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MECHANISMS OF BIOGENIC AMINE TRANSPORT AND STORAGE

IV. RELATIONSHIP BETWEEN K^+ AND THE Na^+ REQUIREMENT FOR TRANSPORT AND STORAGE OF 5-HYDROXYTRYPTAMINE AND NOREPINEPHRINE IN SYNAPTOSOMES

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SUMMARY

The transport of the biogenic amines, 5-hydroxytryptamine and norepinephrine, was studied using isolated nerve endings (synaptosomes) prepared from rat and rabbit brains. The transport was absolutely dependent upon Na^+, but maximum transport required K^+. In the absence of K^+, transport increased with increasing $[Na^+]$ up to about 75 mM, then decreased. High $[K^+]$ decreased transport in the presence of Na^+. In the absence of K^+, a lapse of time preceded the blockade of transport. Moreover, preliminary incubation of synaptosomes in K^+-free media containing 150 mM Na^+ blocked initial transport more than media containing 50 mM Na^+. It is concluded that K^+-lack blocks transport as a result of changes in transmembrane ion gradients secondary to the inhibition of $(Na^+ + K^+)$-ATPase. K^+-lack slightly increased the efflux of stored norepinephrine.

INTRODUCTION

Recent reports have shown that biogenic amines are taken up by nerve endings by means of a saturable[1-3] Na^+-dependent[4,5] process obeying Michaelis–Menten kinetics[35]. A model transport mechanism similar to that developed by CRANE[6] for sugar transport has been described for nerve endings[3]. Amines released by the nerve impulse are recaptured by this process, located in the nerve membrane and utilized in the synaptic transmission of subsequent nerve impulses[7,9]. High $[K^+]$ antagonizes transport, probably by competing with Na^+ for a receptor on the amine carrier mechanism[3,10,11].

In contrast to the inhibitory effects of high $[K^+]$ on transport, low $[K^+]$ facilitates transport[3,10,12,13], suggesting that K^+ is implicated in this process in more than one way. The present report concerns the facilitative function of K^+ in trans-

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port and storage of biogenic amines by synaptosomes (isolated nerve endings prepared from brain) and the relationship between K^+ and $[Na^+]$ in these processes. Evidence is presented to indicate that K^+ exerts its facilitative effect indirectly, by activating the $(Na^+ + K^+)$-ATPase, thus maintaining optimum Na^+ and K^+ gradients across the membrane.

MATERIALS AND METHODS

Synaptosomes were prepared from rat and rabbit brain stems by the method of RODRIGUEZ DE LORES ARNAIZ AND DE ROBERTIS[14]. For the measurement of transport, a quantity of synaptosomes equivalent to a concentration of 0.25 mg protein per ml was suspended in 16 ml of various media containing either 20 ng/ml 5-[^{14}C]hydroxytryptamine (Nuclear Chicago, 39 mC/nmole) or 5 ng/ml (±)-[^{3}H]-norepinephrine (New England Nuclear, 7 C/mmole). After incubation 15 or 20 min at 37°, 4 ml of the incubation mixture were cooled on ice and the synaptosomes were separated from the medium by centrifugation at 10000 × *g* for 5 min. The pellet was washed and assayed for radioactive amine and protein. Protein was estimated by the method of WARBURG AND CHRISTIAN[15]. In some experiments, radioactive amines and deaminated metabolites were assayed both in the synaptosomes and the incubation fluid. Additional details of the methods used in this laboratory for the preparation and incubation of synaptosomes are published elsewhere[16] or are given in the text under RESULTS.

For efflux measurement of [^{3}H]norepinephrine from synaptosomes, rats were given 100 ng [^{3}H]norepinephrine injected into a lateral brain ventricle and killed 3 h after injection. Synaptosomes were prepared and incubated at 37° in Krebs bicarbonate solution. During the incubation samples of fluid (0.5 ml) were taken at 20-min intervals and assayed for the radioactivity lost from the synaptosomes. At the end of the incubation the synaptosomes were collected by centrifugation and assayed for radioactivity. The total amount of radioactive amine originally present was calculated from the final concentration of radioactivity in the medium times volume *plus* the radioactivity removed in earlier samples *plus* that remaining in the synaptosomes. The efflux of [^{3}H]norepinephrine from the synaptosomes during each 20-min time interval was determined from the increase in the total amount of radioactivity in the fluid from one sampling to the next *plus* radioactivity removed in previous samplings. The radioactivity lost from the tissue was expressed as a percentage of total radioactivity originally present in the synaptosomes and was plotted logarithmically against time on semilogarithmic graph paper. The linear portions were used to calculate the half-life of amine in brain and the rate constant of efflux.

The control medium in all experiments was Krebs bicarbonate solution equilibrated with O_2–CO_2 (95:5, v/v) (pH 7.4). Na^+-deficient media prepared with or without K^+ were made isotonic with sucrose and contained all other ions normally present in Krebs bicarbonate solution. Other media are described under specific experiments in RESULTS.

Radioactivity was estimated by scintillation counting after isolation of the various compounds to be estimated. 5-[^{14}C]Hydroxytryptamine was isolated by the method of BOGDANSKI *et al.*[17]. Deaminated metabolites of 5-[^{14}C]hydroxy-

tryptamine were estimated by the method of TISSARI *et al.*[16]. Tritiated norepinephrine and deaminated metabolites were estimated by the method of HAGGENDAL[18].

For the measurement of ion concentrations, synaptosomes were incubated in media containing 0.05 μC [^{14}C]inulin and 0.1 μC tritiated water per ml. The synaptosomes were collected by centrifugation at 10000 $\times$ *g* for 5 min at room temperature. Supernatant water was decanted and residual supernatant water was aspirated off. After resuspension in 2 ml water, 0.5-ml aliquots were removed for the estimation of ^{14}C and ^{3}H. The volume of intracellular water was estimated as the difference between total water (tritium-labeled) and extracellular water containing [^{14}C]inulin. Na^+ and K^+ in the synaptosomes were measured by flame photometry and estimates of intracellular concentrations were made after correction for the electrolytes contained in the extracellular water. Since intracellular volumes were measured in μl and large multiplication factors were involved, and a 50 % correction was made for inulin binding, more emphasis is placed on the ratios $[Na^+]:[K^+]$, than on the actual concentrations.

RESULTS

Accumulation of 5-[^{14}C]*hydroxytryptamine versus* [Na^+] *with* 6 *mM* K^+ *and in* K^+*-free media*

Fig. 1 shows the accumulation of 5-[^{14}C]hydroxytryptamine by rat synaptosomes, expressed as the concentration ratio of intracellular to extracellular amine.

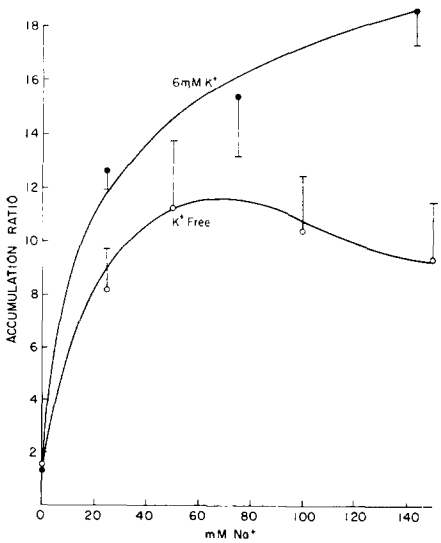

Fig. 1. Effect of K^+-lack on the accumulation of 5-[^{14}C]hydroxytryptamine by synaptosomes incubated in media containing various [Na^+]. (○), media containing 6 mM K^+. (○), K^+-free media. Standard medium, Krebs bicarbonate solution (pH 7.4) equilibrated with O_2–CO_2 (95:5, v/v). Isotonicity in Na^+-deficient media was maintained by sucrose. 20 ng/ml 5-[^{14}C]hydroxytryptamine were incubated in the various media immediately after transfer of the synaptosomal suspensions to the incubator. Incubation time, 15 min. The accumulation ratio (ordinate) is the ratio of the concentration of unchanged 5-[^{14}C]hydroxytryptamine in synaptosomes to that in the medium. Averages of four experiments $\pm$ S.E.

When K^+ was present in the medium at the normal concentration of 6 mM, the accumulation increased with increasing $[Na^+]$ up to at least 150 mM. In the K^+-free media, accumulation increased almost in parallel with controls with increasing $[Na^+]$ up to about 75 mM but then decreased as $[Na^+]$ was raised to 150 mM.

Accumulation of [^{3}H]norepinephrine versus [Na^+] at 6 mM K^+ and in K^+-free media

The accumulation of [^{3}H]norepinephrine by rat synaptosomes incubated in media containing various $[Na^+]$ is recorded in Table I. The accumulation of [^{3}H]norepinephrine, like 5-[^{14}C]hydroxytryptamine, was increasingly stimulated by Na^+ up to a $[Na^+]$ of 150 mM, when K^+ was present in the media. Accumulation was stimulated by $[Na^+]$ up to about 75 mM in the K^+-free medium. However, the accumulation of [^{3}H]norepinephrine was decreased as the $[Na^+]$ was increased from 75 to 150 mM in the K^+-free media. The inhibition of [^{3}H]norepinephrine accumulation by the lack of K^+ was more pronounced than in the case of 5-[^{14}C]-hydroxytryptamine. For example, at 143 mM Na^+, the accumulation ratio of 5-[^{14}C]hydroxytryptamine was 18 in the presence of 6 mM K^+ compared to 9.3 when K^+ was absent. In contrast, the accumulation ratio of [^{3}H]norepinephrine was 35 in the medium containing 6 mM K^+ compared to 6, when K^+ was absent.

TABLE I

EFFECT OF K^+ LACK ON THE ACCUMULATION OF [^{3}H]NOREPINEPHRINE BY RAT SYNAPTOSOMES INCUBATED IN MEDIA CONTAINING VARIOUS $[Na^+]$

Conditions of incubation and K^+-free media as described for Fig. 1. 5 ng/ml [^{3}H]norepinephrine. Averages of four experiments ± S.E.

$[Na^+]$ (mM)	*Accumulation ratios*	
	6 mM K^+	*K^+-free*
0	1.5	1.7
25	5.8	4.0 ± 0.2
50		6.4 ± 1.4
75	16.8	—
100		6.2 ± 1.6
150	33	5.2 ± 0.9

This difference may reflect the fact that more 5-hydroxytryptamine than norepinephrine was metabolized during the incubation (Table II).

Transport of 5-[^{14}C]hydroxytryptamine and [^{3}H]norepinephrine

Accumulation of 5-[^{14}C]hydroxytryptamine and [^{3}H]norepinephrine accounts for only a fraction of the amine that has penetrated the plasma membrane[10,16], since the amine may be deaminated inside the cell and the ^{14}C-labeled products then released back into the incubation medium. Thence, total deaminated metabolites, intracellular and extracellular, in addition to accumulated amine must be determined in order to assess the quantity of amine that has entered the cell. Furthermore, in order to estimate the quantity of amine carried into the cell by the transport mechanism a correction must be made for nonsynaptosomal metabolism and accumulation and for passive diffusion. This correction was made by estimating amine

TABLE II

EFFECT OF $[Na^+]$ ON UPTAKE AND TRANSPORT OF 5-[^{14}C]HYDROXYTRYPTAMINE BY RAT SYNAPTOSOMES INCUBATED IN K^+-FREE MEDIUM CONTAINING VARIOUS $[Na^+]$

Conditions of incubation and K^+-free media are described in Fig. 1. Total uptake and metabolism are expressed as a percentage of the 5-[^{14}C]hydroxytryptamine originally present in the medium and is calculated according to the following equation:

$$\text{Total uptake and metabolism} = \frac{\text{Accumulated 5-[}^{14}\text{C]hydroxytryptamine + total deaminated metabolite}}{\text{Total 5-[}^{14}\text{C]hydroxytryptamine + total deaminated metabolite}} \times 100$$

Total deaminated metabolite and total 5-[^{14}C]hydroxytryptamine represent accumulated substance *plus* that recovered in the medium. Transport was estimated as the difference between total uptake and metabolism as calculated above and nonsynaptosomal accumulation and metabolism as estimated in the $(Na^+ + K^+)$-free medium (accumulation ratio $< 2{\cdot}0$, transport $\cong 0$).

Incubation medium (mM)	*Total uptake and metabolism ± S.E.*		*Transport*	
	5-[^{14}C]Hydroxytryptamine (%)	*[^{3}H]Norepinephrine* (%)	*5-[^{14}C]Hydroxytryptamine* (%)	*[^{3}H]Norepinephrine* (%)
Krebs' bicarbonate	85.3 ± 4	32.6 ± 6	60.2	20.4
6 K^+; 50 Na^+	74.2 ± 6	24.6 ± 2.8	49.7	12.4
0 K^+; 0 Na^+	25.1 ± 1	12.2 ± 3.7	0.0	0.0
0 K^+; 25 Na^+	48.0 ± 7	14.2 ± 2.2	22.9	2.0
0 K^+; 50 Na^+	57.2 ± 6	19.2 ± 1.0	32.1	7.0
0 K^+; 100 Na^+	40.1 ± 9	19.7 ± 2.3	15.0	7.5
0 K^+; 150 Na^+	39.5 ± 7	15.3 ± 3.6	14.4	3.1

accumulation and metabolism in synaptosomes suspended in $(Na^+ + K^+)$-free media or in synaptosomes previously incubated for 10 min with 1 mM ouabain, conditions which eliminate carrier-mediated transport across the synaptosomal membrane[16]. In the present experiments the correction amounted to 25.1 % of the 5-[^{14}C]hydroxytryptamine, and 12.2 % of the [^{3}H]norepinephrine originally present (Table II).

Table II shows that the transport of 5-[^{14}C]hydroxytryptamine or [^{3}H]-norepinephrine by synaptosomes incubated in media containing various $[Na^+]$ responded to the lack of K^+ in a manner completely analogous to accumulation. To illustrate this, transport increased as the $[Na^+]$ was increased up to about 50–100 mM and decreased above this concentration. In Krebs solution transport of 5-[^{14}C]-hydroxytryptamine and [^{3}H]norepinephrine were about 4 and 7 times that in a K^+-free counterpart (150 Na^+, 0 K^+).

Transport of 5-hydroxytryptamine and norepinephrine versus $[K^+]$ at normal $[Na^+]$

Table III shows the effects of various K^+ levels on 5-hydroxytryptamine and norepinephrine transport by synaptosomes that were incubated in media containing 143 mM Na^+. The transport of 5-[^{14}C]hydroxytryptamine and [^{3}H]norepinephrine was enhanced by 6 mM K^+ compared to transport in the absence of

K^+, but as the $[K^+]$ was increased above 6 mM the transport of amine was progressively decreased. It should be pointed out, however, that even with 75 mM K^+, transport was greater than in K^+-free media. This finding indicates that high $[K^+]$ inhibits maximum amine accumulation but the presence of K^+ still fulfills some requirement of the transport process. Hence the facilitative and inhibitory functions of K^+ may be exerted at different points in the transport process.

TABLE III

THE EFFECT OF VARIOUS K^+ LEVELS ON THE TRANSPORT OF 5-[^{14}C]HYDROXYTRYPTAMINE AND [^{3}H]NOREPINEPHRINE BY RAT AND RABBIT SYNAPTOSOMES

Synaptosomes were incubated 10 min in media containing the various K^+ and Na^+ levels given in the table, *plus* all other ions normally present in Krebs bicarbonate solution. The solution containing 50 mM Na^+ was made isotonic with sucrose, then K^+ was added to a concentration of 100 mM. 5-[^{14}C]Hydroxytryptamine (20 ng/ml) was then added, and the incubations continued for 20 min. Transport was estimated as described for Table I. Transport by synaptosomes incubated in normal Krebs bicarbonate solution (6 mM K^+) was arbitrarily assigned the value of 100%, and transport by synaptosomes incubated in the other media was expressed as a percentage of that in the control solution. Inhibition was estimated by subtracting transport from 100. The numbers in parentheses refer to numbers of experiments.

$[K^+]$ (*mM*)	$[Na^+]$ (*mM*)	5-[^{14}C]*Hydroxytryptamine* (% *inhibition* ± *S.E.*)		[^{3}H]*Norepinephrine* (% *inhibition* ± *S.E.*)
		Rat	*Rabbit*	*Rat*
0	150	78 ± 0.3 (2)	76 ± 4.7 (4)	85 ± 4.3 (3)
3	143	24 ± 0.8 (2)	—	—
6	143	0	0	0
12	143	20 ± 1.4 (2)	—	—
25	143	32 ± 0.8 (2)	—	14 ± 1.4 (3)
75	143	—	24 ± 1.9 (3)	32 ± 3.2 (3)
100	50	—	52 ± 2.8 (4)	—
6	50	—	29 ± 2.2 (3)	—

Time lapse for the inhibition of accumulation and transport by K^+-lack

The transport of amines by synaptosomes incubated in control media is progressive for at least 20 min. By contrast, the transport of amines by synaptosomes incubated in K^+-free media begins normally but is almost completely blocked after 5 min (Table IV). This delayed inhibition of the transport process caused by the lack of K^+, an essential extracellular ion, necessitates the conclusion that the inhibition in K^+-free media is the result of a secondary time consuming process caused by the lack of K^+. This situation differentiates from that in which the time delay might be caused by the primary reaction between a receptor and a foreign chemical. The following experiments show that the time-dependent secondary process is dependent upon the presence of Na^+ in the external medium. Thus, a 10-min preliminary incubation of synaptosomes in a K^+-free medium containing 150 mM Na^+ completely blocked the accumulation and transport of 5-[^{14}C]hydroxytryptamine introduced into the tissue suspension at the end of the preliminary

incubation period. By contrast, a 10-min preliminary incubation of synaptosomes in a K^+-free medium containing 50 mM Na^+ blocked transport by about 60% (Table III).

The effect of K^+-lack on the concentration of cytoplasmic Na^+ and K^+

The inhibitory effect of Na^+ on transport in K^+-free media might be related to changes in the concentrations of intracellular Na^+ and K^+, as a result of the inhibition of ($Na^+ + K^+$)-ATPase. The inhibition of that enzyme by K^+-free media has been demonstrated in preparations of synaptosomes.[16] The increase in intracellular [Na^+] and decrease in intracellular [K^+] resulting from the incubation of synaptosomes in K^+-free media are shown in Table V.

TABLE IV

THE EFFECT OF K^+-FREE MEDIUM ON THE TIME COURSE OF ACCUMULATION AND TRANSPORT OF 5-[^{14}C]HYDROXYTRYPTAMINE BY SYNAPTOSOMES

20 ng/ml 5-[^{14}C]hydroxytryptamine were introduced into suspensions of synaptosomes in normal or in K^+-free Krebs bicarbonate media in the cold. The suspensions were then transferred to the incubator and sampled for accumulation and transport at 0, 5 and 15 min. For comparison synaptosomes were incubated at 37° in the K^+-free medium 15 min before substrate was introduced into suspension. The inhibition in this instance was reversible upon the addition of K^+ at 6 mM (accumulation 80% of that in Krebs bicarbonate solution after 15 min). Average of 4 experiments ± S.E.

Medium	*Accumulation ratios ± S.E.*			*Transport (% of original amine at 0 min)*		
	0 min	*5 min*	*15 min*	*0 min*	*5 min*	*15 min*
Krebs	0.5 ± 0.2	6.2 ± 1.0	17.5 ± 1.1	0.9 ± 0.4	12.2 ± 4.5	61.2 ± 7.4
150 mM Na^+; 0 K^+	0.4 ± 0.3	5.5 ± 1.2	7.2 ± 1.8	0.6 ± 1.8	6.1 ± 1.9	11.5 ± 3.0
150 mM Na^+; 0 K^+ 15 min prior incubation	0.3 ± 0.2	1.7 ± 0.3	2.3 ± 0.5	0.2 ± 0.1	1.0 ± 0.8	1.5 ± 1.0
50 mM Na^+; 0 K^+ 15 min prior incubation	0.4 ± 0.2		7.0 ± 1.6			

TABLE V

EFFECT OF VARIOUS MEDIA ON SYNAPTOSOMAL [K^+] AND [Na^+]

Synaptosomes were incubated in the various media listed on the table for varying durations of time given in the column headings. Aliquots of synaptosome suspension were removed at the indicated time intervals. Averages of four experiments ± S.E.

Media (mM)	*5 min*			*15 min*		
	Na^+ (mM)	*K^+ (mM)*	*$[Na^+]_i/[K^+]_i$ ratio*	*Na^+ (mM)*	*K^+ (mM)*	*$[Na^+]_i/[K^+]_i$ ratio*
Krebs	119 ± 16	20 ± 4	6	185 ± 20	31 ± 6	6
0 K^+, 150 Na^+	120 ± 12	6 ± 2	20	190 ± 21	4 ± 2	47
0 K^+, 50 Na^+	35 ± 4	7 ± 2	5	74 ± 5	5 ± 1	15
6 K^+, 150 Na^+, 0.1 ouabain	130 ± 20	12 ± 2	11	190 ± 25	14 ± 3	14

The effect of K⁺-lack on the efflux of stored [³H] norepinephrine

The following experiments were carried out to determine whether K⁺-lack increased the efflux of stored [³H]norepinephrine, analogous to the previously reported effects of Na⁺-lack, which not only increased the efflux of stored [³H]-norepinephrine but also prevented the uptake of exogenous [³H]norepinephrine[4,10]. Fig. 2 shows that the omission of K⁺ from normal Krebs bicarbonate solution increased the rate constant of efflux from a control value of 0.0026–0.0037 min^{-1}. Although significant, this change in k_{efflux} is relatively slight compared to the effect of the absence of Na⁺, which increased k_{efflux} from the control 0.033 min^{-1} to 0.0100 min^{-1} (ref. 10).

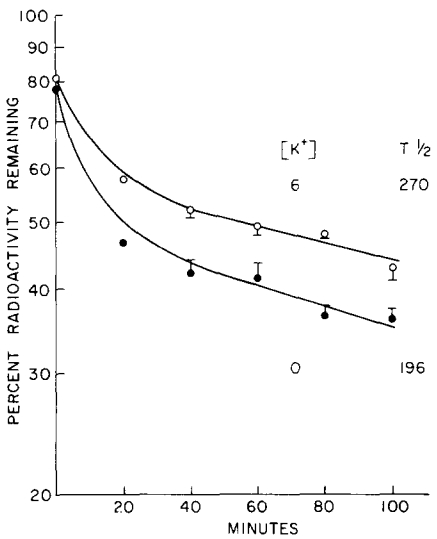

Fig. 2. Efflux of [³H]norepinephrine from synaptosomes. Rats were injected with 50 ng [³H]-norepinephrine by way of brain ventricle and killed 3 h later. Synaptosomes were incubated either in normal Krebs bicarbonate solution (○) or in K⁺-free Krebs bicarbonate solution (●). Ordinate: [³H]norepinephrine remaining in synaptosomes plotted as the percentage of the [³H]norepinephrine originally present (logarithmic scale). Abscissa: time in minutes. Averages of three experiments ± S.E.

DISCUSSION

The facilitative effects of low [K⁺] on the transport of amine into synaptosomes is similar to that shown for the uptake of norepinephrine by heart slices[3,13], the uptake of 5-hydroxytryptamine and norepinephrine by synaptosomes[10,12], the uptake of amino acids and sugars by a variety of tissues[19–22] and the uptake of 5-hydroxytryptamine by platelets[23].

The mechanism by which K⁺ facilitates transport of amino acids and glucose has not been clearly defined. It has been suggested[24] that the increased transport of glucose was secondary to increased tissue respiration when [K⁺] was raised above normal. RIGGS *et al.*[25] have postulated that transport depends upon the energy provided by the downhill gradient of K⁺ by means of which the carrier mechanism

returns to the exterior surface of the plasma membrane. Extracellular K^+ maintains the normal level of intracellular K^+. As an alternative possibility, it was suggested that amine could enter the cell by means of energy provided by the downhill movement of Na^+. Evidence has been reported in support of the idea that gradients of both Na^+ and K^+ may participate in glycine transport by Ehrlich mouse ascites tumor cells[26–28]. A study of particular interest demonstrated that K^+ stimulated absorption of only those sugars that are actively transported[29]. Because K^+ stimulated active transport in conjunction with Na^+, a possible link between ($Na^+ + K^+$)-ATPase and the transport of sugars[29], inorganic phosphate[30] and glycine[31] was suggested. No details of the possible link-up were described.

We have shown that K^+-free media, like ouabain, block synaptosomal ($Na^+ + K^+$)-ATPase, suggesting that K^+ participates in an energy-producing aspect of transport. The enzyme was blocked almost instantly, whereas the development of transport block could be measured temporally. These facts suggest that the inhibition of transport is the secondary result of the primary inhibition of ($Na^+ + K^+$)-ATPase, perhaps the result of redistribution of electrolytes. In support of this argument is the fact that a Na^+ gradient facilitates transport of 5-hydroxytryptamine, whereas high $[Na^+]_0$ increases the transport inhibiting effect of both K^+-lack and ouabain[16]. Moreover, freshly prepared synaptosomes, nearly emptied of Na^+ and K^+, fill up with Na^+ when incubated in K^+-free Krebs solution while losing some of their remaining K^+. These synaptosomes transport amines for about 5 min then stop. Prior incubation of synaptosomes in an identical medium blocks the transport of subsequently added substrate, but significant transport occurs under identical conditions in a medium containing only 50 mM Na^+. Thus, the inhibition of transport in K^+-free media (and ouabain[16]) is related to $[Na^+]_0$, the transport becoming blocked as the corresponding $[Na^+]_i$ increases. By contrast transport takes place, increasing with time, in identical media containing 6 mM K^+, although $[Na^+]_i$ was also high, but $[K^+]_i$ increased to about 25 mM. These results indicate that transport is inhibited by high $[Na^+]_i$ but not if $[K^+]_i$ is maintained by electrolyte transport. Since the intracellular metabolism of amine is blocked when accumulation is blocked or both are correspondingly increased by the various alterations of media, the reaction site must be at the cell membrane, not at possible intracellular binding sites (see refs. 10 and 16).

The question to be answered is: how do all these facts fit into our adaptation of the Na^+ gradient hypothesis[6,25] that the energy for transport is provided for by the Na^+ gradient across the cell membrane? In synaptosomes $[K^+]_i$ and $[Na^+]_i$ relative to each other are the reverse of those found in most types of cells. This raises the question of whether or not the tissue is functioning normally; but the same question faces all who work with any cell fraction. The synaptosomes transport K^+ in the normal direction[32], and we are sure that amine transport occurs under the conditions we describe. It would be extremely difficult to measure the ion content of nerve endings *in situ*. Being functionally specialized to release neurotransmitter, the nerve ending might very well differ in this regard from the rest of the nerve.

The dependence of transport of organic solutes upon Na^+ or $Na^+ - K^+$ gradients has been amply demonstrated by numerous workers[6,25–28,33,34]. KOSTYO AND SCHMIDT[32] presented evidence which they interpreted as establishing the independence of the transport of α-aminoisobutyric acid from $[Na^+]_i$ and $[K^+]_i$. They have shown

that the transport of α-aminoisobutyric acid by rat diaphragm is blocked by 1 mM ouabain at 30 min, but not by 10 μM ouabain at 4 h, although $[Na^+]_i$ and $[K^+]_i$ are the same in both cases. Because of the time element it seems apparent that considerable cation transport occurred while the [ions]$_i$ were changing, indicating that $(Na^+ + K^+)$-ATPase was not completely blocked by the low [ouabain]. The fact is that in these and the present experiments, organic substances are transported when electrolytes are in the process of being transported regardless of the [ions] in the total cytoplasm. Otherwise transport of organic molecules must be directly mediated by $(Na^+ + K^+)$-ATPase, an hypothesis that has been previously tested[6,27]. What follows is our interpretation of the data.

Transport of α-aminoisobutyric acid (or amines in our experiments) could have occurred with low concentrations of ouabain if transport is controlled by the $[Na^+]$ and $[K^+]$ at the transport site, that is, the carrier site on the membrane. It is known that local [ions] at the membrane can differ from those in the cytoplasm. Thus, movements of ions during the passage of a nerve impulse take place at the membrane. More to the point, KAYE *et al.*[31] have shown, using a Na^+ precipitant and electron photomicrography, that ouabain produces local accumulations of Na^+ at the cell membrane. The accumulations were interpreted as being Na^+ bound to the Na^+ carrier. Regardless of the interpretation of their photographs, their work points up the fact that incoming Na^+ will raise $[Na^+]_i$ at the membrane before it being distributed throughout the cytoplasm and in position to be quickly removed. In the presence of low concentrations of ouabain, there may be sufficient transport of ions to maintain ion gradients in the immediate vicinity of functional pumps, presumably very near to the carriers for organic molecules, even though ion gradients are small throughout the cytoplasm as a whole. A likely site of residual gradients would be at the junction of membrane and storage vesicle.

All the effects of K^+-lack can possibly be explained in terms of a Na^+ gradient at the carrier site for organic molecules and maintained with the aid of extracellular K^+. The $[Na^+]_0$ will have variable effects on transport depending upon the resultant $[Na^+]_i$, provided that K^+_i also participates in transport. Intracellular K^+ may participate in the transport process by reducing the affinity of the carrier for amine, acting in competition with Na^+, or by some mechanism related to a K^+ gradient[31]. If K^+ is as weak a competitor of Na^+ at the interior of the membrane as it is at the exterior (Table III and ref. 3), then K^+_i could not compete effectively against high $[Na^+]_i$ (Table III) and the affinity gradient at the carrier in the presence of 150 mM Na^+, no K^+, would be less than in the presence of 50 mM Na^+, no K^+. Conversely, Na^+ deficiency inhibits transport by slowing the rate of the initial attachment of amines to carrier at the outside surface of the membrane. The above interpretation does not preclude the possible role of the K^+ gradient[25–28] (described above) in amine transport, a possible modification of our earlier model[3].

The effect of K^+ lack and ouabain[16] is significantly greater on norepinephrine than on 5-hydroxytryptamine transport. Why this is so is not known, but qualitatively the two amines show nearly identical responses to the various experimental procedures we have employed. One possible reason for the difference is that the K^+ gradient is a less important factor in 5-hydroxytryptamine transport than in norepinephrine transport.

K^+-lack also has a slight effect on the storage of norepinephrine in adrenergic

nerve endings in heart slices[3] and in synaptosomes. The small increase in the rate of efflux of amine from tissues incubated in K^+-free Krebs media can be explained by the failure of the pump mechanism to recapture amines tending to escape.

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While this manuscript was in preparation, a paper by WHITE AND KEEN[37] appeared reporting that a Na^+ gradient was not the sole source of energy for the transport of norepinephrine by synaptosomes. The basis of their conclusion was that raising the Na^+ concentration from 156 to 286 mM increased the accumulation of norepinephrine by synaptosomes incubated in control medium but not by synaptosomes treated with metabolic inhibitors. Under the conditions of their experiments, ion transport was probably abolished[32]. The experiments of WHITE AND KEEN[37] were therefore similar to our experiments on the effect of K^+-lack and ouabain on the uptake of norepinephrine (above and ref. 16) which we have discussed.

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