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Studies on the mechanism by which tryptophan efflux from isolated synaptosomes is stimulated by depolarization

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1 The efflux and influx of tryptophan across the synaptosomal plasma membrane has been studied under a variety of experimental conditions, in order to examine the mechanism by which depolarization enhances the efflux of tryptophan from superfused synaptosomes.

2 Efflux of [^{3}H]-tryptophan from preloaded superfused synaptosomes was found to be enhanced by K^+ depolarization in a Ca^{2+} and dose-dependent manner. In contrast, [^{3}H]-phenylalanine efflux was only poorly stimulated by depolarization and only by very high concentrations of K^+.

3 Tryptophan efflux was also enhanced by decreasing the extracellular Na^+ concentration, but this effect was not dependent on extracellular Ca^{2+}.

4 Influx of [^{3}H]-tryptophan into synaptosomes was stimulated by extracellular Na^+ removal, but the uptake of [^{3}H]-phenylalanine was unaffected by this procedure.

5 Both the induced influx and efflux of tryptophan observed under these experimental conditions was inhibited by immobilizing the plasma membrane carrier with parachlorophenylalanine. This implied that both the enhanced influx and efflux arose as a consequence of the activation of the membrane tryptophan carrier, the direction of the observed effect being dependent upon the manner in which the experiments were conducted.

6 The relationship between depolarization, the activation of the membrane tryptophan carrier and the significance of this to the *in vivo* situation is discussed.

Introduction

Previous studies in this laboratory have demonstrated that [^{3}H]-tryptophan, which had been accumulated by synaptosomes *in vitro*, may be released by K^+ depolarization in an essentially Ca^{2+}-dependent manner (Collard *et al.*, 1982). A number of interpretations of this observation are possible. Firstly, as implied in the previous paper, tryptophan may be released by a process similar to that which operates for conventional transmitters, and that the released tryptophan may have some neuromodulatory function at the synapse. Support for this was obtained by the finding that low, but not high concentrations of tryptophan could potentiate the K^+-evoked release of [^{3}H]-5-hydroxytryptamine (5-HT) from synaptosomes (Collard & Lewis, 1985). An alternative interpretation of the evoked release of tryptophan is that the events which occur during depolarization activate the plasma membrane tryptophan carrier. Because of the way in which the experiments were conducted, this activation is expressed as an increase in the efflux of the amino acid, although it could equally well have been expressed as an increase in influx under the appropriate experimental conditions. In this study we have attempted to determine which of the above mechanisms is most likely to account for the observed release of tryptophan. The synaptosomal transport of tryptophan has been compared with that of the established transmitter 5-HT and with the structurally related amino acid phenylalanine, under conditions which are known to cause the depolarization-dependent release of transmitters and in other non-depolarizing conditions, in which the plasma membrane tryptophan carrier is known to be activated. The involvement of the plasma membrane tryptophan carrier was examined further, by determining the effect of an inhibitor of the carrier on the efflux of tryptophan under both depolarizing and non-depolarizing conditions.

Methods

Efflux studies

Preparation of synaptosomes, incubation and perfusion Male Albino Wistar rats weighing between 200 and 250 g were used in all studies. Synaptosomes were prepared from whole forebrain by the method of Gray & Whittaker (1962), as described previously (Collard *et al.*, 1981). The final synaptosomal pellet (P_2-B) was resuspended in 20 ml of Krebs solution of the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 0.75, glucose 10, $NaHCO_3$ 26. Two 4.95 ml portions of the synaptosomal suspension were preincubated at 37°C for 10 min, after which time 0.05 ml of either [^{3}H]-5-HT (specific activity 13–16 Ci mmol^{-1}), [^{3}H]-tryptophan (specific activity 8 Ci mmol^{-1}), or [^{3}H]-phenylalanine (specific activity 28–29 Ci mmol^{-1}) was added, to give final concentrations of 0.1 μM 5-HT, and 4 μM tryptophan or phenylalanine. Incubation was then continued for a further 10 min at 37°C. Beds of synaptosomes were prepared from 4.5 ml portions of incubated synaptosomes, set up in perfusion chambers (Collard *et al.*, 1981), and perfused at 8.0 ml min^{-1} with oxygenated (95% O_2 + 5% CO_2) Krebs solution of the following composition (mM): NaCl 118, KCl 4.85, KH_2PO_4 1.15, $MgSO_4$ 1.15, $CaCl_2$ 2.5, glucose 11.1, $NaHCO_3$ 25. The application of the depolarizing pulses of K^+, collection of fractions and processing of the tissue at the end of perfusion were carried out as described previously (Collard *et al.*, 1981; 1982).

In a second series of experiments, the effect of an application of a 2 min pulse of Na^+-free Krebs solution on tryptophan efflux was examined. Na^+ was replaced isotonically with Tris and sucrose as described previously (Evans *et al.*, 1985). The experiments were conducted essentially as described above with the pulse of Na^+-free Krebs solution being given at fraction 25.

In studies which examined the effect of an inhibitor of the tryptophan carrier on tryptophan efflux, the drug was applied at the onset of perfusion. This allowed the synaptosomes to be loaded with [^{3}H]-tryptophan before perfusion.

The separation and measurement of released 5-HT, tryptophan and phenylalanine Because the preparations used in these studies are entirely drug-free, the [^{3}H]-5-HT, [^{3}H]-tryptophan and [^{3}H]-phenylalanine present in the collected fractions and in the tissue extracts prepared at the end of perfusion had to be separated from their metabolites. This was accomplished by ion-exchange chromatography. 5-HT was separated by a modification of the method of Smith *et al.* (1975) with Bio-Rex 70 (Na^+ form) as described in detail elsewhere (Collard *et al.*, 1981). The amino acids were separated with Dowex 1-X4 ($HCOO^-$ form) (Collard *et al.*, 1982). Portions (2.0 ml) of collected perfusate and tissue extract were layered onto 5 × 30 mm columns of Dowex 1-X4 200–400 mesh ($HCOO^-$ form) which had been equilibrated with 0.02 M sodium phosphate buffer pH 7.5. The column effluent was discarded. The columns were then washed with 1.5 ml of 0.02 M sodium phosphate buffer pH 7.5, followed by 3.0 ml of deionised water. All washings were discarded. The tryptophan or phenylalanine bound to the column was eluted with 4.0 ml of 1 M HCl. A 1.0 ml portion was taken for measurement of radioactivity by liquid scintillation counting. The mean recovery of tryptophan and phenylalanine from the columns was 71% and 57%, respectively.

From measurements of the amount of [^{3}H]-5-HT, [^{3}H]-tryptophan or [^{3}H]-phenylalanine present in the collected fractions and in the tissue extract, the efflux of each compound from the superfused synaptosomes was expressed as a percentage of tissue content released per fraction. The release in response to a pulse of K^+ or Na^+-free Krebs solution was calculated as the total amount of [^{3}H]-5-HT, [^{3}H]-tryptophan or [^{3}H]-phenylalanine released above the basal efflux during the application of the pulse.

The identity of the material eluted from the columns was confirmed by ascending paper chromatography by use of techniques similar to those described by Denizeau & Sourkes (1977) and Lane & Aprison (1978). Samples of the column eluate, adjusted to pH 7.5 were spotted onto strips of Whatman No. 1 chromatography paper together with a series of unlabelled standards. The chromatograms were developed in isopropanol:0.88 ammonia:deionised water (20:1:2 by volume) for 12 h, then air dried, and those containing column fractions cut into 10 mm strips and eluted with 1.0 ml of 0.1 M HCl. A 0.5 ml portion of eluate was removed for liquid scintillation counting. The positions of the standards on the chromatograms were visualised by staining with either Ehrlich's reagent (for indoles and hydroxyindoles) or ninhydrin (for amino acids).

Approximately 80% of the radioactivity present in the Bio-Rex 70 eluate migrated in a single band with a mean R_F value of 0.41 which was comparable to that of the standard 5-HT. No other bands were detectable. In columns in which Dowex was used to separate tryptophan, 91% of the radioactivity present in the eluate migrated in a single band with a mean R_F value of 0.12 which corresponded with that of standard tryptophan, and when used to separate phenylalanine, 71% of the eluate migrated as a single band with a mean R_F value of 0.24, which again was comparable to that of the standard. No other bands of radioactivity were detected. The other unlabelled standards used were 5-hydroxyindole acetic acid and tryptamine which gave R_F values of 0.1 and 0.63, respectively. These results

provided confidence in the separation techniques by confirming that the majority of the radioactive material eluted from the respective columns was indeed 5-HT, tryptophan and phenylalanine.

Influx studies

Synaptosomes were prepared as described in the previous section and suspended in either a Krebs solution containing (mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 0.75, glucose 10, $NaHCO_3$ 26, or in a Na^+-deficient Krebs solution in which NaCl and $NaHCO_3$ had been replaced isosmotically with sucrose and Tris/HCl, respectively (Wilkinson & Collard, 1984). The pH of both solutions was 7.4 at 37°C. Aliquots (1.0 ml) of synaptosome suspensions were pre-incubated for 5 min at 37°C or 4°C. [^{3}H]-tryptophan or [^{3}H]-phenylalanine (specific activity 0.08 Ci mmol^{-1}, and 0.146 Ci mmol^{-1}, respectively) was added to give a final concentration of 10 μM, and incubation continued for 1 min. Incubated synaptosomes were separated from the incubation medium by Millipore filtration. The synaptosomes were poured onto pre-wetted Millipore filters (0.65 μm pore size) under gentle suction and washed with either three aliquots (5 ml) of Krebs solution or three aliquots (5 ml) of deionised water at room temperature. The water wash lysed the synaptosomes and released transported amino acids. The amount of amino acid transported was computed by subtraction of the radioactivity remaining on the filters washed with water from that on the filters which were washed with Krebs solution. Filter blanks were prepared by pouring 1.0 ml of 10 μM [^{3}H]-tryptophan or [^{3}H]-phenylalanine in Krebs solution over pre-wetted Millipore filters followed by the washing procedures described above. The filters were air dried overnight and their radioactivity content determined by liquid scintillation counting. Homogeneous counting conditions were obtained by the addition of 1.0 ml of ethanol/methanol (4:1 v/v) to the filters followed by 10 ml of Unisolve E and shaking for 4 min.

The protein concentration of the synaptosomal suspensions was measured by the method of Lowry *et al.* (1951). On the basis of the specific activity of the radiolabel added, and the amount of protein present in each sample, the uptake of amino acid was expressed as pmol accumulated mg^{-1} protein min^{-1}.

Results

Efflux studies

The basal efflux and K^+-evoked release of [^{3}H]-5-HT, [^{3}H]-tryptophan and [^{3}H]-phenylalanine In this experiment, the efflux of [^{3}H]-5-HT, [^{3}H]-tryptophan and [^{3}H]-phenylalanine from beds of superfused synaptosomes which had been preloaded with either the labelled transmitter or an amino acid was studied. A number of differences between the characteristics of 5-HT, tryptophan and phenylalanine efflux were observed. The basal efflux of tryptophan and phenylalanine were significantly higher than that of 5-HT, indicating that a greater proportion of tissue phenylalanine and tryptophan entered the superfusion fluid than did 5-HT under resting (non-depolarized) conditions. The release of [^{3}H]-tryptophan, [^{3}H]-5-HT and [^{3}H]-phenylalanine in response to a range of depolarising pulses of K^+ (7.5–50 mM) is shown in Figure 1. It can be seen that the release of tryptophan in response to K^+ depolarization was much smaller than that of 5-HT. However, the induced release of both substances increased in a dose-related manner as the concentration of the K^+ pulse increased from 7.5 mM towards 25 mM, whereupon some evidence of saturation of the response was apparent. In contrast, no release of phenylalanine could be elicited with K^+ pulses of 10 mM and 15 mM, although a small non-Ca^{2+}-depen-

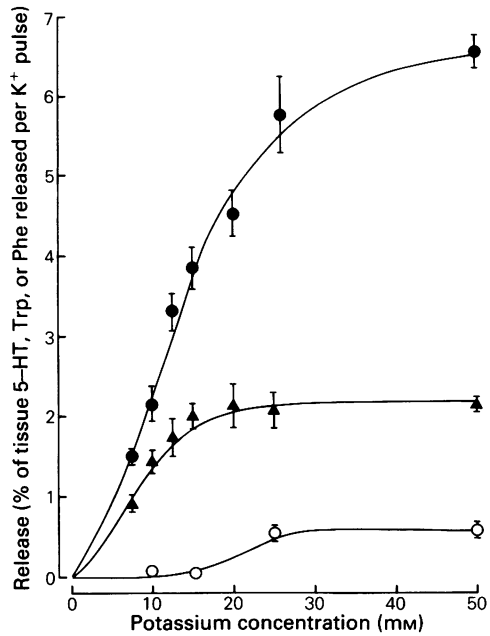

Figure 1 The release of [^{3}H]-5-hydroxytryptamine (5-HT), [^{3}H]-tryptophan and [^{3}H]-phenylalanine from superfused preloaded synaptosomes in response to depolarizing pulses of various concentrations of K^+. Release is expressed as the percentage of tissue [^{3}H]-5-HT (●), [^{3}H]-tryptophan (Trp; ▲) and [^{3}H]-phenylalanine (Phe; ○) released per K^+ pulse. Results are given as the mean ($n = 5–10$); vertical lines indicate s.e.mean. K^+ concentration is given in mM.

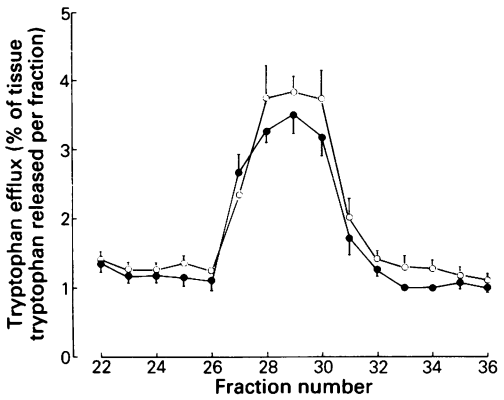

Figure 2 The release of [^{3}H]-tryptophan in response to the application of a 2 min pulse of Na^+-free Krebs solution in the presence (●) and absence (○) of extracellular Ca^{2+} (2.5 mM). Efflux is expressed as the % of tissue [^{3}H]-tryptophan released per fraction. Results are given as the mean ($n = 5$); vertical lines indicate s.e.mean.

dent response was obtained with pulses of 25 mM and 50 mM.

The release of [^{3}H]-tryptophan in response to the application of a 2 min pulse of Na^+-free Krebs solution

Decreasing extracellular Na^+ appears to activate the synaptosomal plasma membrane tryptophan carrier (Wilkinson & Collard, 1984). This study examined whether such a procedure could also stimulate tryptophan efflux under the superfusion conditions employed in these experiments.

The effect of the application of a 2 min pulse of Na^+-free Krebs solution on [^{3}H]-tryptophan efflux from preloaded synaptosomes, in the presence and absence of extracellular Ca^{2+}, is shown in Figure 2. It can be seen that the Na^+-free pulse caused a large increase in [^{3}H]-tryptophan efflux, and that this occurred whether extracellular Ca^{2+} was present or not.

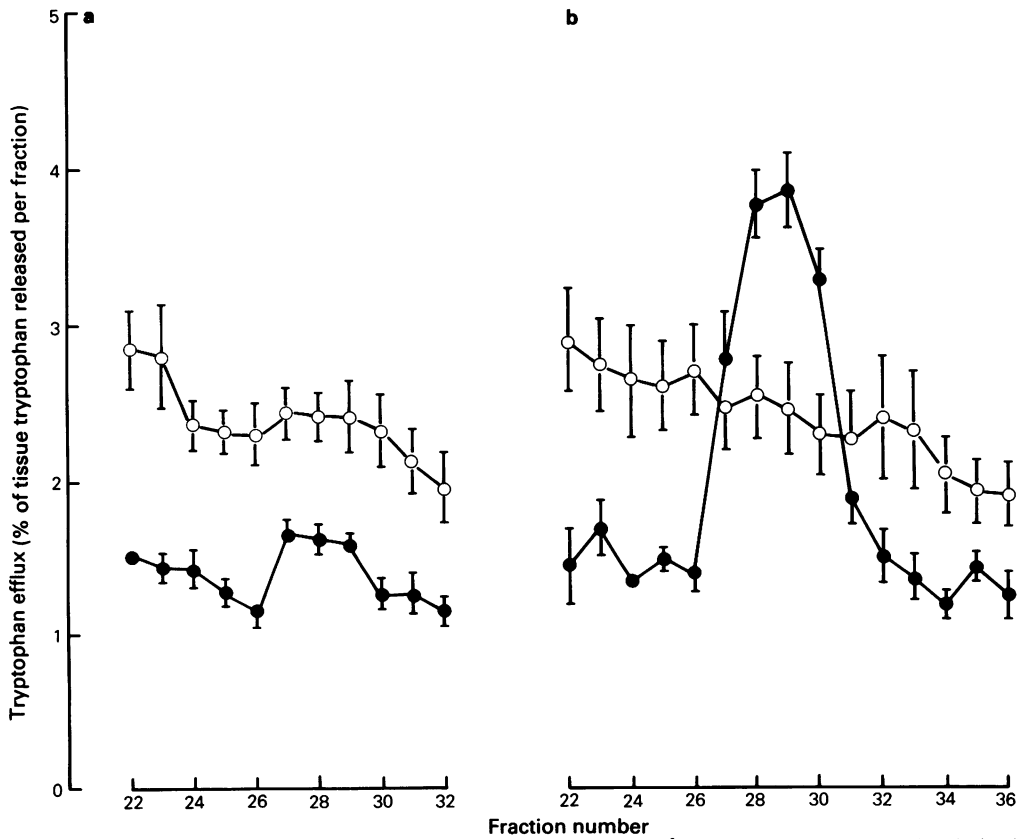

Figure 3 The effect of parachlorophenylalanine (PCPA) on the release of [^{3}H]-tryptophan evoked by depolarization (a) or the application of a pulse of Na^+-free Krebs solution (b). In both (a) and (b), (●) represents the control response and (○) the effect of PCPA. Efflux is expressed as the % of tissue [^{3}H]-tryptophan released per fraction. Results are given as the mean of $n = 3$ (Na^+-free study) or 4 (K^+ study); vertical lines indicate s.e.mean.

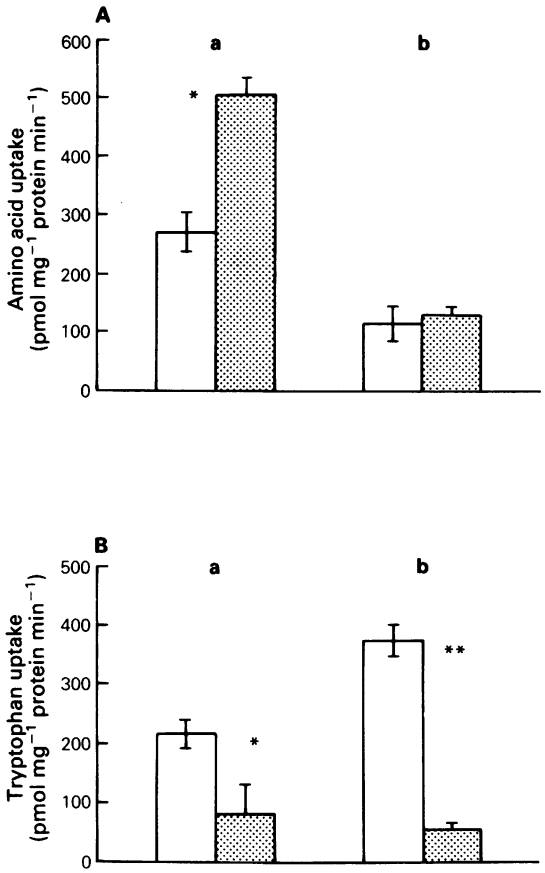

Figure 4 (A) The influx of [^{3}H]-tryptophan (a) and [^{3}H]-phenylalanine (b) in the presence (open columns) and absence (shaded columns) of extracellular Na^+. (B) The effect of parachlorophenylalamine (PCPA; shaded columns) on the influx of [^{3}H]-tryptophan in the presence (a) and absence (b) of extracellular Na^+. Influx is expressed as the amount (pmol mg^{-1} protein) of [^{3}H]-tryptophan or [^{3}H]-phenylalanine actively accumulated over a 1 min incubation period at 37°C. Results are expressed as the mean ($n = 5$); vertical lines indicate s.e.mean. Significant differences between treatments are shown by $*P<0.01$, $**P<0.001$ (Student's t test).

The effect of parachlorophenylalanine on the release of [^{3}H]-tryptophan induced by K^+ depolarization or the application of a pulse of Na^+-free Krebs solution In addition to being an inhibitor of tryptophan hydroxylase (Jequier *et al.*, 1967), parachlorophenylalanine (PCPA) is a powerful inhibitor of the plasma membrane tryptophan carrier (Kiely & Sourkes, 1972). This study examined the effect of PCPA, at a dose which had been shown to immobilize the carrier (see influx studies), on the efflux of tryptophan induced by K^+ depolarization and the application of Na^+-free pulses.

The experiments were conducted essentially as described in the previous sections, but with the presence of 500 μM PCPA in the perfusion fluid of one set of synaptosomes. The results of the studies are shown in Figure 3. It can be seen that the basal efflux of tryptophan was elevated in the presence of PCPA. The reason for this is not clear, but it could indicate that PCPA is causing some form of displacement of [^{3}H]-tryptophan from the intracellular compartment of the synaptosomal preparation. However, despite this shift in the basal efflux, it was quite clear that PCPA strongly inhibited the response to both K^+ depolarization and the applicationof the pulse of Na^+-free fluid.

Influx studies

The uptake of [^{3}H]-tryptophan and [^{3}H]-phenylalanine in the presence and absence of extracellular Na^+ The uptake of [^{3}H]-tryptophan and [^{3}H]-phenylalanine under identical experimental conditions in the presence and absence of extracellular Na^+ is shown in Figure 4A. Two important differences between tryptophan and phenylalanine uptake may be seen. Firstly, significantly greater amounts of tryptophan were taken up compared with phenylalanine. Secondly, tryptophan transport was stimulated by the removal of extracellular Na^+ but that of phenylalanine was not.

The effect of parachlorophenylalanine on the uptake of [^{3}H]-tryptophan in the presence and absence of extracellular Na^+ This experiment was conducted essentially as described above except that in one set of synaptosomes 500 μM PCPA was added with the [^{3}H]-tryptophan. This was done in preference to adding the PCPA in the preincubation phase, in order to limit the possibility of secondary effects of PCPA on tryptophan hydroxylase activity. With only 60 s contact with the tissue, the major effect of PCPA is likely to be on transport phenomena. The results of the study are shown in Figure 4B. It can be seen that PCPA at the concentration used in the efflux experiments caused a profound inhibition of tryptophan uptake. Furthermore, removing extracellular Na^+ in the presence of PCPA failed to elevate tryptophan uptake.

Discussion

The results of this study complement the recent findings of Wolf & Kuhn (1986) that endogenous tryptophan may be released from synaptosomes by veratridine-induced depolarization, and confirm our original observation that K^+ depolarization also

causes the Ca^{2+}-dependent release of exogenous [^{3}H]-tryptophan from superfused synaptosomes (Collard *et al.*, 1982). The comparison of the characteristics of the K^+-evoked release of [^{3}H]-tryptophan, [^{3}H]-5-HT and [^{3}H]-phenylalanine conducted in this study revealed that although quantitative differences exist, the response to K^+ depolarization was qualitatively similar for both 5-HT and tryptophan. The major differences observed were that the basal efflux of tryptophan was significantly higher than that of 5-HT and the response to a given degree of depolarization was smaller. The large basal efflux of tryptophan indicated that a greater proportion of the tryptophan present in the tissue was released in each fraction. This may be due to the differences in the way in which the transported tryptophan or 5-HT is actually stored within the nerve terminal. Over incubation periods of the duration used in this study, and at the concentration of 5-HT used, the majority of transported [^{3}H]-5-HT will enter 5-HT neurones. Within those neurones, that which is not metabolized will be sequestered by some form of intraterminal storage mechanism from which it would have difficulty in crossing the terminal membrane in the non-depolarized state (Ross, 1982). In contrast, if transported tryptophan is not sequestered within the synaptosomes it may have greater access to the extracellular medium and lead to the observed higher basal efflux. The tryptophan which is transported by individual synaptosomes may be used for different purposes such as protein synthesis (Wedege *et al.*, 1977), 5-HT synthesis (Mandell & Knapp, 1977) and other metabolic needs (Gal & Sherman, 1978). The small response to depolarization superimposed onto a large basal efflux, observed in this study, would suggest that only a small proportion of the synaptosomes which have accumulated tryptophan may actually release the amino acid when depolarized.

In contrast to the similarities observed between tryptophan and 5-HT release, phenylalanine efflux did not seem to be increased by depolarization except by very large concentrations of K^+, and the effect was not Ca^{2+}-dependent.

The depolarization-induced efflux of tryptophan could occur either by a release process similar to that which operates for conventional transmitters, or as a consequence of some other change which occurs within the nerve terminal during depolarization, and which under the conditions of these experiments is expressed as an increase in efflux. The studies on the influx and efflux of tryptophan in the presence and absence of extracellular Na^+ can help to answer this question. Tryptophan influx was clearly increased by removing extracellular Na^+, but that of phenylalanine uptake was not. Thus two experimental procedures employed in this study have clearly shown specificity towards tryptophan over phenylalanine i.e. efflux induced by K^+ depolarization, and influx induced by reducing extracellular Na^+. The effect of Na^+ removal on tryptophan efflux also demonstrated that a procedure shown to enhance specifically tryptophan influx can also enhance the efflux of the amino acid from the preloaded synaptosomes.

A common element in all cases where tryptophan efflux and influx are activated seems to be an elevation of intracellular Ca^{2+}. K^+ depolarization is known to elevate intrasynaptosomal Ca^{2+} (Adam-Vizi & Ligeti 1986), and to trigger release. The mechanism by which a reduction in extracellular Na^+ activates tryptophan transport is unknown, but the possibility that an increase in intracellular Ca^{2+} is responsible has been tentatively proposed (Wilkinson & Collard, 1985). While there is some disagreement (Akerman & Nicholls, 1981), there is good evidence that one of the processes by which intracellular Ca^{2+} may be maintained at a low resting level is a plasma membrane Na^+/Ca^+ exchange process by which Na^+ entering the nerve terminal down the concentration gradient drives Ca^{2+} out of the cell (Blaustein *et al.*, 1980, Nachshen *et al.*, 1986). Dissipation of the Na^+ gradient is believed to decrease Ca^{2+} efflux and possibly cause the uptake of Ca^{2+} into the cell if the gradient is reversed (Blaustein & Oborn, 1975). A consequence of this would be an increase in intracellular Ca^{2+} concentration (Nachshen *et al.*, 1986). Thus the plasma membrane tryptophan carrier could be activated by an increase in intracellular Ca^{2+}. In experiments in fixed volume incubation conditions with high extracellular tryptophan concentrations this would be reflected as an increase in influx. However, in the superfusion conditions employed in these experiments with preloaded synaptosomes, extracellular tryptophan is rapidly washed away by the perfusion fluid so that the net transport of tryptophan by the activated carrier is likely to be in the outward direction. The finding that inhibition of the carrier with PCPA blocked both the enhanced influx and efflux observed in these studies would support such a view, despite the fact that the drug can inhibit 5-HT synthesis as well as tryptophan transport. The experiments of the effect of PCPA on tryptophan influx were conducted in such a way that the drug was unlikely to be influencing 5-HT synthesis. However, in the efflux experiments the drug was in contact with the tissue for a sufficient period of time to reduce 5-HT synthesis. Reducing the availability of one metabolic route for the amino acid might modify the amount of amino acid available for efflux. However, only a very small amount of transported tryptophan is actually converted to 5-HT over the time periods used in these experiments (Karobath, 1972; Collard *et al.*, 1982), and the availability of transported tryptophan is unlikely to be altered significantly during the course of the experiment.

Overall, the most parsimonious interpretation of these experimental observations is that, the release of

tryptophan seen under depolarizing conditions in these experiments is more likely to be due to the activation of the tryptophan carrier, possibly as a consequence of the elevated intracellular Ca^{2+}, rather than a true release mechanism such as that used by conventional transmitters. The physiological significance of activation of the membrane tryptophan carrier by depolarization depends, to some extent, on the identity of the neurones in which the effect is observed and on the direction in which the tryptophan is transported *in situ*. Although extracellular tryptophan has been shown to potentiate 5-HT release from synaptosomes (Collard & Lewis, 1985), suggesting some modulatory effect of the released amino acid, the depolarization-induced activation of the influx of tryptophan may make more sense physiologically. In addition to causing the release of 5-HT, depolarization activates 5-HT synthesis in 5-HT nerve-endings (Boadle-Biber, 1978). This is brought about to some extent by a depolarization-induced activation of tryptophan hydroxylase. However, an increase in the supply of the substrate by activating the influx of tryptophan, would provide a ready source of tryptophan to the activated enzyme and ensure that synthesis of new 5-HT could proceed unhampered by the shortage of substrate.

In conclusion, the results of the present study suggest that the plasma membrane tryptophan carrier in some nerve endings may be activated by depolarization, and some other procedures known to elevate intracellular Ca^{2+} levels. The physiological significance of this process, with respect to the synthesis of 5-HT and nerve terminal proteins, will remain unclear until further studies have established the identity of the nerve terminals in which the activation of the tryptophan carrier occurs, and confirmed whether, as predicted, the carrier is turned on by an increase in intracellular Ca^{2+}.

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References

ADAM-VIZI, V. & LIGETI, E. (1986). Calcium uptake of rat brain synaptosomes as a function of membrane potential under different depolarising conditions. *J. Physiol.*, **372**, 363–377.

AKERMAN, K.E.O. & NICHOLLS, D.G. (1981). Calcium transport by intact synaptosomes. The voltage-dependent calcium channel and a re-evaluation of the role of Na^+/Ca^{2+} exchange. *Eur. J. Biochem.*, **117**, 491–497.

BLAUSTEIN, M.P., McGRAW, C.F., SOMLYO, A.V. & SWEITZER, E.S. (1980). How is cytoplasmic calcium concentration controlled in nerve terminals? *J. Physiol. (Paris)*, **76**, 459–470.

BLAUSTEIN, M.P. & OBORN, C.J. (1975). The influence of sodium on calcium fluxes in pinched-off nerve terminals *in vitro*. *J. Physiol.*, **247**, 657–686.

BOADLE-BIBER, M.C. (1978). Activation of tryptophan hydroxylase from central serotonergic neurones by calcium and depolarisation. *Biochem. Pharmacol.*, **27**, 1069–1079.

COLLARD, K.J., CASSIDY, D.M., PYE, M.A. & TAYLOR, R.M. (1981). The stimulus-induced release of 5-hydroxytryptamine from superfused rat brain synaptosomes. *J. Neurosci. Meth.*, **4**, 163–179.

COLLARD, K.J., EVANS, T.N.W., SUTER, H.A. & WILKINSON, L.S. (1982). The stimulus-induced release of 5-hydroxytryptamine and tryptophan from superfused rat brain synaptosomes. *J. Neural Transm.*, **53**, 223–230.

COLLARD, K.J. & LEWIS, D.J. (1985). The modulation of 5-hydroxytryptamine release from rat forebrain synaptosomes by low concentrations of tryptophan and 5-hydroxytryptamine. *J. Physiol.*, **361**, 51P.

DENZEAU, F. & SOURKES, T.L. (1977). Regional transport of tryptophan in rat brain. *J. Neurochem.*, **28**, 951–959.

EVANS, S.M., WILKINSON, L.S. & COLLARD, K.J. (1985). Studies on the mechanism by which extracellular Na^+ depletion causes 5-hydroxytryptamine release from synaptosomes. *Biochem. Soc. Trans.*, **13**, 1209.

GAL, E.M. & SHERMAN, A.D. (1978). Synthesis and metabolism of L-Kynurenine in rat brain. *J. Neurochem.*, **30**, 607–613.

GRAY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenisation and centrifugation. *J. Anat.*, **96**, 79–87.

JEQUIER, E., LOVENBERG, W. & SJOERDSMA, A. (1967). Tryptophan hydroxylase inhibition: the mechanism by which p-chlorophenylalanine depletes rat brain serotonin. *Mol. Pharmacol.*, **3**, 274–278.

KAROBATH, M. (1972). Serotonin synthesis with rat brain-synaptosomes, effects of serotonin and monoamine oxidase inhibitors. *Biochem. Pharmacol.*, **21**, 1253–1263.

KIELY, M. & SOURKES, T.L. (1972). Transport of L-tryptophan into slices of rat cerebral cortex. *J. Neurochem.*, **19**, 2863–2872.

LANE, J.D. & APRISON, M.H. (1978). The flux of radioactive label through components of the serotonergic system following the injection of [^{3}H]-tryptophan. Product-precursor anomalies providing evidence that serotonin exists in multiple pools. *J. Neurochem.*, **30**, 671–678.

LOWRY, O.H., ROSEBROUGH, N.H., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.

MANDELL, A.J. & KNAPP, S.J. (1977). Regulation of serotonin biosynthesis in brain: role of the high affinity uptake of tryptophan into serotonergic neurones. *Fed. Proc.*, **36**, 2142–2148.

NACHSHEN, D.A., SANCHEZ-ARMASS, S. & WEINSTEIN, A.M. (1986). The regulation of cytosolic calcium in rat brain synaptosomes by sodium-dependent calcium efflux. *J. Physiol.*, **381**, 17–28.

ROSS, S.B. (1982). The characteristics of serotonin uptake systems. In *Biology of Serotonergic Transmission*. ed.

Osborne N.N. pp. 159–195. Chichester: John Wiley and Sons.

SMITH, J.E., LANE, J.D., SHEA, P.A., McBRIDE, W.J. & APRISON, M.B. (1975). A method for concurrent measurement of picomole quantities of acetylcholine, choline, dopamine, norepinephrine, serotonin, 5-hydroxytryptophan, 5-hydroxyindoleacetic acid, tryptophan, tyrosine, glycine, aspartate, glutamate, alanine and gamma-aminobutyric acid in single tissue samples from different areas of rat central nervous system. *Analyt. Biochem.*, **54**, 149–169.

WEDEGE, E., LUQMANI, Y. & BRADFORD, H.F. (1977). Stimulated incorporation of amino acids into proteins of synaptosomal fractions induced by depolarising treatments. *J. Neurochem.*, **29**, 527–537.

WILKINSON, L.S. & COLLARD, K.J. (1984). Opposite effects of extracellular sodium removal on the uptake of tryptophan into rat cortical slices and synaptosomes. *J. Neurochem.*, **43**, 274–275.

WILKINSON, L.S. & COLLARD, K.J. (1985). Kinetics of L-tryptophan uptake into rat forebrain synaptosomes in the presence and absence of extracellular Na^+. *Biochem. Soc. Trans.*, **13**, 1208.

WOLF, W.A. & KUHN, D.M. (1986). Uptake and release of tryptophan and serotonin: An HPLC method to study the flux of endogenous 5-hydroxyindoles through synaptosomes. *J. Neurochem.*, **46**, 61–67.

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