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THE ROLE OF INTERNAL AND EXTERNAL Na^+ AND K^+ ON THE UPTAKE OF [^{3}H]NORADRENALINE BY SYNAPTOSOMES PREPARED FROM RAT BRAIN

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SUMMARY

1. Rat-brain synaptosomes prepared on a Ficoll density gradient took up [^{3}H]noradrenaline. This uptake was depressed by poisons (2,4-dinitrophenol and NaCN) and virtually abolished in the absence of K^+.

2. Raising the external Na^+ concentration increased the uptake of [^{3}H]-noradrenaline into non-poisoned synaptosomes but not into poisoned synaptosomes. Raising the external K^+ concentration decreased the uptake of [^{3}H]noradrenaline by non-poisoned synaptosomes.

3. Synaptosomes prepared in a high-Na^+ medium had a high internal Na^+/K^+ ratio whereas those prepared in a high-K^+ medium had a lower internal Na^+/K^+ ratio. These differences in internal ionic composition had no effect on the uptake of [^{3}H]noradrenaline by these synaptosomes.

4. Poisoning reduced the uptake of [^{3}H]noradrenaline by synaptosomes to the same extent whether they initially contained a high Na^+/K^+ ratio or a low Na^+/K^+ ratio.

5. Synaptosomes having a high internal Na^+/K^+ ratio throughout incubation took up [^{3}H]noradrenaline to a much greater extent than did poisoned synaptosomes having a lower internal Na^+/K^+ ratio.

6. These findings suggest that an inward-directed Na^+ concentration gradient and/or an outward-directed K^+ concentration gradient cannot be the only sources of energy for the uptake of [^{3}H]noradrenaline by synaptosomes.

INTRODUCTION

Pinched-off nerve endings (synaptosomes) prepared from brain[1,2] provide a means of investigating transport processes in nervous tissue free from glial and other cells. Brain slices[3] and synaptosomes[4,5] accumulate noradrenaline; this uptake into synaptosomes is Na^+- and K^+-dependent and ouabain-sensitive[4,5]. Similar properties have been found for the transport of 5-hydroxytryptamine[4], choline[6] and γ-amino-butyric acid[7] into synaptosomes, and for the uptake of amino acids by brain slices[8]. The conditions for noradrenaline uptake are very similar to those for $(Na^+ + K^+)$-dependent ATPase activity and BOGDANSKI *et al.*[4] have postulated that the energy

for transport of noradrenaline into synaptosomes is derived from the inward-directed Na^+ concentration gradient across the synaptosomal membrane which is maintained by ($Na^+ + K^+$)-dependent ATPase. This mechanism is similar to that proposed by CRANE[9] for the Na^+-dependent transport of sugars and amino acids by the intestine. We have attempted to test the hypothesis of BOGDANSKI *et al.*[4] by studying the effect of changes of internal and external Na^+ and K^+ concentrations on accumulation of [^{3}H]noradrenaline by synaptosomes. A preliminary report of a part of this work has been made[10].

MATERIALS AND METHODS

Preparation of synaptosomes

Preparation was at 4° throughout and centrifugation was performed on a MSE 40 ultracentrifuge using angle heads. Male Wistar rats, Porton strain, 200–250 g, were killed by cervical dislocation and the whole brains removed to 0.32 M sucrose. Each brain was homogenised in 12 ml of 0.32 M sucrose by 30 strokes of a loose-fitting teflon–perspex homogeniser. The homogenate from four rat brains was divided into eight aliquots and centrifuged at 1000 × *g* for 10 min and the resulting supernatants recentrifuged at 12000 × *g* for 20 min. Each pellet was resuspended with 2.5 ml of 0.32 M sucrose and carefully layered onto a discontinuous density gradient consisting of 6 ml 3% Ficoll on 6 ml 13% Ficoll in 0.32 M sucrose. The gradients were centrifuged at 50000 × *g* for 60 min and the material at the 3%/13% interface removed in a volume of approx. 6 ml using a syringe and a needle with a curved tip. This material was diluted with 10 ml of either high-Na^+ or high-K^+ washing solution and centrifuged at 13000 × *g* for 20 min. The high-Na^+ washing solution contained (mM): Na^+, 162; Mg^{2+}, 3; Ca^{2+}, 2.5; phosphate (pH 7.4), 10; Cl^-, 160; glucose, 10. In the high-K^+ washing solution 150 mM Na^+ was replaced by 150 mM K^+. Each pellet was washed with a further 10 ml of the appropriate solution, recentrifuged at 13000 × *g* for 10 min and finally resuspended with washing solution. All incubations were performed on the same day as the material was prepared.

Determination of DL-[7-^{3}H]*noradrenaline uptake by synaptosomes*

An aliquot of the synaptosomal suspension was added to 3 ml of the appropriate incubation medium in a 10-ml cellulose nitrate centrifuge tube; 0.01 ml of 150 μC/ml [^{3}H]noradrenaline was added and the tube incubated at 37° in a Dubnoff shaking incubator. The reaction was stopped by centrifugation for 10 min at 13000 × *g* at 4°, the pellet allowed to drain for 30 min and the inside of the tube carefully wiped dry. [^{3}H]Noradrenaline was extracted from the pellet with 0.5 ml of 0.4 M $HClO_4$ for 60–90 min, the tube centrifuged and 0.2 ml of the supernatant added to 10 ml of scintillation fluid containing: toluene, 7 ml; ethylene glycol monomethyl ether, 3 ml; 2,5-diphenyloxazole, 0.4%; 1,4-bis-(5-phenyloxazolyl-2)benzene, 0.01%. Radioactivity was determined in a Packard Tricarb spectrometer. The counts/min per mg protein were calculated and the values for zero time subtracted from the values for 15 and 30 min after the start of incubation.

Determination of internal Na^+ and K^+ of synaptosomes

Synaptosomes were incubated as above except that the [^{3}H]noradrenaline was replaced by an equivalent amount of non-radioactive DL-noradrenaline. The

reaction was stopped by the addition of 3 ml ice-cold 0.32 M sucrose and the mixture centrifuged for 10 min at 13000 × *g* at 4°. The pellets were drained briefly, gently resuspended with 5 ml of cold 0.32 M sucrose and recentrifuged at 13000 × *g* for 10 min. The resulting pellet was drained, the inside of the tube wiped dry, and the pellet resuspended with 5 ml of 0.01 M HNO_3. The tubes were capped, and left overnight at room temperature. Na^+ and K^+ concentrations were then determined on the HNO_3 supernatant with an EEL flame photometer by reading against standard solutions containing both Na^+ and K^+ in the molar ratio of 2:1 through the range 0.1 to 0.01 mM Na^+ (0.05 to 0.005 mM K^+). The total $Na^+ + K^+$ of each pellet was calculated.

Contamination of the pellet with ions from the incubation medium was determined by adding 0.01 ml of [^{35}S]sulphate (100 μC/ml) to each tube immediately before the addition of 3 ml cold 0.32 M sucrose and centrifuging as described above. The final washed and drained pellet was resuspended with 0.5 ml of 0.4 M $HClO_4$ for 60–90 min and radioactivity determined in 0.2 ml of the supernatant by liquid scintillation counting. Total counts/min originally added to the tube were determined by measuring the radioactivity of 0.01 ml of the stock [^{35}S]sulphate solution in the presence of 0.2 ml 0.4 M $HClO_4$. The $HClO_4$ was added to quench the standard to the same extent as the pellet extract. Per cent contamination of the pellet was calculated as [(counts/min in pellet)/(total counts/min added)] × 100. Graphs of per cent contamination of pellet against mg protein in pellet were made for the various preparations and incubations at time zero and at 30 min. For each pellet the per cent contamination could then be determined from the appropriate graph and the correction for contamination of the pellet by extracellular ions (C_{Na} or C_K) calculated as

$$\frac{\%\ \text{contamination} \times \text{total ion in incubation medium } (\mu\text{moles})}{100}$$

The μmoles internal Na^+ per mg protein = (μmoles Na^+ in pellet $-C_{Na}$)/mg protein and the μmoles internal K^+ per mg protein = (μmoles K^+ in pellet $-C_K$)/mg protein.

Descending chromatography of [^{3}H]noradrenaline

[^{3}H]Noradrenaline was spotted with 20 μg DL-noradrenaline onto Whatman P81 cellulose phosphate paper and chromatographed in a solvent system consisting of 2 parts 0.2 M ammonium acetate (pH 6) and 1 part isopropanol as described by ROBERTS[11]. The noradrenaline was detected in iodine vapour, the chromatogram cut into equal strips and radioactivity determined by liquid scintillation counting.

Protein was determined by the method of LOWRY *et al.*[12]; DL-[7-^{3}H]noradrenaline (10 C/mmole) was obtained from New England Nuclear and was periodically checked for purity by chromatography as described above. [^{35}S]Sulphate was obtained from Radiochemical Centre, Amersham.

RESULTS

1. Electron microscopy of synaptosomal preparation

The Na^+-washed synaptosomal pellets were fixed for 90 min with 10 mg/ml OsO_4 in 100 mM sodium phosphate buffer (pH 7.4), dehydrated and embedded in araldite. Sections were cut, stained with uranyl acetate and lead citrate[13] and viewed

with a Hitachi HS 7S electron microscope. The Na^+-washed pellet was predominately composed of synaptosomes (Fig. 1). Most of these contained one or more mitochondria and small vesicles similar to those seen in synaptic junctions in brain. Some free myelin was also present.

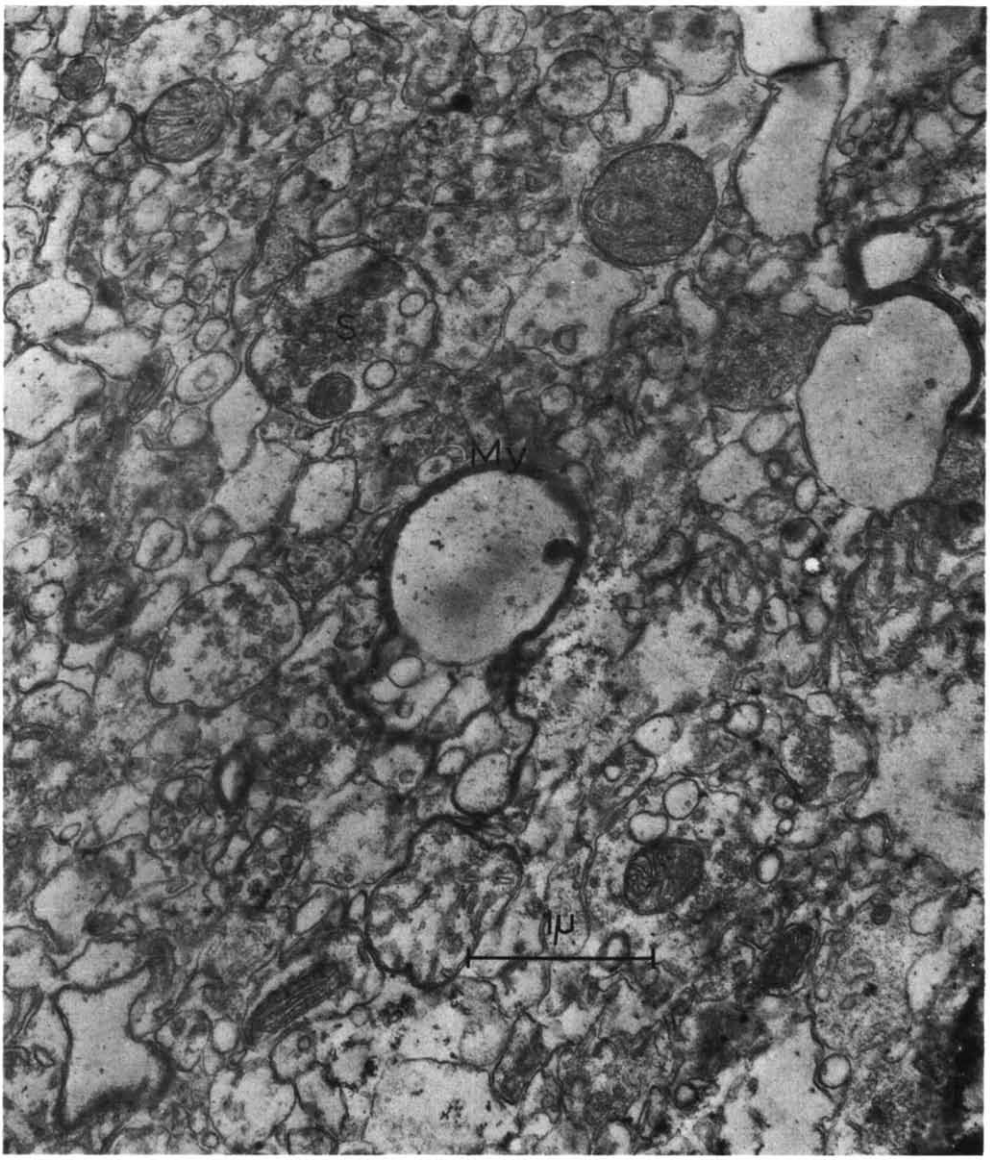

Fig. 1. Electron micrograph of a Na^+-washed synaptosomal fraction prepared on a Ficoll density gradient. Fixed with OsO_4 and stained with lead citrate/uranyl acetate. s, synaptosome; my, myelin.

2. The effect of 2,4-dinitrophenol, NaCN and K^+-free media on the uptake of [^3H]-noradrenaline by synaptosomes

A suspension of Na^+-prepared synaptosomes (0.6 ml) containing about 1 mg protein was added to 3 ml of medium so that the final incubation medium was as described in Fig. 2. Both 2,4-dinitrophenol and NaCN significantly decreased the uptake of [^{3}H]noradrenaline by synaptosomes and, in the absence of K^+, uptake was virtually abolished (Fig. 2).

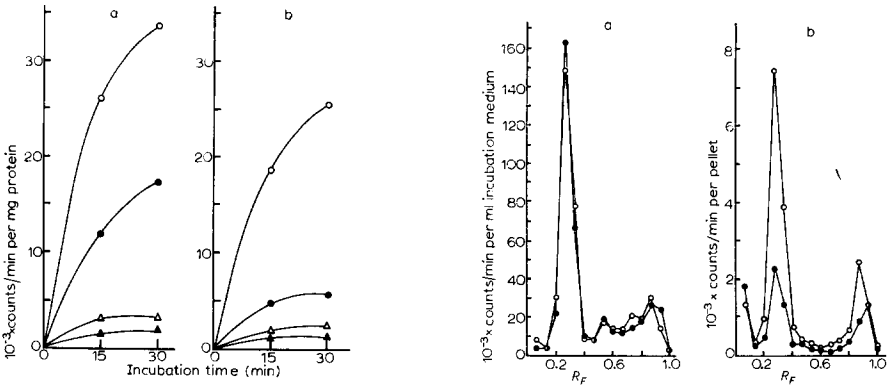

Fig. 2. The effect of poisons and K^+-free media on the uptake of [^{3}H]noradrenaline by synaptosomes. Incubation medium contained (mM): Na^+, 156; K^+, 6; Mg^{2+}, 3; Ca^{2+}, 2.5; PO_4^{3-} (pH 7.4), 10; Cl^-, 160; glucose, 10. When K^+ was omitted it was replaced by an equivalent amount of Na^+. (a) ○, normal medium; ●, 0.81 mM 2,4-dinitrophenol; △, K^+-free medium; ▲, K^+-free medium *plus* 0.81 mM 2,4-dinitrophenol. Each point represents the mean of four determinations. (b) ○, normal medium; ●, 2.1 mM NaCN; △, K^+-free medium; ▲, K^+-free medium *plus* 2.1 mM NaCN. Each point represents the mean of two determinations.

Fig. 3. Chromatography of radioactivity in incubation medium and synaptosomes after 30 min incubation at 37°, in the presence and absence of 2,4-dinitrophenol. Synaptosomes incubated with [^{3}H]noradrenaline as described in Section 2 of RESULTS. The R_F of the carrier noradrenaline was 0.25. ○, normal medium; ●, 0.81 mM 2,4-dinitrophenol. (a) Incubation medium. (b) Synaptosomal pellet.

3. Chromatography of [^3H]noradrenaline in synaptosomes after 30 min incubation

To determine whether total radioactivity was a valid measure of [^{3}H]noradrenaline uptake the incubation medium and a pellet extract were chromatographed. Synaptosomes were prepared and then incubated with and without 2,4-dinitrophenol as described in Section 2. After 30 min incubation the reaction was stopped by centrifugation and 0.01 ml of the supernatant was chromatographed as described in MATERIALS AND METHODS. The drained pellet was extracted in 0.2 ml $HClO_4$ for 60 min and 0.02 ml of the extract chromatographed. Most of the radioactivity both in the pellet and in the incubation medium had the same R_F as the carrier noradrenaline (Fig. 3). 2,4-Dinitrophenol decreased the [^{3}H]noradrenaline content of the pellet but did not alter the nature of the radioactivity in the incubation medium (Fig. 3). This justifies the use of total radioactivity to measure [^{3}H]noradrenaline uptake. The [^{3}H]noradrenaline in the incubation medium was stable over the 30-min incubation period.

4. The effect of increasing the external Na^+ or K^+ concentrations on the uptake of [^3H]noradrenaline by poisoned and non-poisoned synaptosomes

If energy is required simply to maintain an inward-directed Na^+ concentration gradient, an induced Na^+ concentration gradient should stimulate noradrenaline uptake by synaptosomes whether they are poisoned or not. To test this, synaptosomes were incubated as described in Section 2, but in some cases the Na^+ concentration of the medium was increased from 155 mM to 286 mM by the addition of 0.1 ml 5 M NaCl immediately before taking the 15-min sample. The addition of Na^+ to the incubation medium significantly increased [^{3}H]noradrenaline uptake by non-poisoned synaptosomes but did not increase uptake by poisoned synaptosomes (Fig. 4).

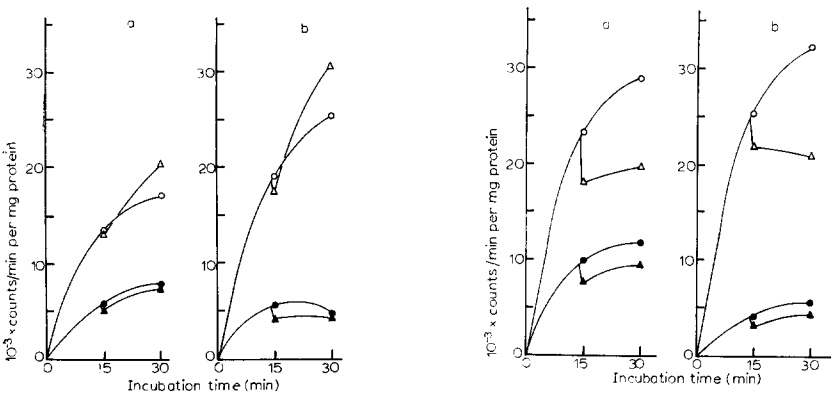

Fig. 4. The effect of increasing external Na^+ concentration on the uptake of [^{3}H]noradrenaline by poisoned and non-poisoned synaptosomes during incubation at 37°. Synaptosomes incubated as described in Section 2 of RESULTS. (a) ○, normal medium; ●, 0.81 mM 2,4-dinitrophenol; △, Na^+ increased to 286 mM; ▲, 0.81 mM 2,4-dinitrophenol and Na^+ increased to 286 mM. Each point represents the mean of four determinations. (b) ○, normal medium; ●, 2.1 mM NaCN; △, Na^+ increased to 286 mM; ▲, 2.1 mM NaCN and Na^+ increased to 286 mM. Each point represents the mean of two determinations.

Fig. 5. The effect of increasing external K^+ concentration on the uptake of [^{3}H]noradrenaline by poisoned and non-poisoned synaptosomes during incubation at 37°. Synaptosomes incubated as described in Section 2 of RESULTS. (a) ○, normal medium; ●, 0.81 mM 2,4-dinitrophenol; △, K^+ increased to 73.5 mM; ▲, 0.81 mM 2,4-dinitrophenol and K^+ increased to 73.5 mM. Each point represents the mean of four determinations. (b) ○, normal medium; ●, 2.1 mM NaCN; △, K^+ increased to 73.5 mM; ▲, 2 1 mM NaCN and K^+ increased to 73.5 mM. Each point represents the mean of two determinations.

When the concentration of K^+ in the medium was increased from 6.3 mM to 73.5 mM by the addition of 0.1 ml 2.5 M KCl immediately before taking the 15-min sample, the uptake of [^{3}H]noradrenaline by both poisoned and non-poisoned synaptosomes was significantly decreased (Fig. 5).

Addition of NaCl or KCl increased the tonicity of the medium and so part of the effect of these additions on noradrenaline uptake could be due to hypertonicity. Unfortunately choline chloride[6], LiCl (ref. 14) and hypertonic sucrose[15] all interact with synaptosomal transport processes and therefore cannot be used to test for solely osmotic effects. However, it is unlikely that the effects of NaCl and KCl on uptake are due to hypertonicity because additions of NaCl and KCl had opposite effects on

uptake into non-poisoned synaptosomes. Furthermore hypertonic NaCl had no significant effect on uptake if the synaptosomes were poisoned whereas if there were an osmotic effect one would expect to see it in both cases.

5. The effects of internal Na^+ and K^+ on the uptake of [^3H]noradrenaline by poisoned and non-poisoned synaptosomes

An attempt was made to alter the internal Na^+/K^+ ratios of synaptosomes by washing them in a high-Na^+ or a high-K^+ medium at 4°. Synaptosomes were prepared as before except that half the material was washed with a high-Na^+ solution and half with a high-K^+ solution (see MATERIALS AND METHODS). Each pellet was suspended in the appropriate washing solution and 0.2 ml of this suspension (containing about 1 mg of protein) added to 3 ml of a medium which, in the case of the Na^+-prepared synaptosomes contained K^+ and in the case of the K^+-prepared synaptosomes lacked K^+, so that the resulting media had identical ionic compositions during the final incubation (see Fig. 6).

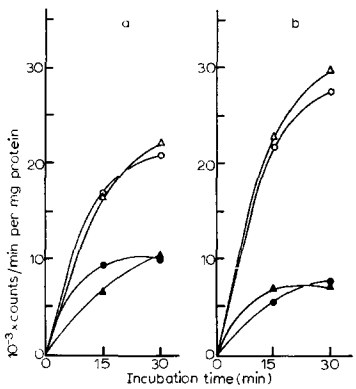

Fig. 6. The effect of internal Na^+ and K^+ on the uptake of [^3H]noradrenaline by poisoned and non-poisoned synaptosomes during incubation at 37°. Synaptosomes incubated as described in Section 5 of RESULTS. Final incubation medium contained (mM): Na^+, 153; K^+, 9; Mg^{2+}, 3; Ca^{2+}, 2.5; PO_4^{3-} (pH 7.4), 10; Cl^-, 160; glucose, 10. (a) ○, K^+-prepared; ●, K^+-prepared *plus* 0.94 mM 2,4-dinitrophenol; △, Na^+-prepared; ▲, Na^+-prepared *plus* 0.94 mM 2,4-dinitrophenol. Each point represents the mean of four determinations. (b) ○, K^+-prepared; ●, K^+-prepared *plus* 2.34 mM NaCN; △, Na^+-prepared; ▲, Na^+-prepared *plus* 2.34 mM NaCN. Each point represents the mean of four determinations.

Synaptosomes prepared in a high-Na^+ medium had a high internal Na^+/K^+ ratio whereas those prepared in a high-K^+ medium had a lower internal Na^+/K^+ ratio (Tables I and II). These differences in internal ionic composition had no significant effect on the uptake of [^3H]noradrenaline by synaptosomes (Fig. 6). Poisoning decreased uptake of [^3H]noradrenaline by Na^+-prepared and K^+-prepared synaptosomes to a similar degree. In the presence of 2,4-dinitrophenol the apparent tendency for K^+-prepared synaptosomes to take up more [^3H]noradrenaline than Na^+-prepared synaptosomes at 15 min (Fig. 6a) was not statistically significant.

The Na^+ and K^+ content of synaptosomes during the uptake of noradrenaline is shown in Tables I and II. Even after washing, the synaptosomal pellets contained some extracellular ions from the incubation media. Initially we used [*carboxy*-^{14}C]inulin to estimate the extent of this contamination, but the corrections so obtained

TABLE I

THE Na^+ AND K^+ CONTENT OF SYNAPTOSOMES DURING INCUBATION AT 37° WITH NORADRENALINE AND IN THE PRESENCE AND ABSENCE OF 2,4-DINITROPHENOL

Incubation as described in Section 5 of RESULTS. Each value represents the mean (± S.E.) of four determinations.

Preparation	*Treatment*	*Na^+ content (μmole/mg protein)*		*K^+ content (μmole/mg protein)*		*Total ions ($Na^+ + K^+$) (μmole/mg protein)*		*Na^+/K^+ ratio*	
		0 min	*30 min*	*0 min*	*30 min*	*0 min*	*30 min*	*0 min*	*30 min*
K^+-prepared	Non-poisoned	0.150 ± 0.008	0.186 ± 0.011	0.123 ± 0.004	0.081 ± 0.004	0.272 ± 0.013	0.267 ± 0.009	1.22 ± 0.03	2.31 ± 0.24
	Poisoned (0.94 mM dinitrophenol)	0.144 ± 0.005	0.201 ± 0.006	0.104 ± 0.004	0.076 ± 0.005	0.248 ± 0.006	0.276 ± 0.009	1.39 ± 0.05	2.68 ± 0.16
Na^+-prepared	Non-poisoned	0.196 ± 0.004	0.239 ± 0.008	0.071 ± 0.006	0.064 ± 0.003	0.267 ± 0.003	0.303 ± 0.007	2.84 ± 0.28	3.78 ± 0.24
	Poisoned (0.94 mM dinitrophenol)	0.196 ± 0.010	0.211 ± 0.005	0.067 ± 0.003	0.052 ± 0.001	0.263 ± 0.009	0.263 ± 0.004	2.95 ± 0.22	4.06 ± 0.17

TABLE II

THE Na^+ AND K^+ CONTENT OF SYNAPTOSOMES DURING INCUBATION AT 37° WITH NORADRENALINE AND IN THE PRESENCE AND ABSENCE OF NaCN

Incubation as described in Section 5 of RESULTS. Each value represents the mean (± S.E.) of four determinations.

Preparation	*Treatment*	*Na^+ content (μmole/mg protein)*		*K^+ content (μmole/mg protein)*		*Total ions ($Na^+ + K^+$) (μmole/mg protein)*		*Na^+/K^+ ratio*	
		0 min	*30 min*	*0 min*	*30 min*	*0 min*	*30 min*	*0 min*	*30 min*
K^+-prepared	Non-poisoned	0.161 ± 0.008	0.209 ± 0.016	0.137 ± 0.012	0.099 ± 0.007	0.297 ± 0.006	0.310 ± 0.022	1.21 ± 0.02	2.12 ± 0.01
	Poisoned (2.34 mM NaCN)	0.172 ± 0.021	0.235 ± 0.013	0.145 ± 0.010	0.085 ± 0.004	0.318 ± 0.025	0.321 ± 0.016	1.20 ± 0.02	2.76 ± 0.01
Na^+-prepared	Non-poisoned	0.224 ± 0.008	0.271 ± 0.012	0.070 ± 0.004	0.080 ± 0.009	0.294 ± 0.010	0.352 ± 0.021	3.24 ± 0.02	3.46 ± 0.26
	Poisoned (2.34 mM NaCN)	0.252 ± 0.006	0.300 ± 0.008	0.061 ± 0.010	0.056 ± 0.004	0.313 ± 0.014	0.356 ± 0.009	4.55 ± 0.93	5.38 ± 0.31

exceeded the total ion contents of the pellets. ESCUETA AND APPEL[16] experienced a similar difficulty when using inulin as a measure of the extracellular volume of a synaptosomal pellet. Therefore we used [^{35}S]sulphate (see MATERIALS AND METHODS) which EDDY[17] showed was a suitable marker for the extracellular space of pellets of Ehrlich ascites cells. ESCUETA AND APPEL[16] found the K^+ content of synaptosomes after 30 sec incubation at 23° in a medium similar to ours to be about 0.08 μmole per mg protein which compares favourably with our value of 0.07 μmole per mg protein for Na^+-prepared synaptosomes at time zero (Tables I and II). No figure for the Na^+ content of synaptosomes under these conditions is available in the literature. There was a general tendency for synaptosomes to gain Na^+ and to lose K^+ during incubation. ESCUETA AND APPEL[16] also observed a decrease in synaptosomal K^+ during incubation at 37°.

The total ($Na^+ + K^+$) content tended to increase in Na^+-prepared synaptosomes but not in K^+-prepared synaptosomes; hence the Na^+/K^+ ratio probably gives the best indication of changes in internal ionic composition. The internal Na^+/K^+ ratio of synaptosomes taken directly from the Ficoll gradient was 0.52 (8 determinations), which compares favourably with the value of 0.61 found for synaptosomes from a sucrose density gradient[18]. Washing in a high-Na^+ medium increased the Na^+/K^+ ratio to 3 whilst washing in a high-K^+ medium (containing 12 mM Na^+) increased the Na^+/K^+ ratio to 1.2. In both preparations the Na^+/K^+ ratios increased during incubation, a tendency which was more marked in the presence of poisons. However, the Na^+/K^+ ratio of the Na^+-prepared non-poisoned synaptosomes was consistently higher than the Na^+/K^+ ratio of the K^+-prepared poisoned synaptosomes.

Despite their higher internal Na^+/K^+ ratio the Na^+-prepared non-poisoned synaptosomes took up much more [^{3}H]noradrenaline than the K^+-prepared poisoned ones (Fig. 6). Thus it appears that the depression of uptake caused by poisons cannot be attributed solely to changes in the internal Na^+ and K^+ content of synaptosomes.

DISCUSSION

Synaptosomes prepared on a discontinuous Ficoll density gradient took up [^{3}H]noradrenaline and this uptake was abolished in the absence of K^+ (Fig. 2). Most of the synaptosomes appeared to be derived from cholinergic neurones (Fig. 1) and it is probable that the [^{3}H]noradrenaline was taken up by these cholinergic synaptosomes as well as by the few adrenergic synaptosomes present since SNYDER AND COYLE[19] have shown that crude homogenates from non-adrenergic areas of the rat brain take up [^{3}H]noradrenaline. Both 2,4-dinitrophenol and NaCN depressed [^{3}H]noradrenaline uptake (Fig. 2), probably by reducing ATP levels because this is their only common effect. Radiochromatography (Fig. 3) showed that 2,4-dinitrophenol did not alter the metabolism of [^{3}H]noradrenaline.

The ouabain-sensitivity and the Na^+- and K^+-dependence[4,5] of noradrenaline uptake by synaptosomes together with the effects of 2,4-dinitrophenol and NaCN (Fig. 2) suggest a central role for ($Na^+ + K^+$)-dependent ATPase in this process. BOGDANSKI *et al.*[4] have suggested that this uptake is carrier mediated, the energy for transport being provided by the inward movement of Na^+ down a concentration gradient into the synaptosome and that ATP supplies energy for the maintenance of this Na^+ gradient. This mechanism was proposed to explain the transport of

sugars and amino acids by the intestine[9] and later to explain the uptake of glycine by pigeon erythrocytes[20] and Ehrlich ascites cells[17]. The following findings, however, suggest that this mechanism does not explain the uptake of noradrenaline by synaptosomes.

(a) Raising the external Na^+ concentration increased the uptake of [^{3}H]-noradrenaline into non-poisoned synaptosomes but not into poisoned synaptosomes (Fig. 4) whereas raising the external K^+ concentration greatly decreased the uptake of [^{3}H]noradrenaline into non-poisoned synaptosomes and slightly decreased uptake into poisoned synaptosomes (Fig. 5). COLBURN *et al.*[5] found that the uptake of [^{3}H]noradrenaline by non-poisoned synaptosomes was increased at high Na^+ concentrations and decreased at high K^+ concentrations. However, if a Na^+ concentration gradient were the sole source of energy for the uptake of noradrenaline one would expect an induced Na^+ concentration gradient to increase uptake into both poisoned and non-poisoned synaptosomes.

(b) The internal Na^+/K^+ ratio of synaptosomes was altered by preparation in a high-Na^+ or a high-K^+ medium (Tables I and II). During incubation for 30 min at 37°, synaptosomes prepared in a high-Na^+ medium took up the same amount of [^{3}H]noradrenaline as did those prepared in high-K^+ medium (Fig. 6), even though the internal Na^+/K^+ ratio of the Na^+-prepared synaptosomes was greater than that of the K^+-prepared synaptosomes throughout incubation (Tables I and II). Similarly, internal Na^+/K^+ ratios have no effect on the uptake of glycine by Ehrlich ascites cells[21].

(c) Poisoning reduced the uptake of [^{3}H]noradrenaline by synaptosomes to the same extent whether they initially contained a high Na^+/K^+ ratio or a low Na^+/K^+ ratio (Fig. 6, Tables I and II). If poisons depressed uptake by raising the internal Na^+/K^+ ratio one would expect synaptosomes prepared in a high-K^+ medium to be less susceptible to their effect.

(d) Na^+-prepared non-poisoned synaptosomes took up [^{3}H]noradrenaline to a much greater extent than K^+-prepared poisoned synaptosomes (Fig. 6) even though the latter had a lower internal Na^+/K^+ ratio (and hence a greater inward-directed Na^+ concentration gradient) throughout incubation (Tables I and II).

All these observations suggest that an inward-directed Na^+ concentration gradient and/or an outward-directed K^+ concentration gradient cannot be the only sources of energy for the uptake of noradrenaline into synaptosomes. Studies are in progress to elucidate further the role of energy in noradrenaline transport.

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