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NORADRENALINE UPTAKE BY SYNAPTOSOMES AND (Na^+-K^+) ATPase

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Abstract—The uptake of [^{3}H]NA by synaptosomes prepared from rat cerebral cortex and brain stem was studied. The results indicate that there are two distinct systems for the uptake of noradrenaline. One system which predominates in cortical tissue has a sodium-dependent maximum rate of transport. This uptake system has a number of characteristics which are similar to the synaptic membrane (Na^+-K^+) ATPase. The activity of this enzyme was studied and the influence of a number of amines determined. Serotonin, tyrosine, *l*-Dopa, dopamine, noradrenaline and adrenaline were all stimulants of the (Na^+-K^+) ATPase. Fenfluramine, phentolamine, chlorpromazine and desipramine antagonized the amine stimulation of (Na^+-K^+) ATPase. Desipramine, which was a more potent inhibitor of noradrenaline uptake than was chlorpromazine, was less effective than chlorpromazine as an antagonist of the amine stimulation of (Na^+-K^+) ATPase. Although there was some similarity between the noradrenaline uptake system and the noradrenaline-stimulated (Na^+-K^+) ATPase, these results did not support the contention that synaptosomal uptake of noradrenaline was a primary active transport process.

Catecholamines released from nerve endings for the purpose of synaptic transmission are believed to have their physiological actions terminated by a presynaptic membrane recapture mechanism. This reuptake process has been studied in synaptosomes and is a saturable process obeying Michaelis–Menten kinetics [1]. There is evidence that the uptake of noradrenaline is sodium-dependent [2, 3], potassium-dependent [4], and ouabain-sensitive [5], and is inhibited by metabolic inhibitors [6], thus suggesting than an energy-dependent, active transport process is involved. The conditions for noradrenaline (NA) uptake are very similar to those for (Na^+-K^+)-stimulated Mg-dependent activity and it has been postulated that the energy for the transport of noradrenaline into synaptosomes is derived from the inward-directed Na^+ concentration gradient across the synaptosomal membrane, which is maintained by (Na^+-K^+) ATPase [5].

The sodium gradient hypothesis explains the time lag between the blockade of noradrenaline uptake by ouabain and the inhibition by the drug of the (Na^+-K^+) ATPase [5]. However, noradrenaline has been reported to antagonize the ouabain inhibition of synaptic membrane (Na^+-K^+) ATPase [7]. The sodium gradient hypothesis has also been challenged by the findings of White and Keen [8], who demonstrated that increasing the external (Na^+) from 155 to 286 mM in the presence of metabolic inhibitors did not result in an increase in NA accumulation.

It is possible that the (Na^+-K^+) ATPase is directly involved in the uptake of noradrenaline since the biogenic amines have been reported to stimulate (Na^+-K^+) ATPase in various brain preparations [7, 9–15]. Schaefer *et al.* [9] reported that the soluble fraction of rat brain homogenates contained a heat stable dialysable fraction which inhibited the ATPase activity of the particular fraction. This inhibition could be overcome by catecholamines. Chlorpromazine was observed to inhibit the ATPases [16]. However, it is the metabolites and not the parent phenothiazine which possess the property of inhibiting the (Na^+-K^+) ATPase.

This paper investigates the role of (Na^+-K^+) ATPase in the uptake of noradrenaline into cerebral synaptosomes. More specifically, it reports on the influence of sodium ions on the kinetics of noradrenaline transport and the nature of the noradrenaline-stimulated (Na^+-K^+) ATPase of cerebral synaptic membranes.

MATERIALS AND METHODS

Preparation of synaptosomes. The synaptosomes were prepared from cerebral cortices of rats of the Wistar strain by the method previously described [17]. The resultant crude P_2 fraction was layered on a discontinuous sucrose density gradient and centrifuged at 35,000 *g* for 3 hr at 4° in an 8 × 50 ml angle head centrifuge. The synaptosomes were collected at the 0.8–1.2 M sucrose interphase and were used within 24 hr of the animal's death.

Determination of the uptake of noradrenaline by synaptosomes. The synaptosomes were incubated for 1 min at 37°. Previously it was determined that the uptake of noradrenaline was linear for the first 90 sec but then rapidly fell off to a plateau after 4 min. The details of the incubation media are given in the text and legends to the figures and tables. The incubations were commenced by adding 1 μl of [^{3}H]NA to the incubation medium. The incubation was stopped 60 sec later by passing through a millipore filter of pore size 0.45 μm. The filters were washed twice with 2 vol. of ice-cold incubation media. The filters were placed in 10 ml of scintillation medium consisting of

4 g of PPO; 0.4 g of PoPoP dissolved in 1 l. of Toluene. The radioactivity was determined using a Tricarb Liquid Scintillation spectrophotometer, and the quenching was corrected for by the automatic external standards ratio method.

Accumulation of NA is the amount of unchanged exogenous amine inside the synaptosomes after a period of incubation. After a 10 min incubation period 80 per cent of the radioactivity recovered from the synaptosomes could be recovered as noradrenaline. Nialamide, a monoamine oxidase inhibitor, increased the percentage recovery to 90 per cent and in some cases 95 per cent. The initial rates of [^{3}H]NA accumulation by synaptosomes after incubation for 1 min with various concentrations of amine in imidazole–HCl buffer, pH 7.4, containing 150 mM Na^+, 10 mM K^+, 5 mM Mg^{2+} and 10 g of glucose/l. of buffer, were determined. The data suggest that accumulation was mediated by more than one process. The initial rates for the first process approached saturation at an amine concentration of approximately 1.5 μM. The second process showed a linear relationship with the amine concentration which suggested that this second process represented simple diffusion of the amine into the synaptosomes. By extrapolating the linear segment of the total uptake back to the y axis one can describe this diffusion process superimposed upon accumulation due to the active transport mechanism. Thus the y axis intercept represent zero accumulation by diffusion. The difference between the extrapolated linear segment and the y intercept describes the accumulation due to diffusion at any given amine concentration as the difference between the total accumulation and the diffusion component.

The measurement of [^{3}H]*NA recoverable from synaptosomes.* Synaptosomes were incubated in 1 ml of incubation medium, pH 7.4, at 37°. The incubation was started by adding 10 μl of [^{3}H]noradrenaline to a final concentration of 5 μM. Incubation was stopped 10 min later by passing through a millipore filter of fine size 0.45 μm. The filters were washed twice with 2 ml of ice-cold medium and extracted with 5 ml of acetone–HCl (1:1). One drop of 9.5% EDTA was added and the pH adjusted to 8.5 with 1 N NaOH. Ten microlitres of this extract was added to 10 ml of scintillation cocktail as before. Four millitres of the extract were passed through an alumina oxide column, and the noradrenaline eluted with 15 ml of 0.4 M acetic acid. The columns were obtained from the Boehringer Corp. The [^{3}H] content of 100 μl of the eluate was determined as before. The efficiency of the columns were determined (45–58%).

Determination of synaptosomal integrity. The degree of intactness of the synaptosomes was assayed by the occluded LDH method [18]. Cerebral synaptosomes precincubated at 0° or 37° had occluded LDH values of 12.3 ± 2.7 and 3.8 ± 1.6 units/mg of protein, respectively. Studies on the effect of temperature on NA accumulation in the range 0–40° revealed an optimal temperature of 27°. This may be related to the structural integrity of the synaptosomes.

Preparation of synaptic membranes. Synaptic membranes were prepared by osmotically rupturing the synaptosomes using distilled water and centrifuging the resultant suspension at 50,000 g for 1 hr. Synaptic membranes which are rich in (Na^+-K^+) ATPase are pelleted by this procedure.

Assay of ATPase. ATPase were assayed by determination of the rate of release of inorganic phosphate (Pi). Membranes were incubated at 37°, unless otherwise stated, in a 50 mM imidazole–HCl buffer, pH 7.4 (unless otherwise stated), for 20 min. Total ATPases activity was assayed in a medium containing Na, K, Mg and ATP, at final concentrations given in text. The Mg ATPase was assayed in a medium containing Mg and ATP. All data presented here was obtained using Boehringer disodium ATP. The membranes were added to a final concentration of 10 g/ml and the incubation was commenced by the addition of ATP. Preliminary experiments showed that the optimum conditions for the assay of rat brain (Na^+-K^+) ATPase were 150 mM [Na^+], 10 mM [K^+], 5 mM [Mg], 5 mM [ATP] pH 7.4 and a temperature of 37°. The ATPase activity was linear for at least 30 min under these assay conditions, and the ATPase activity increased linearly with concentrations ranging from 5 to 20 μg of protein.

Determination of Pi. The incubations were stopped by adding 4 ml of colour solution prepared by dissolving 10 g of ammonium molybdate and 10 g of Lubrol WX in 1000 ml of 0.9 M H_2SO_4. The colour was left to develop for 30 min and was stable for 3 hr at room temperature. NA (10^{-4}) affects the estimation of Pi by this method. When possible this was overcome by using NA at concentrations not greater than 20 M. However, if [NA] greater than 20 μM were required, standard Pi graphs were prepared for each [NA] used. The psychotropic drugs also affected the estimation of Pi. Standard graphs were prepared for each drug.

Protein assay. Protein was assayed by the method of Lowry *et al.* [19].

RESULTS

The influence of Na^+ *on noradrenaline uptake system and ATPase of rat cerebral synaptic membranes.* The uptake of monoamines into synaptosomes is a saturable sodium-dependent process obeying Michaelis–Menten kinetics. The influence of sodium ions on the kinetic constants for noradrenaline uptake has been studied in whole rat brain and rabbit brain stem [3, 8, 20]. The initial rate of uptake is linear over the first 90 sec of incubation (Fig. 1). The transport process found in brain stem has a sodium-dependent affinity constant, K_m (0.25 μM in medium containing 150 mM Na^+), and a sodium-independent maximum rate of transport (6 nmoles/mg/min). However, the amine transport system which predominates in cerebral cortex homogenates has a sodium-dependent V_{max} (25 nmoles/mg/min) and a sodium-independent K_m (0.9 μM NA) (Fig. 2). In cerebral synaptosomes at low amine concentrations there is a linear relationship between the 60 sec accumulation of [^{3}H]NA and the external sodium concentration (60–160 mM). However, at higher amine concentrations the response is less linear with respect to the external sodium concentration. These results are interesting because they validate the

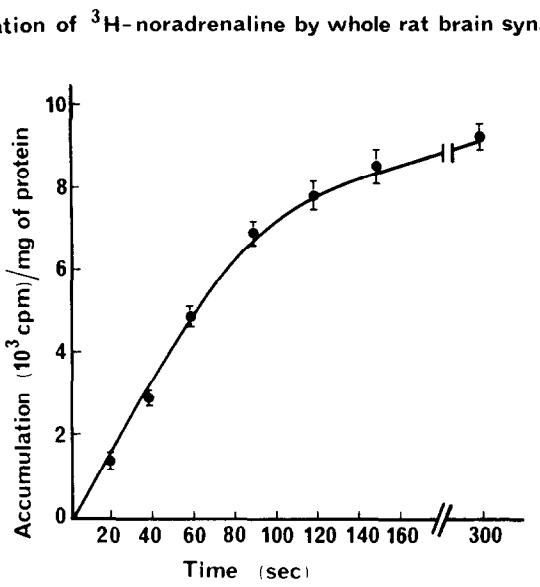

Fig. 1. Synaptosomes, 2 mg of protein, prepared from rat cerebral cortex, were incubated in 20 ml of imidazole buffer, pH 7.4, at 37° gassed with 95% O_2 and 5% CO_2 and containing 150 mM Na^+, 10 mM K^+, 5 mM Mg^{2+}, 200 mg of glucose, 0.02 M NA. At various intervals, a 1 ml sample was removed and filtered through a millipore filter of pore size 0.45 μm. The filters were washed twice with ice-cold medium and the ^{3}H content determined as described. The accumulation was expressed at 10^3 cpm per mg of synaptosomal protein. Each point is the mean of three experiments, the bars represent the S.D.

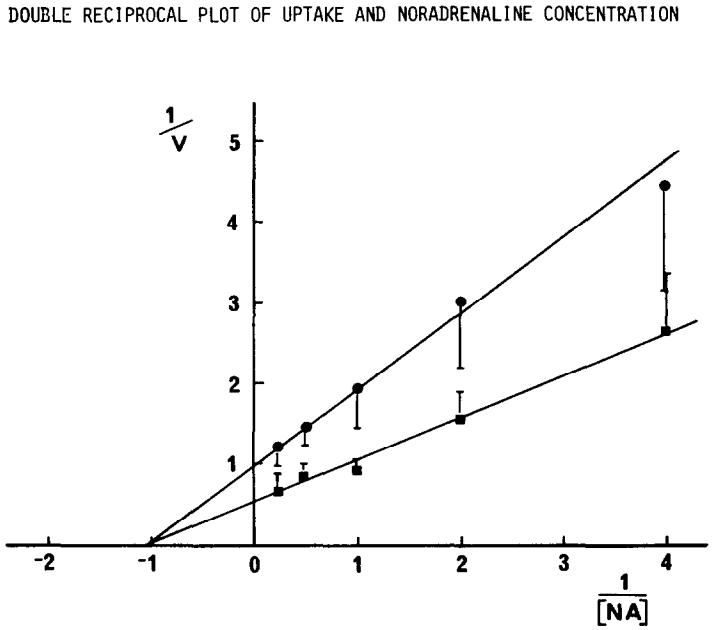

Fig. 2. Synaptosomes, 100 g of protein, prepared from rat cerebral cortex, were incubated for 60 sec in 1 ml of imidazole–HCl buffer, pH 7.4, at 37° and containing 10 mM K^+, 5 mM Mg^{2+}, 10 mg glucose and either 150 mM Na^+ or 112 mM Na^+. Incubation was commenced by the addition of ^{3}H NA to the tubes and the medium was gassed $t = 0$ with 95% O_2 and 5% CO_2. All points are the mean of five experiments and the bars represent the S.D.

assumption that the Briggs–Haldane–Michaelis theory adequately describes the noradrenaline transport mechanism found in synaptosomes,

i.e.

$$v = \frac{V_{max}[S]}{K_m + [S]}. \quad (1)$$

At low $[S]$ equation 1 tends to

$$v = \frac{V_{max}[S]}{K_m}.$$

Differentiating

$$\frac{dv}{dx} = \frac{dv}{d[Na]} \cdot \left[\frac{dx}{d[Na]}\right]^{-1},$$

i.e.

$$\frac{dv}{d[Na]} = \frac{dv}{dx} \cdot \frac{dx}{d[Na]}.$$

Substituting K_m for x,

$$\frac{dv}{d[Na]} = \frac{-V_{max}S}{K_m^2} \times \frac{d\,K_m}{d[Na]}, \quad (2)$$

i.e. a non linear response as observed in brain stem. Substituting V_{max} for x,

$$\frac{dv}{d[Na]} = \frac{S}{K_m} \cdot \frac{d\,V_{max}}{d[Na]}. \quad (3)$$

Since in cerebral cortex dV_{max}/dNa is a constant (Fig. 3), and K_m is sodium-independent (Fig. 2) and

$$\frac{dv}{d[Na]}$$

is constant at low amine concentrations, then it is clear that the assumption underlying the data plot in Fig. 2 is valid.

Cyanide inhibited the accumulation of [^{3}H]NA by the synaptosomes. Increasing the external sodium ion concentration to 295 mM did not result in an increase in the rate of [^{3}H]NA accumulation. However, synaptosomes are relatively leaky to sodium ions, thus there is a large increase in the sodium content of synaptosomes incubated in solutions containing high concentrations of sodium ions (Fig. 4). The rate at which synaptosomes accumulate sodium is dependent on the permeability of the synaptosomal membrane to sodium and also on the rate of sodium extrusion. The efflux of sodium from synaptosomes has been studied and there are apparently two components of efflux [21]. There is a fast component of efflux with a time constant of 2.387 hr^{-1}, which lasts for only three or four minutes, and a slower efflux. Neither efflux is stimulated by noradrenaline. The slower component of efflux could imply contamination of the synaptosomal fraction with gliasomes,

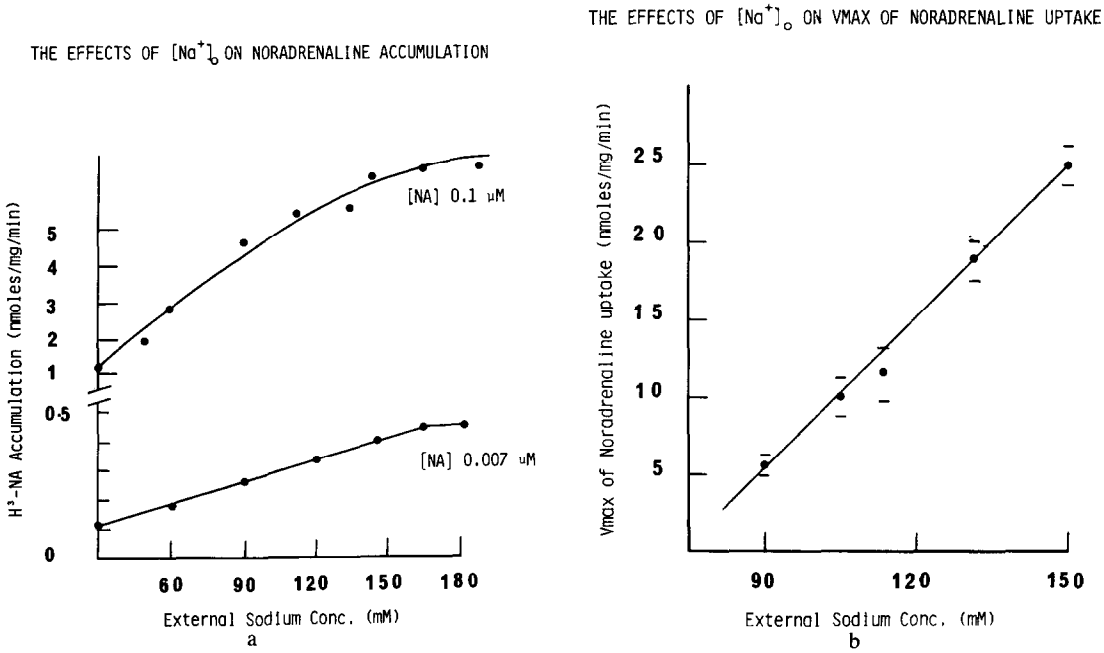

Fig. 3. Panel a. The accumulation of [^{3}H]noradrenaline by rat cerebral cortical synaptosomes incubated in medium containing various concentrations of sodium ions. The isotonicity was maintained by replacing the sodium removed with sucrose. The accumulation in the absence of sodium is thought to be due to diffusion. The slope of the line is assumed to represent the rate of increase in synaptosomal uptake with respect to the external sodium concentration, dv/dNa, see equation 2. The incubation medium was essentially that used in studies reported in Fig. 1. Panel b. The effects of external sodium ion concentrations on V_{max}. The V_{max} was computed from the intercept of the y axis as in Fig. 2. The data from experiments with external sodium concentrations in the range 90–150 mM could be fitted to a straight line. Thus it is concluded that dV_{max}/dNa is a constant.

membrane vesicles from glial cells. In view of this, it would be wrong to interpret the data in Fig. 4 as evidence that there was an inward directed sodium gradient across the synaptosomal membrane, even in the initial period following the addition of high external sodium concentrations. It has been suggested that an ATPase may be directly involved in the uptake of noradrenaline by synaptosomes. ATPases are characterized by their requirement of a divalent ion for the enzyme–substrate interaction. The Mg-dependent ATPases of rat cerebral synaptic membrane were studied. In the absence of potassium ions, sodium stimulated Mg ATPase activity. The sodium stimulation of Mg ATPase had a maximum response at 60 mM Na^+ and the half maximum response around 40 mM (Fig. 4). (Na^+-K^+) ATPase of rat cerebral synaptic membranes has a K_{Na} value of 80 mM.

The effect of neurotransmitters on Mg *ATPase.* The effects of neurotransmitters and phenethylamine derivatives on Mg ATPase were studied. Acetylcholine, noradrenaline, adrenaline, serotonin and amphetamine have no effect on Mg ATPase; however, fenfluramine and norfenfluramine, fluoride-substituted derivatives of amphetamine, inhibited Mg ATPase; Mg ATPase activity 26.3 ± 1.27 was reduced to 22.7 ± 0.8 and 20.3 ± 1.51 moles Pi/mg/hr (S.E.M. N = 6) with 10^{-4} M fenfluramine and norfenfluramine, respectively.

Phentolamine, an α-adrenergic receptor blocking agent, and propanalol, a β-blocker, did not affect Mg ATPase. Noradrenaline, serotonin and dopamine (10^{-5} M) stimulated Na-Mg ATPase. Noradrenaline did not influence the optimal conditions for the Na-Mg ATPase which were 100 mM Na, 1 mM Mg and 1 mM ATP. Thus since the optimum [Na^+] for Na-Mg ATPase is not similar to the optimum [Na^+] for noradrenaline uptake, then it should be concluded that the amine stimulation of Na-Mg ATPase is not an enzymic representation of the monoamine transport mechanism.

The influence of amine on (Na^+-K^+) *ATPase.* It is well known that amino acids and amines may act as chelating agents for divalent ions and Godfraind *et al.* [11] suggested that catecholamine-induced

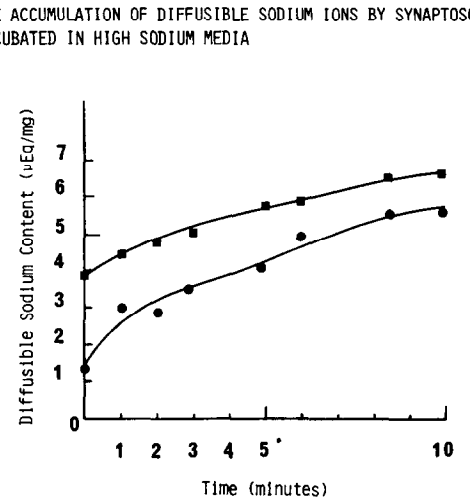

Fig. 4. Synaptosomes prepared from rat cerebral cortex and incubated at 37° for 8 min in 50 mM histidine buffer, pH 7.4, containing 150 mM Na, 10 mM K, 2 mM Mg, and 1 g/l. of glucose and in the presence or absence of 2 mM NaCN. The synaptosomal protein concentration was 7.5 mg/ml at 8 min, $t = 0$, 1 ml of the suspension was removed and 1 ml of ^{3}M NaCl was added to the incubation medium. The media were gassed throughout with 95% O_2 and 5% CO_2. Samples were removed at various time intervals and filtered as before. Filters were washed with 2 vol. 0.25 M sucrose and then placed in 10 ml of 15 mM SiCl overnight to elute the diffusible sodium ions. The sodium content of the elute was determined by atomic emission spectroscopy.

stimulation of (Na^+-K^+) ATPase was due to the calcium chelating effects of the amines. However, in this study aliphatic amino acids, e.g. lysine, serine, alanine, leucine, glutamic acid, glycine and aspargine, had no significant effect on Mg ATPase, Na-Mg ATPase or (Na^+-K^+) ATPase at concentrations in the range 10^{-8} to 10^{-3} M. Similarly, the indoleamines tryptamine and tryptophan had no effects on the ATPases, although 5-OH-tryptamine (serotonin) had a pronounced stimulating effect on (Na^+-K^+) ATPase at 5×10^{-7} M ($P < 0.01$). Serotonin

Table 1. Effects of phenethylamine derivatives on (Na-K) ATPase*

	Enzyme activity as percentage of control	Drug concn. producing maximum stimulation (M)
Control	100 ± 3.9	
Dopamine	123 ± 2.2	5×10^{-7}
Noradrenaline	136 ± 5.1	1×10^{-5}
Adrenaline	125 ± 2.7	1×10^{-4}
Isoprenaline	94 ± 0.8	—
pHO phenethylamine	105 ± 2.6	—
Amphetamine	123 ± 1.5	5×10^{-6}
Norfenfluramine	117 ± 3.7	5×10^{-7}
Fenfluramine	117 ± 1.6	1×10^{-7}
Phenylephrine	95 ± 3.7	—

* Specific activity of (Na^+-K^+) ATPase was 15.7 ± 0.37 μmoles Pi.mg^{-1}hr^{-1} (S.E.M., N = 15). The percentage enzyme activity was calculated for each experiment. The data presented are the means ± S.E. of at least five experiments at the drug concentration giving maximum reponse.

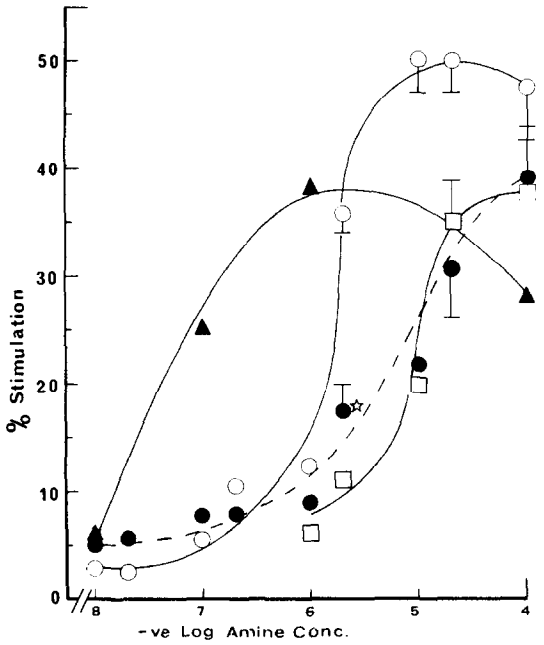

Fig. 5. The stimulation of (Na^+-K^+) ATPase by phenethylamine derivatives. (—▲—) fenfluramine, —○— noradrenaline, —□— adrenaline, —●— noradrenaline + 10^{-8} fenfluramine. The data represents the mean percentage increase over control (Na-K) ATPase, 15.7 ± 0.37 moles Pi/mg/hr (S.E.M., N = 15). Each point is the mean of two experiments in triplicate, the bars represent S.E. ★fenfluramine (10^{-8}M) inhibits the noradrenaline stimulation of (Na^+-K^+) ATPase ($P < 0.01$).

(10^{-5} M) produced a 100 per cent increase in enzyme activity. Phenylalanine (10^{-5} M) had no effect on the ATPases. However, 10^{-5} M tyrosine (hydroxyphenylalanine) and *l*-dihydroxyphenylalanine were capable of stimulating (Na^+-K^+) ATPase. The catecholamines dopamine, noradrenaline and adrenaline stimulated (Na^+-K^+ ATPase (Table 1). Noradrenaline was a more potent stimulant than adrenaline (Fig. 5). Isoprenaline and phenylephrine had no significant effect on (Na^+-K^+) ATPase. The order of potency of adrenergic agonists was noradrenaline = adrenaline > isoprenaline = phenylephrine which is not consistent with the classic adrenoreceptor classification; however, this may indicate that a catecholic moiety may be an important factor for the stimulation of (Na^+-K^+) ATPase since phenylephrine exhibits only a very weak effect if at all. OH-phenethylamine had no effect on the ATPases, although amphetamine, *p*-chloramphetamine, fenfluramine and norfenfluramine stimulated (Na^+-K^+) ATPase. The amphetamine analogues were stimulants of (Na^+-K^+) ATPase at 10^{-7} M. However, their maximum effects were less than the maximum effect of noradrenaline ($P < 0.001$). At 10^{-8} M fenfluramine antagonized the noradrenaline stimulation of (Na^+-K^+) ATPase.

The in vitro *effects of psychotropic drugs on* (Na^+-K^+) *ATPase.* Harmaline (10^{-5} M), a monoamine oxidase inhibitor (MAOI) inhibits (Na^+-K^+) ATPase; however, the other MAOI, harmine, which is structurally similar to harmaline and nialamide, did not affect (Na^+-K^+) ATPase. Noradrenaline (10^{-5}–10^{-4} M) did not affect the degree of inhibition of (Na^+-K^+) ATPase by harmaline. Chlorpromazine (10^{-5} M), which has both adreno- and dopaminergic receptor antagonist properties, did not affect (Na^+-K^+) ATPase. However, chlorpromazine was a potent inhibitor of noradrenaline stimulation of (Na^+-K^+) ATPase. The tricyclic antidepressants desipramine and imipramine, which are potent inhibitors of adrenaline uptake, were less potent than chlorpromazine as inhibitors of noradrenaline-stimulated (Na^+-K^+) ATPase. The IC_{50} values/drug concentration required to inhibit the noradrenaline effect by 50 per cent were chlorpromazine, $5.7 \pm 0.7 \times 10^{-7}$ M; desipramine, $1.1 \pm 0.2 \times 10^{-5}$; imipramine, $2.7 \pm 0.3 \times 10^{-5}$ M (S.E.M.).

The effects of chlorpromazine and desipramine on noradrenaline uptake. Synaptosomes (crude P_2 fraction) were incubated at 0° and at 27°; the optimal temperature for noradrenaline accumulation. This is a lower optimum temperature than that described by Sachs. However, in these studies, the synaptosomes were considerably more leaky at 37° than at 27°–estimated by changes in occluded LDH values. Noradrenaline accumulation was markedly reduced at 0°, which confirms the findings of Dengler *et al.* [22]. Noradrenaline uptake was estimated as the difference in 60 sec accumulation of [^{3}H]NA by synaptosomes incubated at 0° and 27°. Chlorpromazine (10^{-6} M) and desipramine (10^{-6} M) had no effect on accumulation of 0°. However, both drugs significantly inhibited the uptake of noradrenaline. The concentration of desipramine required to inhibit noradrenaline uptake by 50 per cent (IC_{50}) was $8.9 \pm 1.1 \times 10^{-7}$ M. The IC_{50} for chlorpromazine $2.8 \pm \pm 0.7 \times 10^{-6}$ M. Similar IC_{50} values for these drugs were obtained at 37°, $7.1 \pm 1.5 \times 10^{-7}$ and $4.7 \pm \pm 1.2 \times 10^{-6}$ M desipramine and chlorpromazine, respectively.

DISCUSSION

A transport system which carries solute against a prevailing concentration gradient requires the expenditure of free energy. Depending on whether the coupling to free energy sources is direct or indirect, the resulting uphill transport is a primary active or secondary active process. Intrasynaptosomal transport of monoamines is complicated by the fact that a second transport system is operating within the synaptosomes transferring the amine into intrasynaptic storage vesicles and which therefore may alter the concentration of free substrate within the axoplasm. When accumulation of the amine in the tissue is taken as a measure of uptake, enzymic degradation of the translocated substrate by MAO and COMT may produce a further difficulty. Accumulation as a parameter of neuronal or extraneuronal net uptake favours contributions by intracellular binding. However, it is important to realize that initial rates of accumulation do reflect initial rates of uptake although initial rates of accumulation are difficult to obtain; the observation that accumulation is linear for the first 90 sec of incubation justifies our use of the 60 sec accumulation as a measure of uptake.

Numerous studies have shown that the neuronal accumulation of noradrenaline and related amines is sodium-dependent [4, 23, 24]. The requirement for sodium is absolute as it cannot be replaced by Li^+, K^+, Rb^+, Cs^+ or choline [4, 25]. It has been shown that accumulation of noradrenaline by synaptosomes is not determined by the intracellular [Na^+] [8] but a reduction in the external [Na^+] impaired the intraneuronal storage of noradrenaline [4] and accelerated the efflux of noradrenaline from the cytoplasm of adrenergic neurones [26]. In this present study, a reduction in external Na^+ impaired the 60 sec accumulation of noradrenaline, a finding consistent with that of White and Paton [2], which suggests that noradrenaline uptake is also Na^+-dependent. The effects of varying the external [Na^+] revealed regional variations in the noradrenaline uptake systems; in cortical tissues, the maximum rate of transport was influenced by [Na^+], whereas in the brainstem the affinity of the carrier was increased by Na^+.

Although ouabain inhibits the accumulation of noradrenaline in a noncompetitive manner [5, 27] there is no conclusive evidence that ouabain inhibits the neuronal uptake of the amine. Ouabain increases the intracellular Na^+ in synaptosomes within 60 sec, yet the inhibitory effects of ouabain take some 4 min to appear [4, 5]. However, noradrenaline initially antagonizes ouabain inhibition of synaptic membrane (Na^+-K^+) ATPase. So it could be postulated that the energy for the uptake of noradrenaline was provided by the transmembrane sodium gradient. To test this hypothesis synaptosomes were poisoned with CN-, then incubated for 60 sec in medium containing 295 mM Na^+ and 1.0 μM [^{3}H]NA. It has been shown that the synaptosomal Na^+ content does not equilibrate under these conditions for at least 6 min (Fig. 4). In view of the failure to promote NA uptake under these conditions, it should be concluded that the sodium gradient is not the sole source of energy for the synaptosomal noradrenaline uptake. It is possible in this situation part of the required energy is provided by a direct link between the transport process and the vectorial chemical reaction. If this is so, then is noradrenaline stimulation of (Na^+-K^+) ATPase an enzymic representation of the noradrenaline uptake system?

The concentration of sodium giving half maximum stimulation of noradrenaline uptake was 80 mM. The K_{Na} of Na-Mg ATPase was 40 mM. The noradrenaline uptake was potassium-dependent. The (Na^+-K^+) ATPase (ouabain-sensitive) has a K_{Na} of 80 mM. There is a superficial similarity between sodium- and potassium-dependent ouabain-sensitive noradrenaline uptake and the ouabain-sensitive (Na^+-K^+) ATPase which is stimulated by noradrenaline.

In this study, none of the amino acids tested had any significant effect on the activity of the ATPases. Dopamine and noradrenaline, adrenaline, amphetamine, norfenfluramine and fenfluramine were able to stimulate (Na^+-K^+) ATPase. The noradrenaline concentration giving half maximum stimulation of (Na^+-K^+) ATPase was 6 μM. The maximum effect was observed at 10^{-5} M noradrenaline. The K_m for NA uptake was 1 μM NA and the uptake system is saturated at NA concentrations greater than 4 μM. The noradrenaline uptake site displays rather low specificity since various adrenergic neurone blocking agents and hydroxylated tryptamines are also substrates for uptake. The uptake of noradrenaline is competitively inhibited by a variety of phenethylamine derivatives [1]. Figure 2 shows that fenfluramine is a partial agonist for (Na^+-K^+) ATPase; at 10^{-8} M, fenfluramine is an inhibitor of noradrenaline stimulation of (Na^+-K^+) ATPase. Chlorpromazine was a more selective inhibitor of the noradrenaline-stimulated (Na^+-K^+) ATPase than desipramine. ID_{50} values were $5.7 \pm 0.7 \times 10^{-7}$ M and $1.1 \pm 0.2 \times 10^{-5}$ M, respectively. The ID_{50} values for inhibition of noradrenaline uptake were $8.9 \pm 1.1 \times 10^{-7}$ M desipramine and $2.8 \pm 0.7 \times 10^{-6}$ M chlorpromazine. These results, although indicating some similarity between the noradrenaline uptake system and the noradrenaline stimulated (Na^+-K^+) ATPase, do not support the contention that synaptosomal noradrenaline uptake is a primary active transport process. However, synaptic membranes have a high specific activity of (Na^+-K^+) ATPase which, from a functional standpoint, is directly proportional to ion flux [28]. The observation that NA and 5HT stimulate (Na^+-K^+) ATPase of cerebral synaptic membranes [7] suggest that (Na^+-K^+) ATPase may have a regulatory role in synaptic events. The amine activated ATPase could be present simultaneously at several sites, both pre- and post-synaptic. Synaptosomes (pinched-off nerve endings) have an internal volume: protein ratio of 2.9 μl/mg [29] and it is estimated that the synaptosomal surface area ranges from 19.6 to 25.6 cm^2/mg of protein [30]. Thus nerve endings have a large surface area to volume ratio and it is conceivable that the amine stimulation of the membrane sodium pump would be necessary to maintain the electrical excitability of the nerve endings and facilitate transmitter release. Gilbert *et al.* [23] suggested that the inhibitory feedback mechanism creating transmitter release, which is ouabain-sensitive, involved a NA-stimulated sodium pump. Noradrenaline (10^{-5} M) has no effect on the active extrusion of ^{22}Na from synaptosomes [24].

The questions to be posed now are "What is the role of (Na^+-K^+) ATPase in the uptake of noradrenaline?" and "Is there a physiological role for the noradrenaline-stimulated (Na^+-K^+) ATPase?" In answer to the latter, let us concede that if there is a physiological role, then it is unlikely to be the presynaptic uptake of noradrenaline. The former question requires a more fundamental analysis of the evidence in support of the contention that noradrenaline uptake into nerve endings of the cerebral cortex is a (Na^+-K^+) ATPase-dependent process. If we concur that the enzyme is involved, then perhaps it generates an electrical potential gradient across the membrane.

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