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EFFECTS OF EXTERNAL Na^+ AND K^+ ON THE INITIAL RATES OF NORADRENALINE UPTAKE BY SYNAPTOSOMES PREPARED FROM RAT BRAIN

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SUMMARY

1. A technique was devised using Millipore filtration to quickly terminate the uptake of noradrenaline by rat brain synaptosomes, so that true initial rates of influx could be determined.

2. The uptake of noradrenaline was linear for approximately 1 min but had significantly declined by 5 min. Uptake after 1 min incubation was therefore taken as an estimate of the initial rate of influx and was employed in all subsequent studies.

3. Noradrenaline influx was inhibited by low concentrations of desmethylimipramine and cocaine.

4. Reducing the concentration of Na^+ in the medium reduced the V but did not alter the K_m for uptake. In the absence of external Na^+, uptake was reduced but still obeyed Michaelis–Menten kinetics and had the same K_m as in the presence of 120 mM Na^+.

5. Removing K^+ from the medium reduced the V but had no significant effect on the K_m for noradrenaline transport.

6. Two models for the transport of noradrenaline across the pre-synaptic neuronal membrane are presented. In the first, Na^+ increases the rate of movement of the carrier–noradrenaline complex across the membrane, whilst in the second, Na^+ increases the total number of active sites for noradrenaline transport.

INTRODUCTION

When brain is gently homogenized in isotonic sucrose, synaptosomes, or pinched-off and resealed nerve-endings of presynaptic origin, are formed[1,2]. These synaptosomes have been shown to accumulate noradrenaline against a concentration gradient [3,4] by a process which requires external Na^+ and K^+ in physiological concentrations[3,4], is ouabain sensitive[4] and is inhibited by cyanide and dinitrophenol[5] thus suggesting that an energy-dependent, active-transport process is involved. Hence preparations of synaptosomes may be used to study the re-uptake process which is thought to terminate the action of noradrenaline at the synaptic cleft *in vivo*[6].

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BOGDANSKI *et al.*[4] have proposed a scheme for noradrenaline transport, based on a model which had previously been postulated by CRANE[7] to explain the transport of sugars and amino acids across intestinal mucosa, in which Na^+ was thought to facilitate transport by increasing the binding affinity of a carrier for noradrenaline. Under equilibrium conditions, this increase in affinity of carrier for noradrenaline in the presence of Na^+ should manifest itself as a reduction in the apparent Michaelis constant (K_m) and there should be no change in the maximal rate of uptake (V).

In order to apply enzyme kinetics to transport processes, the initial rate of transport must be observed before significant concentrations of substrate have accumulated at the inner surface of the membrane. In the study reported here, a technique was devised using Millipore filtration to quickly terminate the uptake of noradrenaline by synaptosomes, so that true initial rates of influx could be measured and the effects of Na^+ and other parameters on the transport kinetics could be determined. A preliminary report of part of this work has been made[8].

MATERIALS AND METHODS

General

(−)-[α-^{14}C]Noradrenaline bitartrate, and (−)-[7-^{3}H]noradrenaline acetate were obtained from Amersham/Searle Ltd., Toronto. Desmethylimipramine was obtained from Geigy (Canada) Ltd., Montreal, and cocaine–HCl was supplied by British Drug Houses (Canada) Ltd., Toronto. Millipore filters of 25 mm diameter and 0.45 μm pore size were purchased from the Millipore Corporation Ltd., Montreal. Protein was determined by the method of LOWRY *et al.*[9].

Preparation of synaptosomes

The preparation and isolation of synaptosomes was performed essentially as described previously[10]. This preparation has been shown to yield osmotically and morphologically intact synaptosomes[10]. All steps in the procedure were carried out in the cold (0–4°). Male Wistar rats weighing from 200–250 g were killed by cervical dislocation and the whole brains removed and chilled in 0.32 M sucrose. Each of four brains was homogenized in 12 ml of 0.32 M sucrose by 15 strokes of a loose-fitting, teflon-glass homogenizer. The homogenate from the four brains was divided into eight portions and centrifuged at 1000 × g for 10 min, and the resulting supernatants recentrifuged at 11000 × g for 20 min. The eight pellets so obtained were resuspended in 16 ml of 0.32 M sucrose and 3 ml layered onto each of six discontinuous density gradients consisting of 5 ml 0.8 M sucrose on 5 ml 1.2 M sucrose. The gradients were centrifuged at 150000 × g for 60 min in a Beckman SW 40 rotor and the material at each 0.8 M: 1.2 M interface removed in 3 ml using a syringe and needle with curved tip. Each suspension was diluted to 10 ml with 0.32 M sucrose and centrifuged at 20000 × g for 30 min. The resulting six pellets were finally resuspended with a total of 2 ml 0.32 M sucrose and immediately used in the uptake studies.

Determination of the uptake of noradrenaline by synaptosomes

50 μl of ice-cold, synaptosomal suspension were added to a 10-ml conical flask containing 1 ml of incubation medium at 37° and preincubated for 1 min in a Dubnoff shaking incubator, following which 10 μl of radioactively labelled (−)-noradrenaline

were added and the contents incubated for either zero min or the times indicated in the text. The normal medium contained: NaCl, 120 mM; KCl, 3 mM; $MgCl_2$, 3 mM; $CaCl_2$, 2 mM; Tris–HCl (pH 7.4), 20 mM. When the concentration of NaCl was reduced it was replaced with equiosomolar sucrose. Noradrenaline uptake was terminated by filtering 0.5 ml of the suspension by suction on a 0.45-μm pore-size Millipore filter and then washing with 10 ml ice-cold, amine-free medium. This washing procedure took approx. 20 sec.

The Millipore filter was then removed and dissolved in 10 ml of scintillation fluid containing 7 ml toluene; 3 ml ethylene glycol monomethyl ether; 0.4 % 2,5-diphenyloxazole; 0.01 % 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]benzene and the radioactivity determined in a Picker Nuclear spectrometer. Quenching was corrected by the channels-ratio method and the moles of noradrenaline per mg protein at time zero calculated and subtracted from the values after incubation to give the actual moles of noradrenaline taken up per mg protein.

RESULTS

The time course of uptake of (−)-noradrenaline at 37°

In order to study the initial rate of a transport process, one must observe the uptake at a time when it is proceeding linearly; therefore, a time course for (−)-noradrenaline uptake was performed. Synaptosomes were incubated for up to 10 min with (−)-[7-^{3}H]noradrenaline in the normal incubation medium containing 120 mM NaCl and 3 mM KCl, conditions which have been reported to result in excellent uptake of the amine[3–5].

Fig. 1 shows that the uptake was linear for approx. 1 min but was beginning to decrease by 3 min and had significantly declined by 5 and 10 min. Therefore, the amount of noradrenaline which had accumulated over 1 min incubation was taken as a measure of the initial rate of influx for this transport process and was employed in all subsequent studies.

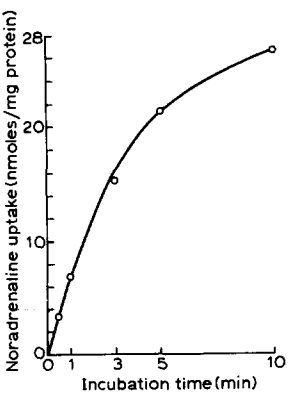

Fig. 1. Time course for noradrenaline uptake by synaptosomes. The incubation medium contained (mM): NaCl, 120; KCl, 3; $MgCl_2$, 3; $CaCl_2$, 2; Tris–HCl (pH 7.4), 20. The concentration of (−)-[7-^{3}H]noradrenaline in the medium was 0.172 μM. Each point represents the mean of 8 determinations.

The uptake of (−)-noradrenaline into synaptosomes prepared from whole brain and from brain stem

Since we wished to use entire brain as our source of synaptosomes, it was necessary to demonstrate that the uptake into synaptosomes prepared from whole brain did not differ quantitatively from the uptake into synaptosomes prepared from areas of the brain known to contain relatively higher concentrations of endogenous noradrenaline.

In separate experiments the uptake of (−)-[α-^{14}C]noradrenaline was determined, firstly, into synaptosomes prepared from whole brain and secondly, into a preparation from brain stem. In this study, brain stem refers to the area bounded dorsally by a horizontal line through the anterior commissure, anteriorally by the optic chiasma and laterally by the internal walls of lateral ventricles[11]. The medulla and pons were also included, but great care was taken to exclude all traces of striatal tissue from the dissection. Synaptosomes were prepared from eight brain stems in the manner described for whole brain in METHODS.

There was no significant difference in the uptake of (−)-noradrenaline by synaptosomes prepared from whole brain or from brain stem (Table I). This finding justifies the use of synaptosomes prepared from entire brain to study the noradrenaline uptake process. As yet, no one has successfully isolated purely noradrenergic synaptosomes and until such time as this is accomplished, heterogeneous preparations are the best available.

TABLE I

UPTAKE OF (−)-NORADRENALINE BY SYNAPTOSOMES PREPARED FROM WHOLE BRAIN AND FROM BRAIN STEM

Incubation medium as described in Fig. 1. The concentration of (−)-[α-^{14}C]noradrenaline in the medium was 0.625 μM. Each value is the mean($\pm$ S.E.) of 8 determinations.

Preparation	*Uptake of noradrenaline (nmoles/mg protein per min)*
Whole brain	15.8 $\pm$ 0.9
Brain stem	18.0 $\pm$ 1.1

The effects of desmethylimipramine and cocaine on (−)-noradrenaline uptake

Both the tricyclic antidepressant, desmethylimipramine, and the local anaesthetic, cocaine, have been shown to specifically inhibit the noradrenaline re-uptake process[6,12,13]. Synaptosomes were incubated in the presence of small concentrations of these compounds to determine whether or not the uptake observed at 1 min in the present study was susceptible to this inhibition.

As little as 50 nM desmethylimipramine caused a slight but significant decrease in uptake, whilst 50 μM desmethylimipramine reduced the uptake to 34 % of the control (Table II). Cocaine (2 μM) inhibited uptake to 55 % fo the control.

The effect of Na^+ on the initial rate for (−)-noradrenaline uptake

In order for a transport process to be considered carrier mediated, it must be saturable and obey Michaelis–Menten kinetics. The double reciprocal plot of the rate

TABLE II

EFFECT OF DESMETHYLIMIPRAMINE AND COCAINE ON NORADRENALINE UPTAKE BY SYNAPTOSOMES

Incubation medium as described in Fig. 1. The concentration of (−)-[7-^{3}H]noradrenaline in the medium was 1.24 μM. Each value is the mean (± S.E.) of 6 determinations. All values differed significantly ($p<0.05$) from the control (F-test).

Treatment	*Uptake of noradrenaline (nmoles/mg protein per min)*	*% of control*
Control	18.3 ± 1.4	100
50 nM desmethylimipramine	15.0 ± 1.3	82
50 μM desmethylimipramine	6.2 ± 1.5	34
2 μM cocaine	10.0 ± 1.4	55

of uptake of (−)-noradrenaline against (−)-noradrenaline concentrations was linear (Fig. 2) suggesting that a carrier-mediated process, which can be described by Michaelis kinetics, might be involved. Saturation of the transport system by substrate was apparent both in the presence and in the absence of external Na^+. However, the concentration of Na^+ immediately adjacent to the transport sites may not actually have equalled zero since the synaptosomes should have been actively extruding Na^+ during the incubation[14,15].

Increasing the Na^+ concentration of the medium from zero to 120 mM increased the V for transport by a factor of 2–3 (Fig. 2 and Table III), but had very little

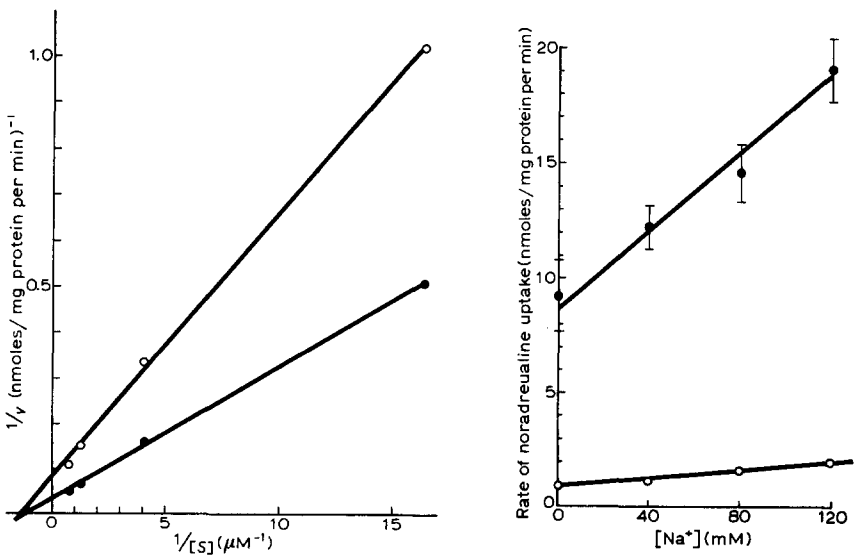

Fig. 2. Double reciprocal plot of the rate of noradrenaline uptake by synaptosomes (v) against the concentration of noradrenaline in the incubation medium ([S]) showing the effect of Na^+. ○, zero Na^+; ●, 120 mM Na^+. The lines were fitted by the method of least squares using an APL/360 computer system. Each point represents the mean of 15 experiments.

Fig. 3. Rate of noradrenaline uptake plotted against the concentration of Na^+ in the medium. When NaCl was reduced, it was replaced with sucrose. The concentrations of (−)-[7-^{3}H]noradrenaline in the media were: ○, 61 nM; ●, 1.24 μM. Each point represents the mean of 15 experiments.

TABLE III

EFFECT OF SODIUM ON THE K_m AND V FOR NORADRENALINE UPTAKE BY SYNAPTOSOMES

Double reciprocal plots of the rate of noradrenaline uptake against the noradrenaline concentration in the medium were performed and the lines fitted by the method of least squares using the the APL/360 computer system. The computer programmes were constructed to provide direct mathematical estimates of K_m and V. Each value is derived from 15 experiments.

Na^+ concn. (mM)	*K_m (μM)*	*V (nmoles/mg protein per min)*
0	0.746	13.0
40	0.764	14.7
80	0.779	22.1
120	0.890	30.8

effect on the K_m which remained virtually constant at about 0.8 μM. The small change in K_m was in the direction of an increase which would indicate a decreased affinity of carrier for noradrenaline, and hence a decreased uptake, as the Na^+ concentration was increased. However this change in K_m probably lies within the experimental error of the procedure employed.

At concentrations of Na^+ up to 120 mM, there was no evidence that Na^+ saturated the noradrenaline transport system, in so far as the plot of uptake of (−)-noradrenaline against Na^+ concentration was linear (Fig. 3). It would be expected that saturation would occur at higher, more hypertonic Na^+ concentrations.

The effect of K^+ on the initial rate for noradrenaline uptake

Several studies have shown that low concentrations of K^+ are required for the optimal uptake of noradrenaline by synaptosomes[3,5] and for the transport of certain

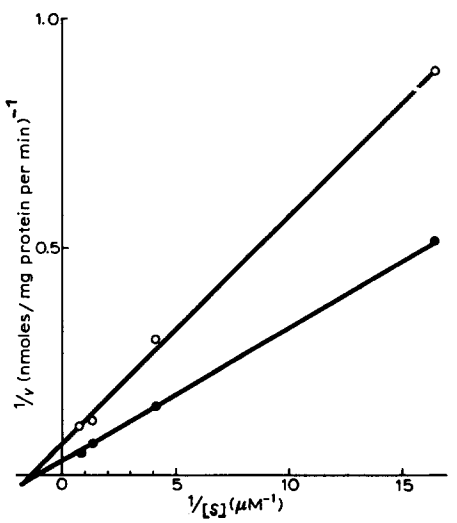

Fig. 4. Double reciprocal plot of the rate of noradrenaline uptake by synaptosomes (V) against the concentration of noradrenaline in the incubation medium ([S]) showing the effect of K^+. ○, zero K^+; ●, 3 mM K^+. The lines were fitted by the method of least squares using an APL/360 computer system. Each point represents the mean of 10 experiments.

amino acids[16–18] and sugars[19–22] into other tissues. Therefore, the effects of low concentrations of K^+ on the initial rates of (−)-noradrenaline uptake into synaptosomes were investigated.

Increasing the K^+ concentration of the medium from zero to 3 mM increased the V for transport by a factor of 2–3 but had essentially no effect on the K_m of the process (Fig. 4 and Table IV). Carrier-mediated transport was also observed in the absence of K^+ in the medium (Fig. 4). This could have been due to the presence of residual external K^+ which was not removed during the preparation of the synaptosomes or, alternatively, small amounts of K^+ might have leaked out of the synaptosomes and created areas of localized higher K^+ concentrations immediately adjacent to the transport sites.

TABLE IV

EFFECT OF POTASSIUM ON THE K_m AND V FOR NORADRENALINE UPTAKE BY SYNAPTOSOMES

Double reciprocal plots of the rate of noradrenaline uptake against the noradrenaline concentration in the medium were performed and the values for K_m and V determined as described in Table III. Each value is derived from 10 experiments.

K^+ concn. (mM)	*K_m (μM)*	*V (nmoles/mg protein per min)*
0	0.715	14.3
0.5	0.845	29.6
1.5	0.726	28.1
3.0	1.035	34.7

A slight increase in the K_m was observed in the presence of 3 mM K^+ when compared with the K_m when 1.5 mM K^+ was present (Table IV). This effect can be accounted for if K^+ at higher concentrations acts as a reversible competitive inhibitor for noradrenaline in the transport process. In any event, the principle role of K^+ at low concentrations was to increase the uptake of noradrenaline by increasing the V for transport. It should be noted that as little as 0.5 mM K^+ gave almost maximal uptake (Table IV).

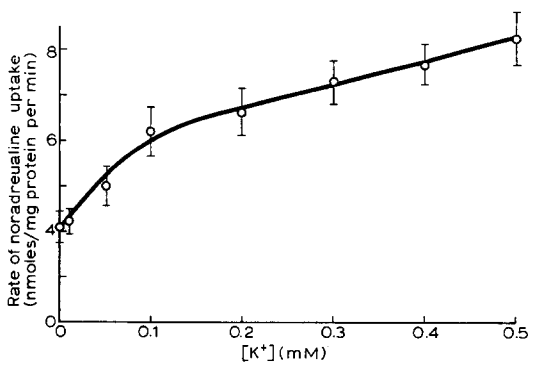

Fig. 5. Rate of noradrenaline uptake plotted against the concentration of K^+ in the medium. The concentration of (−)-[7-^{3}H]noradrenaline in the medium was 0.172 μM. Each point represents the mean of 6 determinations.

As the concentration of K^+ in the medium was increased above 0.1 mM, the rate of increase of noradrenaline uptake by synaptosomes began to diminish (Fig. 5), suggesting that K^+ might be saturating the transport system.

DISCUSSION

In this study we determined the effects of various conditions on the initial rates of noradrenaline uptake by synaptosomes. It was hoped that by observing uptake after short pre-incubations and incubations (1 min each), and by quickly halting the reaction by filtration through Millipore discs, any effects of the various external conditions on synaptosomal ion and energy contents would be minimized. Uptake was inhibited by both desmethylimipramine and cocaine suggesting that the transport observed at 1 min was occurring via the neuronal-membrane uptake process thought to be responsible for the termination of the synaptic actions of noradrenaline[6].

Recently COYLE AND SNYDER[23] have observed an uptake of (+,−)-[7-^{3}H] noradrenaline by the predominantly dopaminergic nerve endings of striatal areas and it could therefore be argued that in our experiments, the uptake of noradrenaline into synaptosomes from whole brain occurred primarily via dopaminergic synaptosomes. This possibility was excluded by our findings that synaptosomes from whole brain took up the same amount of (−)-noradrenaline as did synaptosomes from brain stem devoid of striatal tissue. Moreover SNYDER AND COYLE[24] found the K_m of noradrenaline uptake into non-striatal areas of the brain to be 0.4 μM whereas the K_m into striatal areas was 2.0 μM. Our value of 0.8 μM is clearly more in keeping with uptake into non-striatal synaptosomes.

Noradrenaline uptake was facilitated by both Na^+ and low concentrations of K^+ added to the medium, thus confirming the findings of others[4,5]. The uptake was saturable and apparently obeyed Michaelis–Menten kinetics, suggesting that a carrier-mediated transport process might be involved. To this extent, these results agree with the recent findings of BOGDANSKI *et al.*[25] using rabbit synaptosomes. However, in our study external Na^+ altered the maximal rate for transport (V) and had no effect on the Michaelis constant (K_m), whereas the above mentioned workers found that external Na^+ changed the K_m but not the V for uptake[25]. This discrepancy might be due to the fact that they observed the uptake of noradrenaline after a 10-min preincubation and 6.5-min incubation and it is possible that they were not observing the true initial rates for transport. Alternatively, the difference might reflect species variation.

Synaptosomes accumulated noradrenaline when no Na^+ was present in the incubation medium and this uptake process was substrate saturable with the same K_m for uptake as in the presence of 120 mM Na^+. This is strong evidence in favour of the two processes sharing a common carrier. However, it is also possible that the external Na^+ concentration in the vicinity of the transport mechanism was not really zero, since the synaptosomes would likely have been extruding Na^+ under these conditions[14,15] and this could result in a localized accumulation of Na^+ immediately adjacent to the outside of the synaptosomal membrane. It thus remains equivocal whether carrier-mediated transport of noradrenaline can proceed in the absolute absence of external Na^+. It should be mentioned that other workers have observed very little uptake of

nordrenaline when synaptosomes were incubated for longer periods of time in the absence of Na^+ [3,4].

The uptake of noradrenaline was linear with Na^+ concentrations up to 120 mM Na^+. Nevertheless, it seems likely from other evidence[25] that higher concentrations of Na^+ can saturate the noradrenaline transport system. In fact, it is difficult to envisage a Na^+-facilitated uptake process which cannot be saturated by Na^+.

Low concentrations of K^+ (up to 3 mM) stimulated the uptake of noradrenaline by synaptosomes by increasing the V for transport but had no significant effect on the K_m. In the absence of K^+ some influx still occurred, apparently by a carrier-mediated process having a very similar K_m to uptake in the presence of K^+. Some authors have proposed that Na^+-dependent uptake processes are inhibited in the absence of external K^+ because of the requirement of K^+ for Na^+-pump activity, and hence for the maintenance of an inward-directed Na^+ concentration gradient to drive uptake[4,7,26]. It seems most unlikely, however, that in the present study removal of K^+ for 2 min could have abolished the Na^+ gradient and inhibited uptake in this way. Any increase in the intracellular Na^+ concentration caused by the absence of external K^+ should stimulate the efflux of noradrenaline and have no effect on the influx of substrate. Since, in the experiments described here, uptake was observed before significant efflux of noradrenaline could occur, any alternations in internal ions should not have influenced uptake. In any event, previous studies have shown that the accumulation of noradrenaline by rat brain synaptosomes during 30 min incubations is unaffected by the internal concentrations of Na^+ and K^+ [5]. Thus it seems more likely that K^+ in low concentrations has a direct, external effect on noradrenaline-transport across the synaptosomal membrane.

In considering possible models for the transport system for noradrenaline by sypnaptosomal membrane, the following observations have to be taken into account: (a) external Na^+ alters the V for transport while the K_m is not altered; (b) there appears to be a mediated influx in the absence of external Na^+; (c) inward-directed, Na^+ concentration gradients cannot stimulate or restore uptake into metabolically poisoned preparations. These characteristics of the process are derived from the present study and that of WHITE AND KEEN[5].

One way in which Na^+ could facilitate noradrenaline transport is by increasing the movement of a carrier–noradrenaline complex across the synaptosomal membrane. This concept is illustrated in Fig. 6 and is derived from the general model described by WEBB[27] for an enzyme reaction involving an activator. The carrier (X) can combine

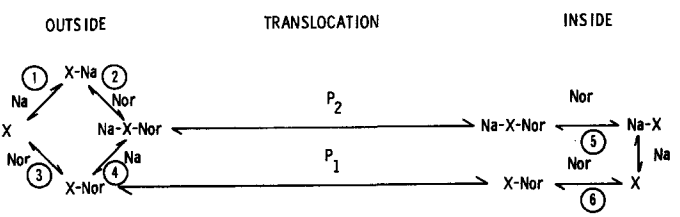

Fig. 6. A model for the transport of noradrenaline across the synaptosomal membrane, in which Na^+ increases the rate of translocation of the carrier–noradrenaline complex. P_1 and P_2 are coefficients for translocation and $P_2 > P_1$. $K_1 = K_4$ and $K_2 = K_3$. Further explanation is found in the text.

with either (or both) Na^+ and amine. However, since Na^+ increases the V for transport, the coefficient of translocation for the Na^+–X–noradrenaline complex (P_2) is greater than that for the X–noradrenaline complex (P_1). Since Na^+ does not alter the K_m, $K_1 = K_4$ and $K_2 = K_3$. This model is similar to that proposed for the transport of sugars across intestinal mucosa[28] but differs in that noradrenaline can apparently be translocated in the absence of Na^+ and hence $P_1 > 0$.

This model predicts that an inward-directed, Na^+ concentration gradient should supply the energy necessary for the active accumulation of noradrenaline by synaptosomes. Unfortunately this has not been observed[5] and it is necessary to postulate that another energy source besides ion-asymmetries is involved in the transport process. For example, it is possible that ATP is required for the phosphorylation of the carrier either before or after Na^+ binding.

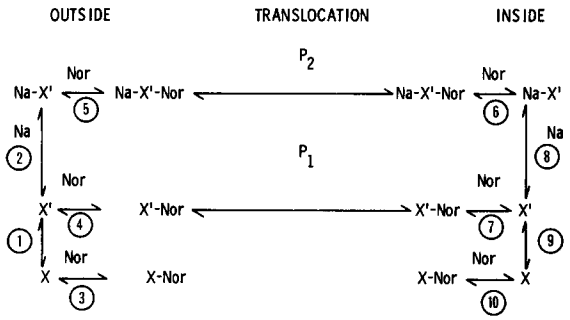

Fig. 7. A model for the transport of noradrenaline across the synaptosomal membrane, in which Na^+ increases the total number of active sites available for transport. $P_1 = P_2$ = coefficients for translocation. $K_3 = K_4 = K_5$. X is an inactive form of the carrier which cannot translocate. X′ is the active form of the carrier. Further explanation is found in the text.

As an alternative to the hypothesis that Na^+ increases the rate of translocation of the carrier–noradrenaline complex across the synaptosomal membrane, it is possible that Na^+ could act by increasing the total number of active transport sites. The scheme shown in Fig. 7 is derived from the "positive V system" described by MONOD *et al.*[29] to explain the allosteric activation of enzymes. In this model, an inactive form of the carrier (X) is in equilibrium with its active form (X′). Noradrenaline has the same affinity for the two states of the carrier but only X′ can transport it across the synaptosomal membrane. Na^+ has a greater affinity for X′ than for X and hence it combines with X′ to form Na–X′, the latter having the same affinity for noradrenaline and the same overall rate of transport as X′. However, by forming the Na–X′ complex, Na^+ shifts the equilibrium between X and X′ towards X′, the end result being an increase in the total number of active sites (X′ and Na–X′) available for transport. It should be noted that if both Na^+ and substrate were to have differential affinities for X and X′, then the presence of Na^+ would modify the apparent affinity of the carrier for substrate and *vice versa*. This "K system"[29] could explain uptake processes in which Na^+ alters the K_m for transport.

As in the previous instance, this model predicts that the internal Na^+ concentration (and hence Na^+ gradients) should influence noradrenaline transport, unless one assumes that the inactive form of the carrier (X) cannot exist at the inner surface

of the membrane. If this is the case, internal Na^+ will not shift an equilibrium between X and X' and the rates of efflux of noradrenaline will be unaffected. Moreover, energy utilization, perhaps as an hydrolysis of ATP at the translocation step, must be introduced into this system for active uptake to occur.

The model outlined above is consistent with the observed characteristics of noradrenaline uptake by synaptosomes. It might also explain the observations by a number of workers that various other transport systems which are not influenced by internal Na^+ and K^+ and cannot be driven by ion gradients nevertheless require external Na^+ for optimal acitivity[30–35].

At present, insufficient evidence is available to choose between these and other models. For example, further studies are required to determine whether Na^+ and noradrenaline fluxes are coupled and, if so, what the coefficient of coupling is. Furthermore, since K^+ has profound effects on the influx of noradrenaline, its role will also have to be incorporated into any model to account for noradrenaline transport by synaptosomes.

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REFERENCES

1 E. G. GRAY AND V. P. WHITTAKER, *J. Anat.*, 96 (1962) 79.
2 E. DE ROBERTIS, A. P. DE IRALDI, G. RODRIGUEZ AND J. GOMEZ, *J. Biophys. Biochem. Cytol.*, 9 (1961) 229.
3 R. W. COLBURN, F. K. GOODWIN, D. L. MURPHY, W. E. BUNNEY AND J. M. DAVIS, *Biochem. Pharmacol.*, 17 (1968) 957.
4 D. F. BOGDANSKI. A. TISSARI AND B. B. BRODIE, *Life Sci.*, 7 (1968) 419.
5 T. D. WHITE AND P. KEEN, *Biochim. Biophys. Acta.*, 196 (1970) 285.
6 J. AXELROD, *Recent Prog. Horm. Res.*, 21 (1965) 597.
7 R. CRANE, *Fed. Proc.*, 24 (1965) 1000.
8 T. D. WHITE AND D. M. PATON, *Proc. Can. Fed. Biol. Soc.*, 14 (1971) 76.
9 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
10 P. KEEN AND T. D. WHITE, *J. Neurochem.*, 17 (1970) 565.
11 J. GLOWINSKI AND L. L. IVERSEN, *J. Neurochem.*, 13 (1966) 655.
12 S. B. ROSS AND A. L. RENYI, *Eur. J. Pharmacol.*, 2 (1967) 181.
13 F. H. LEITZ AND F. J. E. STEFANO, *Eur. J. Pharmacol.*, 11 (1970) 278.
14 C.-M. LING AND A. A. ABDEL-LATIF, *J. Neurochem.*, 15 (1968) 721.
15 A. H. TISSARI, P. S. SCHONHOFFER, D. F. BOGDANSKI AND B. B. BRODIE, *Mol. Pharmacol.*, 5 (1969) 593.
16 M. FOX, S. THIER, L. ROSENBERG AND S. SEGAL, *Biochim. Biophys. Acta.*, 79 (1964) 167.
17 T. R. RIGGS, L. M. WALKER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 233 (1958) 1497.
18 P. N. ABADOM AND P. G. SCHOLEFIELD, *Can. J. Biochem. Physiol.*, 40 (1962) 1603.
19 E. RIKLIS AND J. H. QUASTEL, *Can. J. Biochem. Physiol.*, 36 (1958) 347.
20 A. KLEINZELLER AND A. KOTYK, *Biochim. Biophys. Acta*, 54 (1961) 367.
21 I. BIHLER AND R. K. CRANE, *Biochim. Biophys. Acta*, 59 (1962) 78.
22 D. M. KIPNIS AND J. E. PARRISH, *Fed. Proc.*, 24 (1965) 1051.
23 J. T. COYLE AND S. H. SNYDER, *J. Pharmacol. Exp. Ther.*, 170 (1969) 221.
24 S. H. SNYDER AND J. T. COYLE, *J. Pharmacol. Exp. Ther.*, 165 (1969) 78.
25 D. F. BOGDANSKI, A. H. TISSARI AND B. B. BRODIE, *Biochim. Biophys. Acta*, 219 (1970) 189.

26 S. G. SCHULTZ AND D. F. CURRAN, *Physiol. Rev.*, 50 (1970) 637.
27 J. L. WEBB, *Enzyme and Metabolic Inhibitors*, Vol. 1, Academic Press, New York, 1963, p. 46.
28 A. M. GOLDNER, S. G. SCHULTZ AND P. F. CURRAN, *J. Gen. Physiol.*, 53 (1969) 362.
29 J. MONOD, J. WYMAN AND J.-P. CHANGEUX, *J. Mol. Biol.*, 12 (1965) 88.
30 H. KROMPHARDT, H. GROBECKER, K. RING AND E. HEINZ, *Biochim. Biophys. Acta*, 74 (1963) 549.
31 R. K. MARGOLIS AND A. LAJTHA, *Biochim. Biophys. Acta*, 163 (1968) 374.
32 J. A. JACQUEZ AND J. A. SCHAFER, *Biochim. Biophys. Acta*, 193 (1969) 368.
33 S. POTASHNER AND R. M. JOHNSTONE, *Biochim Biophys. Acta*, 203 (1970) 445.
34 A. KLEINZELLER, *Biochim. Biophys. Acta*, 211 (1970) 277.
35 G. A. KIMMICH, *Biochemistry*, 9 (1970) 3669.