New England Nuclear Corporation. The following drugs and chemicals were used and obtained from the sources indicated: 2,4-dinitrophenol (DNP) (Fisher Scientific Company); iodoacetic acid (IAA) (Eastman Organic Chemicals); reserpine (Ciba Company); and ouabain (U.S.P.).

Media

The ionic composition of the solutions used is shown in Table 1. When the buffer was Na HCO_3, the solutions were gassed with 95% O_2 and 5% CO_2; when Tris(hydroxymethyl)aminomethane, with 100% O_2. All solutions contained 0·03 mM Na_2 EDTA.

Statistical analysis

All results are shown as mean ± standard error of the mean. When the significance of data was evaluated, Student's *t*-test was the method used.

Results

Ability of ions to substitute for Na⁺ and K⁺

Uptake of [^{3}H]-metaraminol in Krebs solution was compared with that in a low Na^+ solution in which 116 mM NaCl was replaced iso-osmotically by one of the following: sucrose, choline chloride, LiCl, KCl, RbCl or CsCl. None of the above could substitute for Na^+, uptake being significantly reduced in all cases, but least by sucrose (Table 2).

The ability of ions to substitute for K^+ was examined by determining the uptake of [^{3}H]-metaraminol in K^+-free Krebs solution to which was added one of the following: KCl, RbCl, CsCl or LiCl, to produce concentrations ranging from 0·5 to 20 mM, isotonicity being maintained by the addition of sucrose as required. Uptake was markedly reduced in the absence of K^+, was increased significantly by the addition of 0·5 mM K^+, was maximal at 3·0 mM K^+ and remained essentially unchanged from 3–20 mM K^+ (Fig. 1). Rb^+ was a good substitute for K^+, uptake of [^{3}H]-metaraminol being significantly greater in the presence of K^+ at 3 and 10 mM only. Cs^+ could substitute for K^+, but to a significantly smaller extent than either K^+ or

TABLE 1. *Composition of media used*

Media	Constituents of media (mM)								
	NaCl	$NaHCO_3$	NaH_2PO_4	KCl	$MgSO_4$	$CaCl_2$	Glucose	Sucrose	Tris
Krebs solution	116	22	1·2	5·0	1·2	2·5	10	—	—
100 mM Na^+ solution	76·8	22	1·2	5·0	1·2	2·5	10	100	—
Low Na^+ solution	—	22	1·2	5·0	1·2	2·5	10	225	—
Na^+-free solution	—	—	—	5·0	1·2	2·5	10	260	1·1
K^+-free solution	116	22	1·2	—	1·2	2·5	10	10	—

TABLE 2. *Inability of ions to substitute for Na^+ in the transport of (^{3}H)-metaraminol by rabbit ventricular slices*

Incubation medium	Distribution ratio (mean ± S.E.)	*t* test
Krebs solution	5·04 ± 0·35 (5)	
Low Na^+ solution containing:		
Sucrose (225 mM)	3·00 ± 0·32 (6)	$P<0·01$
Choline chloride (116 mM)	2·12 ± 0·26 (5)	$P<0·001$
LiCl (116 mM)	2·06 ± 0·21 (6)	$P<0·001$
KCl (116 mM)	1·97 ± 0·21 (6)	$P<0·001$
RbCl (116 mM)	1·83 ± 0·14 (6)	$P<0·001$
CsCl (116 mM)	1·96 ± 0·26 (6)	$P<0·001$

All slices were preincubated for 30 min in the solution shown. (^{3}H)-metaraminol was then added for an additional 30 minutes. The number of slices in each group is shown in brackets.

Rb^+ ; uptake was maximal in the presence of 5–10 mM Cs^+, being reduced at 20 mM. Li^+ was unable to substitute for K^+ at concentrations up to 20 mM (Fig. 1). The ability of cations to maintain [^{3}H]-metaraminol uptake in the presence of 139·2 mM Na^+ was thus K^+=or $>Rb^+>Cs^+>>Li^+$.

Uptake of [^{3}H]-metaraminol in a modified Krebs solution, containing 100 mM Na^+ and 100 mM sucrose (i.e., 100 mM Na^+ medium in Table 1) was compared with those in which 100 mM sucrose was replaced by one of the following: KCl, RbCl, CsCl, or LiCl at a concentration of 50 mM. All these ions at this concentration significantly reduced uptake (Fig. 2).

Reversibility of effects of omission or reduction of Na^+ and K^+

Heart slices preincubated for 30 min in Na^+-free solution did not subsequently recover their ability to accumulate [^{3}H]-metaraminol when incubated in Krebs

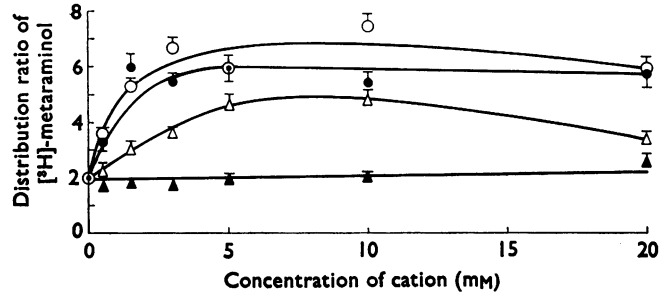

FIG. 1. Ability of cations to substitute for K^+ in the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were exposed to K^+-free solution to which was added one of the following: ○——○, KCl; ●——●, RbCl; △——△, CsCl; ▲——▲, LiCl to give concentrations ranging from 0·5 to 20 mM. The distribution ratios were determined after 30 min incubation with the amine and are shown as the mean±S.E. The number of determinations for each point ranged from six to fourteen. The uptake curves with CsCl and LiCl were significantly different from that for KCl. The uptake curve with RbCl only differed significantly from that for KCl at 3 and 10 mM.

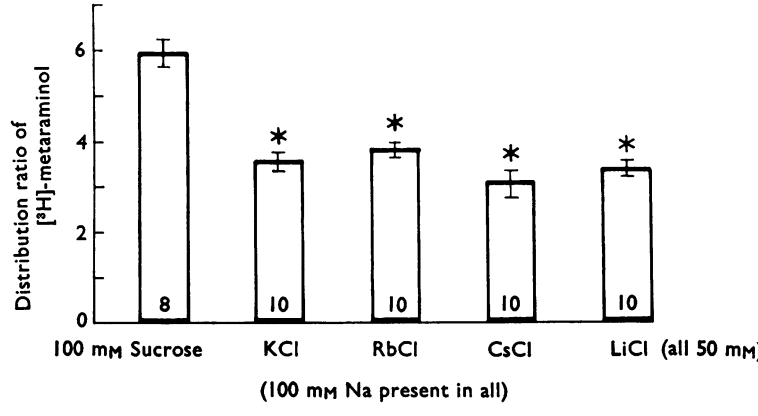

FIG. 2. Inhibitory effect of cations on the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were exposed to a 100 mM Na^+ solution to which was added 100 mM sucrose or 50 mM KCl, RbCl, CsCl or LiCl. The distribution ratios were determined after 30 min incubation with the amine and are shown as the mean±S.E. The number of determinations are indicated in the columns. The values marked with an asterisk differ significantly from those obtained in the presence of 100 mM sucrose.

medium. However, the inhibitory effect on the uptake of [^{3}H]-metaraminol of incubation in 23 mM Na^+ was rapidly reversed on exposure to Krebs solution (Fig. 3). Slices were preincubated for 30 min in either Krebs solution containing 139·2 mM Na^+ or low Na^+ solution containing 23·2 mM Na^+, then transferred to one of the above solutions to which [^{3}H]-metaraminol had been added. Inhibition of uptake was present after 6 min exposure to low Na^+ medium. Conversely slices preincubated in low Na^+ solution rapidly recovered their ability to transport [^{3}H]-metaraminol when exposed to Krebs medium (Fig. 3).

The ability of slices to recover from the omission of K^+ was also determined. Following preincubation in either Krebs solution or K^+-free solution for 60 min, during which time the media were changed three times, slices were placed in either Krebs medium or K^+-free medium containing [^{3}H]-metaraminol. Uptake of [^{3}H]-metaraminol was reduced after omission of K^+ for only 6 minutes. Conversely the ability of K^+ depleted slices to transport [^{3}H]-metaraminol was rapidly restored by exposure to 5·0 mM K^+. A 60 min period of K^+ depletion during preincubation combined with omission of K^+ during the subsequent incubation with the amine produced the greatest inhibition of uptake (Fig. 4).

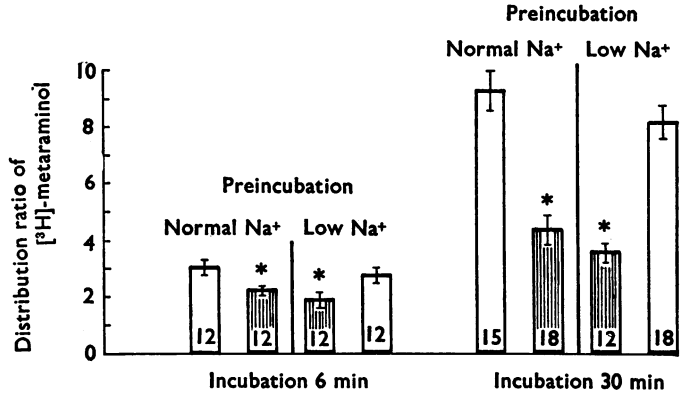

FIG. 3. Effect of Na^+ on the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were preincubated for 30 min in either Krebs solution (normal Na^+) or low Na^+ solution (low Na^+) and then incubated with [^{3}H]-metaraminol in either Krebs solution (□) or low-Na^+ solution (|||). Distribution ratios were determined after 6 and 30 min incubation and are shown as the mean ± S.E. The values marked with an asterisk differ significantly from those obtained following exposure to normal Na^+ during preincubation and incubation.

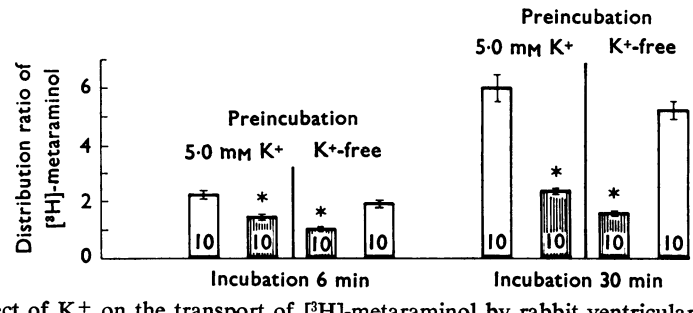

FIG. 4. Effect of K^+ on the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were preincubated for 60 min in either Krebs solution (5 mM K^+) or K^+-free solution and then incubated with [^{3}H]-metaraminol in either Krebs solution (□) or K^+-free solution (|||). The values marked with an asterisk differ significantly from those obtained following exposure to Krebs solution during preincubation and incubation. Other details as in Fig. 3.

Effect of duration of exposure on action of ouabain

The effect of 10^{-7}–10^{-4}M ouabain on [^{3}H]-metaraminol transport was studied, ouabain being present either during incubation with [^{3}H]-metaraminol only or during the period of 30 min preincubation in addition. It can be seen (Table 3) that the inhibitory effect of ouabain on uptake after 6 and 30 min exposure to the amine was markedly time dependent, being increased significantly by 30 min preincubation. Ouabain produced a concentration dependent inhibition of uptake, the maximal effect being produced by concentrations of 10^{-5}M or greater and 50% inhibition being produced by $2–3 \times 10^{-6}$M.

Effect of an inwardly directed Na^+ gradient on uptake

An analysis of the model for amine transport proposed by Bogdanski & Brodie (1969) leads to the prediction that an inwardly directed Na^+ gradient should produce net inward amine transport even in the absence of a functioning Na^+ extrusion mechanism so long as the net Na^+ gradient continues to be inwardly directed (Stein, 1967). In designing experiments to test this postulate it had to be borne in mind that (1) slices did not recover their ability to transport [^{3}H]-metaraminol after exposure to Na^+-free solution; (2) the effects of ouabain were time dependent; and (3) abolition of Na^+ pumping would result in a rise in intracellular Na^+ thus abolishing the normal inwardly directed Na^+ gradient. The following experimental design was therefore adopted: all slices were exposed during preincubation to a low $-Na^+$ solution containing 23 mM Na^+ to prevent a marked increase in intracellular Na^+ and subjected to one of the following procedures known to inhibit the membrane ATPase: (1) 10^{-5}M ouabain for 30 min or (2) 4° C for 30 min or (3) K^+-free medium for 60 min or (4) metabolic inhibition for 60 min (see Table 4 for full details). Thereafter slices were incubated with [^{3}H]-metaraminol for 6 and 30 min in Krebs medium (139·2 mM Na^+) or low Na^+ medium (23·2 mM Na^+), Na^+ pumping being inhibited by either (1) 10^{-5}M ouabain, (2) 4° C, (3) omission of K^+ or (4) omission of glucose, depending on the condition prevailing during preincubation (see Table

TABLE 3. *Effect of time on action of ouabain on transport of (^{3}H)-metaraminol by rabbit ventricular slices*

Ouabain (M)	Distribution ratio (mean±S.E.) Ouabain: Present during preincubation	Ouabain: Absent during preincubation	*t* test
(a) 6 min incubation			
—	—	2·37±0·11 (10)	—
10^{-7}	2·13±0·09 (5)	2·32±0·15 (4)	NS
10^{-6}	2·38±0·24 (5)	2·45±0·29 (5)	NS
5×10^{-6}	1·34±0·08 (10)	2·35±0·19 (10)	$P<0·001$
10^{-5}	0·99±0·05 (10)	2·06±0·15 (10)	$P<0·001$
10^{-4}	1·14±0·18 (5)	1·80±0·08 (5)	$P<0·01$
(b) 30 min incubation			
—	—	9·10±0·46 (9)	—
10^{-7}	8·80±0·65 (5)	8·82±1·08 (5)	NS
10^{-6}	7·89±0·65 (5)	9·90±0·89 (5)	NS
5×10^{-6}	2·69±0·17 (5)	5·62±0·37 (5)	$P<0·001$
10^{-5}	1·73±0·08 (5)	2·96±0·30 (5)	$P<0·01$
10^{-4}	1·59±0·06 (5)	1·69±0·03 (5)	NS

In all cases slices were preincubated for 30 min in Krebs solution. (^{3}H)-metaraminol was then added and the incubation continued for 6 or 30 minutes. The number of slices in each group is given in brackets.

TABLE 4. *Influence of Na^+ gradient on the transport of (^3H)-metaraminol by rabbit ventricular slices*

Conditions during preincubation	Conditions during incubation with (^{3}H)-metaraminol	Distribution ratio (mean ±S.E.) 6 min	30 min
Low Na^+ solution (30 min)	Low Na^+ solution	1·86±0·27 (12)	3·52±0·32 (18)
	Krebs solution	2·73±0·26 (12)	8·15±0·59 (18)
Low Na^+ solution plus 10^{-5}M ouabain (30 min)	Low Na^+ solution plus 10^{-5}M ouabain	1·20±0·13 (6)	2·47±0·10 (7)
	Krebs solution plus 10^{-5}M ouabain	1·13±0·13 (6)	1·75±0·07 (9)
Low Na^+, K^+-free solution (60 min)	Low Na^+, K^+-free solution	1·58±0·10 (8)	3·49±0·21 (8)
	K^+-free Krebs solution	1·20±0·16 (8)	1·97±0·13 (8)
Low Na^+ solution at 4° C (30 min)	Low Na^+ solution at 4° C	0·68±0·04 (6)	1·18±0·10 (6)
	Krebs solution at 4° C	0·68±0·16 (6)	0·96±0·07 (6)
Low Na^+ glucose-free solution (60 min) with 10^{-3}M dinitrophenol (40 min) and 10^{-3}M iodoacetic acid (20 min)	Low Na^+, glucose-free solution	1·09±0·04 (10)	1·79±0·07 (10)
	Glucose-free Krebs solution	0·96±0·08 (10)	1·55±0·07 (10)

In all cases slices were preincubated in low Na^+ solution then transferred to either low Na^+ solution or to Krebs solution containing [^{3}H]-metaraminol for 6 or 30 minutes. The number of slices in each group is in brackets.

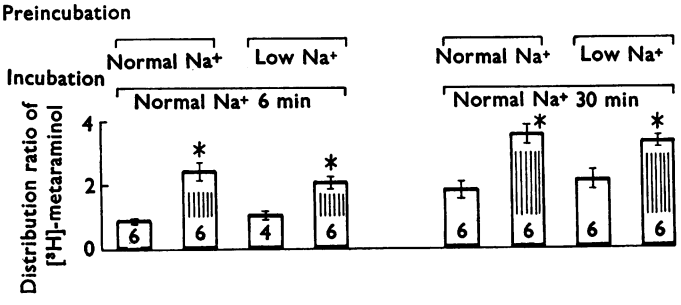

FIG. 5. Effect of Na^+ gradient on the action of ouabain on the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were preincubated for 30 min in either Krebs solution (normal Na^+) or low Na^+ solution (low Na^+) in the presence (□) or absence (|||) of 10^{-5}M ouabain. All slices were then incubated with [^{3}H]-metaraminol and 10^{-5}M ouabain in Krebs solution (normal Na^+) for 6 or 30 minutes. The values marked with an asterisk differ significantly from those obtained following exposure to Krebs solution and 10^{-5}M ouabain during preincubation and incubation. Other details as in Fig. 3.

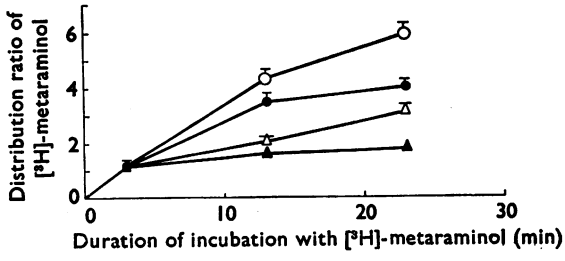

FIG. 6. Effect of Na^+-free solution on the transport of [^{3}H]-metaraminol by rabbit ventricular slices. Slices were incubated for 3 min with [^{3}H]-metaraminol in Krebs solution. They were then either removed to determine the distribution ratio or the incubation with the amine was continued in one of the following: Krebs solution (control), ○——○; presence of 10^{-5}M ouabain, ●——●; presence of 5×10^{-6}M noradrenaline, △——△; or Na^+-free solution, ▲——▲. The mean values±S.E. are indicated. The number of determinations for each point ranged from nine to twelve. The uptake curves with noradrenaline and Na^+-free solution were significantly different from the control curve as was the curve with ouabain after 23 minutes.

4). It can be seen that an induced inwardly directed Na^+ gradient did not increase the uptake of [^{3}H]-metaraminol after 6 or 30 min when Na^+ pumping was inhibited.

The relationship of the Na^+ gradient to amine uptake was studied further using 10^{-5}M ouabain. Slices were preincubated for 30 min in Krebs solution or in low Na^+ solution with or without the addition of 10^{-5}M ouabain. All slices were then transferred to Krebs solution containing 10^{-5}M ouabain and [^{3}H]-metaraminol. It can be seen (Fig. 5) that the degree of inhibition of uptake of amine produced by ouabain was not affected by exposing the slices to 23·2 or 139·2 mM Na^+ during the preincubation period, but was much greater if ouabain was present during preincubation.

Effect of an outwardly directed Na^+ gradient on uptake

An analysis of the model for amine transport proposed by Bogdanski & Brodie (1969) also leads to the prediction that an outwardly directed Na^+ gradient should produce net outward amine transport (Stein, 1967). To test this postulate slices were exposed to [^{3}H]-metaraminol in Krebs solution for 3 min, then incubation with the amine continued for a further 20 min in either Krebs medium, with or without the addition of ouabain to a concentration of 10^{-5}M or noradrenaline to a concentration of 5×10^{-6}M, or in Na^+-free medium. It can be seen (Fig. 6) that uptake was significantly reduced by ouabain (10^{-5}M) or noradrenaline (5×10^{-6}M) or by exposure to Na^+-free medium. It is important to note, however, that uptake in Na^+-free medium continued to increase slowly over the 20 min period, never falling below the control level attained after 3 minutes.

Discussion

In the present study no ion was able to substitute for Na^+ in maintaining [^{3}H]-metaraminol transport, while Rb and Cs^+ but not Li^+ could substitute for K^+. These ionic requirements are similar to those of the Na^+ and K^+ activated membrane ATPase (Skou, 1965) providing further evidence for the suggested role of the membrane ATPase in catecholamine transport (Berti & Shore, 1967 ; Bogdanski & Brodie, 1969).

Addition of Na^+ to slices preincubated in Na^+-free medium did not restore [^{3}H]-metaraminol transport. Similar findings have been reported in studies of [^{3}H]-noradrenaline transport by synaptosomes (Tissari, Schönhöfer, Bogdanski & Brodie, 1969). Na^+ deficient solutions produced non-specific damage to the intracellular ultrastructure of platelets (Prada, Tranzer & Pletscher, 1967) and similar alterations may account for the long lasting effects of Na^+ omission on amine transport.

Transport of noradrenaline and metaraminol has been generally reported to be K^+ dependent (Gillis & Paton, 1967 ; Colburn, *et al.*, 1968 ; Paton, 1968 ; Bogdanski & Brodie, 1969 ; Sugrue & Shore, 1969a) although the uptake of [^{3}H]-noradrenaline by perfused cat spleen was reported not to require K^+ (Kirpekar & Wakade, 1968). This finding may have resulted from a failure adequately to deplete the tissue of K^+ before determining the magnitude of amine uptake. Sugrue & Shore (1969a) have recently reported that the addition of K^+ to K^+ depleted heart slices restored metaraminol transport but these workers, in contrast to the present study, found that prolonged depletion of K^+ was required before transport was reduced. This

difference may reflect differences in experimental design since Sugrue & Shore (1969a) used larger slices which were incubated in a smaller volume and exposed to a larger concentration of amine.

It is noteworthy that the addition of K^+ rapidly reversed the inhibition of transport produced by K^+ depletion since this is not what would be anticipated from a consideration of the model of Bogdanski & Brodie (1969). According to their model, K^+ omission prevents amine transport by inhibiting the membrane ATPase which in turn results in an increase in intracellular Na^+. For transport to recover on the addition of K^+, Na^+ ions would have to be actively extruded and it seems unlikely that this process would have been completed within 6 min (see Fig. 4).

In the present study the inhibitory action of ouabain on [^{3}H]-metaraminol transport was found to be markedly time dependent as was reported recently for [^{3}H]-noradrenaline uptake by synaptosomes (Tissari, *et al.*, 1969). These workers found that inhibition of amine uptake by ouabain was delayed for 5–10 min whereas the membrane ATPase was inhibited almost immediately. This finding led Tissari, *et al.* (1969) to propose that ouabain blocks amine transport by inhibition of the membrane ATPase thus increasing intracellular Na^+ and thereby abolishing the usual inward Na^+ gradient. These workers were unable, however, to restore noradrenaline transport by adding 143 mM Na^+ to synaptosomes that had been previously incubated with ouabain in the presence of 25 mM Na^+ as would be predicted from the model proposed by Bogdanski & Brodie (1969).

An analysis of the model for amine transport described by Bogdanski & Brodie (1969) leads to the prediction that a Na^+ gradient should drive a net [^{3}H]-metaraminol flux even in the absence of a functioning Na^+ pump. Studies using pigeon erythrocytes (Vidaver, 1964), ascites tumour cells (Eddy, Mulcahy & Thomson, 1967) and rabbit ileum (Hajjar, Lamont & Curran, 1970) have shown that the Na^+ concentration gradient alone provides at least part of the energy required for the active transport of amino-acids in these tissues, suggesting that transport does not involve a direct input of metabolic energy. In the present study, however, an induced inwardly directed Na^+ gradient failed to increase [^{3}H]-metaraminol transport when Na^+ pumping was inhibited by ouabain, omission of K^+, by cold or by metabolic inhibition. Uptake of [^{3}H]-noradrenaline by synaptosomes was similarly not increased by an induced inward Na^+ gradient after ouabain (Tissari, *et al.*, 1969) or metabolic inhibition (White & Keen, 1970). A complicating factor in such studies, however, is the rapidity with which the intracellular Na^+ increases in the absence of Na^+ pumping. The intracellular Na^+ content of ascites tumour cells approximately doubled within 10 min under these conditions and this was associated with a marked decline in the ability of the cells to transport glycine (Eddy, *et al.*, 1967). The intracellular Na^+ content has not been determined in studies designed to examine the effect of the Na^+ gradient on amine transport when Na^+ pumping was inhibited (Tissari, *et al.*, 1969; White & Keen, 1970; present study). Such estimations were not performed in the present study because the adrenergic innervation forms a very small fraction of each slice thus making interpretation of values difficult. In an attempt to overcome the possibility of rapid dissipation of the Na^+ gradient [^{3}H]-metaraminol transport was determined after 6 min in the present study.

Crane (1954) showed that 6-deoxyglucose was extruded from villus cells of hamster intestine when the cellular Na^+ concentration was made to exceed that in

the bathing medium. This phenomenon of uphill efflux could not be demonstrated in the present study (see Fig. 6).

White & Keen (1970) found that synaptosomes prepared in a high Na^+ medium had a high internal Na^+/K^+ ratio whereas those prepared in a high K^+ medium had a lower internal Na^+/K^+ ratio. These differences in internal ionic composition had no effect, however, on uptake of [^{3}H]-noradrenaline.

A reduction of the external Na^+ concentration did not change the apparent K_m of the carrier for metaraminol but lowered the apparent V_{max} (Sugrue & Shore, 1969b). Na^+ had a similar effect on the transport of sugars by rabbit kidney slices (Kleinzeller, Kolinska & Benes, 1967) and by rabbit ileum (Goldner, Schultz & Curran, 1969) but a reduction in Na^+ altered the K_m and left the V_{max} unchanged for sugar transport by hamster intestine (Crane, Forstner & Eichholz, 1965) and for amino-acid transport by rabbit lymph node cells (Kipnis & Parrish, 1965). It is possible that the adrenergic neurone membrane transport site for noradrenaline and metaraminol combines with both Na^+ and amine to form a ternary complex. The translocations of the binary complexes formed by amine carrier and Na^+ carrier are probably much slower than that of the ternary complex, amine-Na^+ carrier.

The findings: (1) that an induced inward Na^+ gradient failed to cause uptake of amine when the Na^+ pump was inhibited (Tissari, *et al.*, 1969 ; White & Keen, 1970 ; present study) ; (2) that an induced outward Na^+ gradient failed to cause uphill efflux of amine (present study) ; and (3) that uptake of [^{3}H]-noradrenaline was not apparently determined by the intracellular Na^+ concentration (White & Keen, 1970) all suggest that the Na^+ gradient cannot be the only driving force or energy required for the uptake of noradrenaline and structural analogues by the adrenergic neurone. The membrane ATPase may be essential for amine transport possibly because the membrane transport site must be phosphorylated before translocation of amine can occur.

This study was supported by a grant (MA2472) from the Medical Research Council of Canada. Dr. Paton was a senior Research Fellow of the Canadian Heart Foundation. The author would like to acknowledge the skilled technical assistance of Miss Alice K. Fryer and the generous gifts of metaraminol bitartrate from Merck Sharpe and Dohme Canada Ltd. and of reserpine (pure substance) from Ciba Company Ltd.

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(*Received August* 24, 1970)