J. exp. Biol. (1975), **62**, 55–67
With 3 figures
Printed in Great Britain

THE UPTAKE OF L-GLUTAMATE BY THE CENTRAL NERVOUS SYSTEM OF THE COCKROACH, *PERIPLANETA AMERICANA*

BY P. D. EVANS*

ARC Unit of Invertebrate Chemistry and Physiology, Department of Zoology, Downing Street, Cambridge

(*Received* 17 *June* 1974)

SUMMARY

A concentrative uptake mechanism for L-glutamate with the following characteristics has been identified in the abdominal nerve cord:

1. The uptake can be divided into Na^+-sensitive and Na^+-insensitive components.

2. The Na^+-sensitive component showed the typical saturation kinetics of a carrier mediate process. It had a V of $15{\cdot}9 \times 10^{-6}$ μM/mg wet weight/min and a K_m of 0·33 mM. Its magnitude was proportional to the first power of the Na^+ concentration of the medium. The uptake was specific for L-dicarboxylic amino acids and was sensitive to the presence of metabolic inhibitors.

3. The Na^+-insensitive component was linearly related to the glutamate concentration of the medium.

An isosmotic saline is described for use with the isolated intact abdominal nerve cord of *P. americana*.

INTRODUCTION

The insect central nervous system possesses a well developed 'blood–brain barrier', which is believed to restrict the intercellular diffusion of water-soluble ions and molecules to the spaces immediately surrounding the axons (cf. Treherne & Pichon, 1972). In the cockroach, the structural basis of this barrier has been well studied (Maddrell & Treherne, 1967; Lane & Treherne, 1970, 1972; Treherne, Lane, Moreton & Pichon, 1970), as has the movement of inorganic ions across it (Treherne *et al.* 1970; Pichon & Treherne, 1970, 1971; Pichon, Moreton & Treherne, 1971; Tucker & Pichon, 1972; Treherne & Pichon, 1972; Treherne, Schofield & Lane, 1973).

We are, however, still largely ignorant of the processes involved in the uptake, metabolism and translocation of nutrient substances, such as sugars and amino acids, within the insect central nervous system. The importance of the role of glial elements in this transfer was suggested by the histochemical study of Wigglesworth (1960) on the uptake of carbohydrate by the CNS of the cockroach. The kinetics of the uptake of radioactive glucose and trehalose by the cockroach abdominal nerve cord were also studied by Treherne (1960, 1961*a*).

* Present address: Department of Neurobiology, Harvard Medical School, 25 Shattuck Street Boston, Mass. 02115, U.S.A.

The present study reports on the characterization of the uptake of the amino acid L-glutamate, by the CNS of the cockroach *Periplaneta americana*. The transport of this amino acid was chosen for study because of its important metabolic role, particularly as the precursor of γ-aminobutyric acid, a potential central transmitter in insects (cf. Gerschenfeld, 1973). Glutamate itself is thought to be the likely excitatory transmitter at the insect neuromuscular junction (cf. Kravitz, Slater, Takahashi, Bownds & Grossfeld, 1970), but as yet no pharmacological studies have been made on the action of glutamate on central neurones of insects. Transport systems for L-glutamate have been studied in nervous tissue from many sources, such as crustacean peripheral nerves (Evans, 1973*a*, 1974; Baker & Potashner, 1971, 1973), cockroach peripheral nerve (Faeder & Salpeter, 1970), frog peripheral nerve (Wheeler & Boyarsky, 1968, 1971) and rat peripheral nerve (Yamaguchi *et al.* 1970).

The amino acid levels in various regions of the CNS of *Periplaneta americana* have been examined in the present study and compared with levels in whole haemolymph samples from the same population of animals, to estimate the concentration gradient for glutamate between the CNS and the haemolymph. The importance of the use of an isosmotic saline medium is stressed, for work on isolated portions of nerve cord, especially where rates of movement of molecules are to be estimated.

The results of the present study are discussed in terms of a current theory for the functioning of the insect blood–brain barrier.

METHODS

The cockroaches were reared at 26–28 °C and fed on a standard diet of crushed dog biscuits and tap water, *ad libidum*. The importance of rearing these insects on a controlled diet has been emphasized by Pichon (1970). Adult male insects were used in all experiments described in this study.

Amino acid measurements were made on samples of nervous tissue from various regions of the central and peripheral nervous system. The nerve cords were dissected from the insects under saline, taking care not to stretch the preparation (Lane & Treherne, 1970). Peripheral nerves were dissected from the 3rd metathoracic legs and pooled prior to analysis, at least ten portions of nerve from five different animals being used for each determination. The techniques for the extraction and estimation of the amino acids, using a Technicon automatic amino acid analyser, have been described previously (Evans, 1973*b*). The results are expressed as mmoles of amino acid per kilogram wet weight of tissue. Haemolymph samples were obtained by bleeding insects at the base of the third metathoracic leg, measured volumes being placed in calibrated microcaps previously cooled to 4 °C. The techniques for deproteinizing the blood sample and extracting the amino acids are described in Evans & Crossley (1974). The results are expressed as mmoles of amino acid per litre of haemolymph.

The saline devised by Yamasaki & Narahashi (1959) was used in preliminary experiments. It was found, however, that this saline resulted in weight losses in the intact tissue when incubated at room temperature (see Fig. 1). Thus a search was initiated for a saline composition that would maintain the tissue at a constant weight during the course of the incubations. Weight changes were noted in abdominal nerve cords

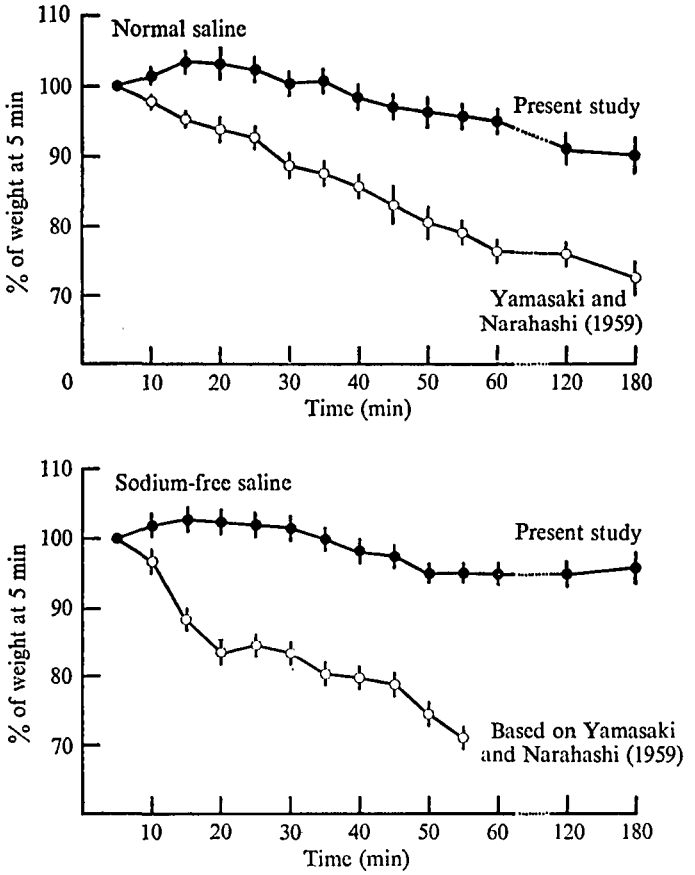

Fig. 1. Weight changes in isolated ligatured abdominal nerve cords of *Periplaneta*, incubated in various salines. The weights are expressed as a percentage of the weight of the nerve cord at 5 min after excision from the animal. The compositions of the salines used are given in Table 2. The bars represent 2 S.E. and $n = 10$.

incubated in salines of varying ionic content. Prior to weighing, on a 1 mg torsion balance, adhering surface moisture was removed by gently drawing the specimen across a glass slide. The results presented in the next section detail the composition of the saline finally chosen for the present investigation.

Isolated ligatured abdominal nerve cords were incubated for various times in saline containing [U^{14}C]glutamate (260 mCi/mmole, Radiochemical Centre, Amersham) at a concentration of 0·01 mM and 2 μCi/ml. Each nerve cord was incubated in 1 ml of solution and agitated in a water bath held at room temperature. The nerve cord was then washed for 1 min, in ice-cold saline to remove superficial radioactivity. Efflux controls showed that this procedure effectively removed all the radioactivity taken up by the nerve cord in a 1 sec exposure to the above radioactive solution, the labelled glutamate being lost with a half-time of 30 sec. At the end of one minute the activity left in the tissue still accounted for 97·5% of the 'tissue component' extrapolated to zero time.

The nerve cords were then blotted as above and weighed, prior to their solubilization,

Table 1. *The free amino acid content of central nervous tissues from* Periplaneta

Amino acid	Whole nerve cord	Thoracic nerve cord	Abdominal nerve cord	6th abdominal ganglion	Peripheral leg nerve	Whole blood
Cysteate	0·52	1·32 ± 0·23	1·01 ± 0·32	1·31 ± 0·12	—	—
Taurine	0·57	1·15 ± 0·30	1·76 ± 0·11	2·77 ± 0·50	0·51 ± 0·07	—
Aspartate	6·88 ± 0·85	5·73 ± 0·81	4·90 ± 0·66	5·53 ± 0·39	2·31 ± 0·26	0·10
Threonine	0·37	0·21	0·51	0·67	0·27	—
Serine	1·56	0·72	3·47	2·71	0·87	—
Glutamine	5·04	—	—	—	—	—
Glutamate	9·38 ± 0·99	9·27 ± 1·26	7·63 ± 1·55	9·68 ± 1·14	5·33 ± 0·94	0·27
Proline	10·96 ± 1·09	12·96 ± 1·06	8·64 ± 1·78	11·26 ± 2·44	2·73 ± 0·44	5·22
Glycine	5·15 ± 1·64	5·02 ± 0·73	2·25 ± 0·68	4·16 ± 0·32	2·60 ± 0·37	4·62
Alanine	4·65 ± 0·56	5·37 ± 0·39	3·12 ± 1·13	5·26 ± 0·89	1·48 ± 0·14	0·77
Valine	0·46 ± 0·15	0·44 ± 0·09	0·34	0·64	0·30 ± 0·06	0·89
Isoleucine	0·04 ± 0·02	0·34 ± 0·10	0·05	—	0·10	—
Leucine	0·07 ± 0·02	0·24 ± 0·05	0·36 ± 0·25	0·28 ± 0·07	0·19 ± 0·07	—
Tyrosine	0·22 ± 0·02	0·28 ± 0·07	0·26 ± 0·02	0·42 ± 0·02	0·20 ± 0·04	—
Phenylalanine	0·07 ± 0·01	0·06 ± 0·01	0·18 ± 0·06	0·24 ± 0·07	0·17 ± 0·06	—
Lysine	0·27 ± 0·11	1·54 ± 0·12	0·87 ± 0·15	0·66 ± 0·13	0·36 ± 0·14	—
Histidine	0·50 ± 0·19	1·28 ± 0·22	0·46 ± 0·08	1·15 ± 0·15	0·56 ± 0·20	—
Arginine	7·44 ± 1·22	11·04 ± 0·78	3·76 ± 0·88	12·46 ± 1·30	5·35 ± 0·25	0·52

The results are expressed as mmoles/kg wet weight ± S.E. for the nerve tissue samples and as mmoles/litre of haemolymph for the whole blood sample. — = present but not estimated.

in 0·5 ml of Hyamine hydroxide (Koch Light Ltd.) at 50 °C for 30 min. After cooling the vials were made up to 10 ml with a scintillation fluid consisting of 0·8 % butyl-PBD (Koch Light Ltd.) in a mixture of Triton X100: toluene (1 : 2 v/v) and the radioactivity estimated in a Packard Tri Carb liquid scintillation spectrometer. Correction for quenching was made by reference to the external standard.

The influx of radioactivity was linear for the first 30 min, and so a short incubation time of 10 min was used to obtain an accurate measure of the initial rate of influx. At this incubation time up to 92 % of the radioactivity extractable in 60 % aqueous ethanol could be shown to be associated with glutamate and under these conditions the uptake showed a pH optimum of 7·3 (Evans, unpublished). The influx is expressed as μM/mg wet weight of tissue/min.

The concentration of glutamate in the medium was varied and a dose-uptake curve, constructed. Estimates of K_m and V_{max} for this system were obtained from plots of V against V/S (see Dowd & Riggs, 1965; Neame & Richards, 1972). The effect of variation in the Na^+ and K^+ levels of the medium has been studied. The specificity of the uptake of glutamate by this preparation was investigated in a series of competition experiments with various analogues and other amino acids. The effect of a series of metabolic inhibitors was also examined.

RESULTS

Amino acid measurements

The amino acid distribution in the various regions of the CNS of the cockroach is shown in Table 1. The whole nerve cord measurements are in close agreement with the partial analysis of Ray (1964). There is a marked similarity between the various regions, an exception being the reduced level of arginine found in the abdominal cord

Table 2. *Composition of salines*

	Normal		Sodium-free	
	Yamasaki & Narahashi (1959)	Present study	Based on Yamasaki & Narahashi (1959)	Present study
NaCl	210·2	150·0	—	—
KCl	3·1	10·0	—	3·1
$CaCl_2$	1·8	1·8	1·8	1·8
Na_2HPO_4	1·8	1·8	—	—
NaH_2PO_4	0·2	0·2	—	—
$NaHCO_3$	—	2·5	—	—
K_2HPO_4	—	—	1·8	1·8
KH_2PO_4	—	—	0·2	0·2
$KHCO_3$	—	—	—	2·5
Tris Cl	—	—	190·0*	126·0
Tris base	—	—	23·0*	14·9

Concentrations are expressed as mmoles/litre.

* Tris (hydroxymethyl)methylammonium ions have been used as a sodium substituent rather than choline (Pichon *et al.* 1971).

samples. The levels of isoleucine and leucine also showed some variability between the regions examined. Proline was the most concentrated amino acid in all CNS samples, being associated with significant amounts of glutamate, aspartate, glutamine, glycine, alanine and arginine. The peripheral nerve samples, however, showed a reduction in the amount of proline, with arginine and glutamate (the proposed excitatory neuromuscular transmitter in insects) being the most abundant. The measurements on whole cockroach haemolymph show proline and glycine to be the most concentrated of the amino acids estimated. A comparison of the levels in whole blood and nervous tissue gives a minimum estimate of the concentration gradient between the nerve cord and the haemolymph, ratios of approximately 30:1 and 60:1 respectively being found for glutamate and aspartate. These ratios must be regarded as minimal estimates for the haemocytes were not removed prior to estimation. The plasma levels, actually bathing the tissues, could be, thus, significantly lower, giving larger distribution ratios, depending on the amounts of these amino acids sequestered in the haemocyte fraction. The importance of this point will be discussed below.

Composition of saline

A comparison of the weight changes of isolated abdominal nerve cords incubated in the saline of Yamasaki & Narahashi (1959), and in the saline developed in the present investigation, is shown in Fig. 1. Table 2 gives the compositions of the salines. The composition of the saline used in the present investigation is similar to that used by Yamasaki & Narahashi (1957) and to that developed by Treherne (1961*b*, *c*, 1962) minus the organic component of amino acids and sugars. A wide range of saline compositions was examined. The final salines selected for use in this study (see Table 2) gave the least weight changes over the time course of the incubations. The values of 156 mM-Na^+ and 10 mM-K^+ approach more closely to the values measured in the haemolymph of this species (Pichon, 1970) than do those used by Yamasaki & Narahashi (1959). It is interesting to note that the Na^+ concentration found to give the least weight loss in the present experiment is of a similar level to that used by Yamasaki

& Narahashi (1957) for experiments on intact neve cords, but when they used the same solution on a desheathed preparation (Yamasaki & Narahashi, 1959), they observed that the axons swelled slightly after being kept in the Ringer's solution for a period of several hours. This gives further evidence for the glial elements of the insect CNS maintaining a higher concentration of Na^+ around the neurones than that normally measured in the haemolymph of the insect.

The addition of 2·5 mM-HCO_3^- to the medium was found slightly to decrease the weight losses of the nerve cord and also to maintain the pH of the solution at 7·3 for extended periods. The presence of bicarbonate ions has been suggested to be an important factor in the maintenance of steady resting potentials in cockroach muscle (Wareham, Duncan & Bowler, 1973). The latter authors suggest this is due to the presence of a bicarbonate-sensitive hyperpolarizing component of the resting potential, which accounts for approximately one third of the normal transmembrane potential.

The inclusion of 20 mM glucose again gave an improved stability to the weight measurement, particularly at long incubations. It was, however, omitted for the purposes of the present investigation as glucose has been shown in other tissues to interfere with the uptake of amino acids. It has been shown to be inhibitory in frog peripheral nerve (Wheeler & Boyarsky, 1971) and crustacean peripheral nerve (Evans, 1973*a*), but to have a stimulatory effect on glutamate uptake in the CNS of vertebrates (Yamaguchi *et al.* 1970; Yamaguchi *et al.* 1972). Preliminary experiments on cockroach abdominal nerve cords indicate that the concentration of glucose present could markedly alter the uptake rates of glutamate, the direction and extent of the effect being dependent on the glucose concentration. A more detailed analysis of the interactions of glucose and amino acid transport systems in this preparation will be reported in a future communication from this laboratory.

In the sodium-free solutions (Table 2) Tris has been used as the sodium substituent (Pichon *et al.* 1971) rather than choline or glucose, which were used by Yamasaki & Narahashi (1959). The weight changes of isolated abdominal nerve cords incubated in the sodium-free saline based on Yamasaki & Narahashi (1959), and those observed during incubations in the sodium-free saline developed in the present study, are shown in Fig. 1.

The importance of choosing the correct isosmotic medium will be discussed below in terms of its possible implications for the findings of previous investigators.

Characterization of L-*glutamate uptake*

Uptake kinetics

The distribution of radioactivity between the abdominal nerve cord and the bathing medium was greater than unity within 10 min in cords incubated in saline containing [^{14}C]glutamate (0·01 mM and 2 μCi/ml). Since the rates of efflux of glutamate from this tissue into a medium containing a high concentration of unlabelled glutamate (20 mM) showed no significant increases over control effluxes into saline, it is suggested that any exchange diffusion component would be trivial (P. D. Evans, unpublished). This distribution ratio may therefore be taken as indicative of a concentrative uptake mechanism for glutamate in this tissue.

It can be seen from Fig. 2 that the influx of L-glutamate can be differentiated into two components, since in the absence of Na^+ from the bathing medium there is still

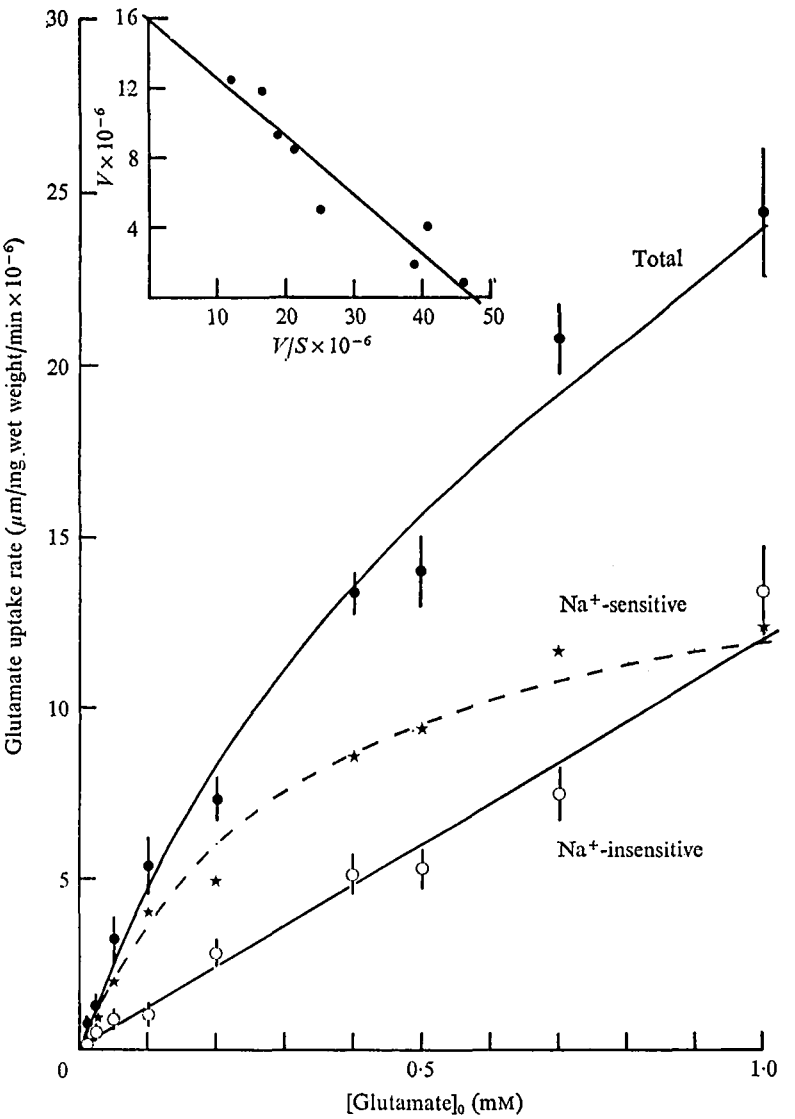

Fig. 2. The rate of uptake of glutamate is plotted against the concentration of glutamate in the bathing medium. The total uptake represents the uptake from normal saline (156 mM-Na^+) and the Na^+-insensitive uptake represents the influx from a Na^+-free medium. The linear portions of the plot were obtained by a linear regression analysis. The Na^+-sensitive plot was obtained by the subtraction of the Na^+-insensitive component from the total uptake at each glutamate concentration. The bars represent 2 S.E. and $n = 5$. The insert shows a plot of V against V/S for the Na^+-sensitive component of the uptake where V is the rate of uptake and S the concentration of glutamate in the bathing medium.

an appreciable influx. The latter Na^+-insensitive component was linearly related to the glutamate concentration of the medium between 0·005 and 1 mM. The Na^+-sensitive plot was obtained by deducting the Na^+-insensitive component from the total uptake in normal saline (156 mM-Na^+) for each of the experimental points. The Na^+-sensitive uptake showed the typical saturation kinetics of a carrier-mediated

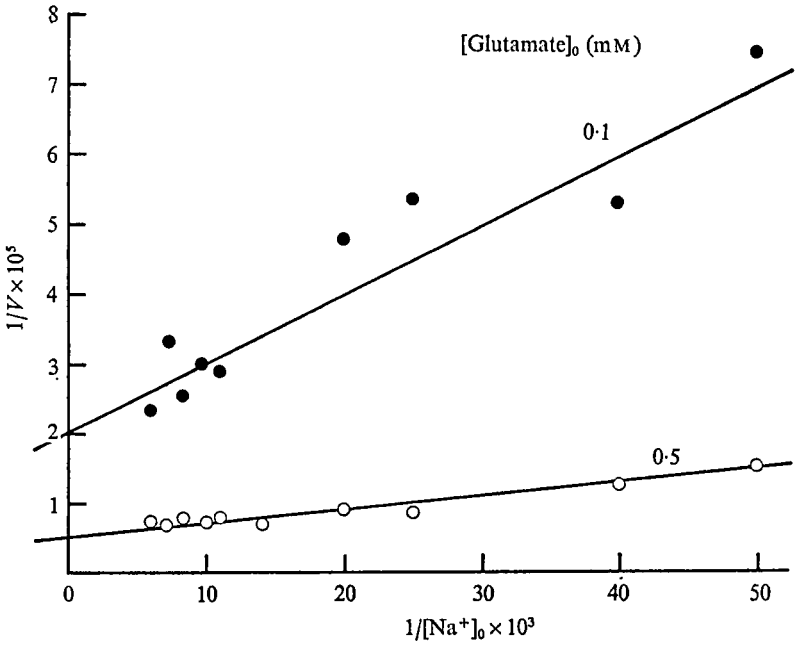

Fig. 3. The variation in the rate of the Na^+-sensitive component of the glutamate influx, V, with the variation in the Na^+ concentration of the bathing medium, $[Na^+]_0$, at two concentrations of glutamate, 0·1 and 0·5 mM. The results are shown as a reciprocal plot. The best fit straight lines were obtained by a linear regression analysis of the data.

process. The measured whole haemolymph content of glutamate for these insects was 0·27 mM which is below the saturation level of this process. Plots of V against V/S gave estimates of 0·33 mM for the K_m of the uptake and a V of $15{\cdot}9 \times 10^{-6}$ μM/mg wet weight of tissue/min (see insert Fig. 2).

Ionic dependence of glutamate uptake

The effect of reduction of the external Na^+ level upon the Na^+-sensitive influx of glutamate is shown in Fig. 3 at two different glutamate concentrations. Plots of $1/V$ against $1/[Na^+]_0$, where V is the initial rate of influx and $[Na^+]_0$ is the Na^+ concentration of the medium, gave a better approximation to linearity than plots of $1/V$ against $1/[Na^+]_0^2$ as judged on the basis of a linear regression analysis of the data. Such a finding has been interpreted as indicating that the Na^+-sensitive uptake mechanism is activated by a single sodium ion.

Varying the potassium ion concentration of the medium between 3 and 50 mM had no significant effect on either the Na^+-dependent or independent components of glutamate uptake at a fixed concentration of glutamate (0·1 mM).

Specificity of uptake system

The most potent competitors of the Na^+-sensitive glutamate influx were other amino acids such as L-aspartate, L-cysteate, OH-glutamate and L-2 amino adipic acid (Table 3). Closely related analogues such as glutamine and γ-aminobutyric acid were

Table 3. *The effect of other amino acids on the influx of glutamate*

Competitors	% Influx ± S.E.	
	Normal Ringer	Na^+-free Ringer
L-2-Aminoadipic acid	58·5 ± 3·4	96·7 ± 6·4
L-allo-γ-Hydroxy glutamic acid	62·1 ± 7·6	85·3 ± 8·8
L-Cysteic acid	72·1 ± 8·8	82·2 ± 5·0
L-Aspartic acid	78·5 ± 9·1	88·0 ± 0·8
L-Glutamine	84·0 ± 7·0	90·3 ± 4·2
DL-threo-β-Hydroxy aspartic acid	85·5 ± 12·0	72·6 ± 8·4
L-Histidine	85·5 ± 20·4	102·6 ± 9·9
L-Leucine	86·5 ± 11·3	101·6 ± 20·0
L-Proline	86·9 ± 10·0	101·0 ± 16·0
L-α-Alanine	90·4 ± 7·2	101·3 ± 14·5
D-Glutamic acid	95·6 ± 13·6	98·8 ± 9·7
γ-Aminobutyric acid	96·9 ± 20·0	95·6 ± 7·0
Glycine	105·4 ± 7·9	98·5 ± 8·5
L-Arginine	105·5 ± 8·9	93·8 ± 5·4
Taurine	113·2 ± 12·0	94·9 ± 9·4
L-Lysine	114·1 ± 23·0	100·1 ± 17·0

The results are expressed as a percentage of the influx of radioactivity from each solution in the absence of competitors. L-[U-^{14}C]glutamate was present at a concentration of 0·5 mM and 1 μCi/ml in the solutions used. The incubations were for 10 min periods. Putative competitors were present as a 10-fold excess, except for DL isomers which were used as a 20-fold excess. Each result is the mean of six determinations and is expressed ± S.E.

Table 4. *Effect of inhibitors on glutamate influx*

	Influx of glutamate (%)	
	Normal Ringer	Na^+-free Ringer
+ Cyanide	101·2 ± 8·7	102·8 ± 12·0
+ Iodoacetic acid	104·4 ± 13·3	111·3 ± 9·1
+ 2,4-Dinitrophenol	67·8 ± 7·4	100·8 ± 8·0
+ Azide	79·2 ± 5·7	—
+ (Cyanide + iodoacetic acid)	101·0 ± 16·5	83·9 ± 14·6
+ (2,4-Dinitrophenol + iodoacetic acid)	58·1 ± 7·9	103·0 ± 10·4

The results are expressed as a percentage of the influx of radioactivity from each solution in the absence of any inhibitors. The radioactive incubations were for 10 min periods and were preceded by 20 min preincubations in non-radioactive salines in the presence of the various inhibitors. The concentrations of the inhibitors used were cyanide 0·5 mM; iodoacetate 1 mM; 2,4-dinitrophenol 0·2 mM; and azide 1 mM. L-[U^{14}C]Glutamate was present at a concentration of 0·5 mM and 1 μCi/ml in the solutions used. Each result is the mean of six determinations and is expressed ± S.E.

not very effective competitors. The stereospecificity of the uptake was confirmed, as D-glutamate was not an effective competitor. The picture was very similar for the Na^+-insensitive component except for the decreased competitor effect of L-2 amino adipic acid.

The effect of inhibitors

It can be seen from Table 4 that only 2, 4-DNP and azide, of the metabolic inhibitors used, significantly reduced the uptake of glutamate in normal saline. The addition of iodoacetic acid (IAA) alone produced no significant inhibition, whereas in combination

with DNP it caused a greater inhibition than with either compound used singly. The uptake seemed to be CN^--insensitive. The combination of CN^- and IAA appeared to be the only significant inhibitor of the Na^+-independent uptake component.

DISCUSSION

The amino acid measurements indicate that the central nervous system of the cockroach is capable of maintaining a concentration gradient for amino acids such as glutamate and aspartate with respect to the haemolymph, the distribution ratios being approximately 30:1 and 60:1 respectively (CNS:haemolymph). These ratios are considerably lower than the corresponding ones found in the peripheral nerves of *Carcinus* (glutamate 933:1 and aspartate 18176:1, Evans, 1973*b*). The present calculations were, however, based on whole haemolymph values, not on plasma levels, and the distributions must therefore be considered as minimal estimates. Previous workers on insect haemolymph from orthopteran insects such as the locust (Miller, Leaf & Usherwood, 1973) and the cockroach (Holden, 1973) have reported that only about 12% of the total free amino acids of the haemolymph of these species could be found in association with the haemocyte fraction. However, Evans & Crossley (1974) found that in the haemolymph of *Calliphora vicina* 69% and 62%, of the aspartate and glutamate respectively, were sequestered by the haemocyte fraction. It is possible that differences in the stability of haemocytes from different orders of insects could account for these discrepancies. Further work on the stabilization of cockroach haemocytes, which are notoriously unstable, is needed before the true extent of the concentration gradients of ions and organic molecules between CNS and plasma can be accurately estimated for this insect.

In the present study the saline devised by Yamasaki & Narahashi (1959) for use with desheathed cockroach nerve cords proved to be hyperosmotic to the intact, unstretched, abdominal nerve cord. Incubation in this saline resulted in rapid weight losses by the tissue. It is suggested that this supports the concept that the perineurial glial sheath maintains a higher internal concentration of Na^+ ions around the neurones than that present in the medium (cf. Treherne & Pichon, 1972). However, many previous workers have used the Yamasaki & Narahashi (1959) saline in studies on the intact abdominal nerve cords of *Periplaneta* (e.g. Pichon & Treherne, 1970; Treherne *et al.* 1970; Pichon, Moreton & Treherne, 1971; Tucker & Pichon, 1972; Treherne, Schofield & Lane, 1973). At present it is uncertain if the exposure to this hyperosmotic saline would produce any significant changes in the parameters measured by the above authors. For example, the rates of ion movements between the different compartments of this tissue could depend upon the composition of the saline. It is possible that the significant increases in Na^+ content, and associated decreases in K^+, after 1·5 h noted in the ionic composition of the abdominal nerve cord by Tucker & Pichon (1972) might in part, at least, be attributable to the composition of the saline used. Also Treherne, Schofield & Lane (1973) report that incubation of abdominal nerve cords in this hyperosmotic saline, prior to fixation for E.M. study, resulted in the production of lacunae between the perineurial cells and, also at times, in some actual cell damage. In nerve cords treated only with fixative, the perineurium displays only occasional lacuna or spaces between the lateral borders of adjacent cells. This observa-

tion is consistent with the findings of weight losses in the present study and also with preliminary electron microscope observations (Evans, unpublished).

The characteristics determined for the uptake of L-glutamate by the abdominal nerve cord of the cockroach are similar to those reported for the uptake of this molecule by other nervous tissues, e.g. in frog peripheral nerve (Wheeler & Boyarsky, 1968, 1971), in *Maia* peripheral nerve (Baker & Potashner, 1971, 1973) and in *Carcinus* peripheral nerve (Evans, 1973*a*). The apparent K_m in cockroach abdominal nerve was 0·33 mM, in comparison to 0·28 mM for *Carcinus* peripheral nerve. The total uptake could be differentiated into Na^+-sensitive and Na^+-insensitive components in both cases. The dependence of the saturable component of the glutamate influx upon the first power of the external Na^+ concentration is also in agreement with the findings for *Carcinus* peripheral nerve (Evans, 1973*a*). This finding conflicts, however, with that of Baker & Potashner (1971, 1973) who reported that the glutamate uptake process into both *Maia* peripheral nerve and squid giant axon required two Na^+ for activation.

The specificity of the uptake site of the Na^+-sensitive L-glutamate carrier is also similar to that found in the analogous system in other tissues (Wheeler & Boyarsky, 1968; Baker & Potashner, 1971; Evans, 1973*a*). However, in the present case the specificity of the carrier seems to be rather broader in terms of the allowable changes in the length of the molecule accepted. The specificity exhibited by the Na^+-independent component again suggests that this part of the uptake is not wholly a free diffusion of glutamate, but that at least a part of it could be a carrier-mediated system with its own separate receptor sites to those of the Na^+-sensitive system (cf. Evans, 1973*a*).

The observation that the Na^+-sensitive glutamate influx in this preparation can be reduced by a variety of metabolic poisons suggests that this process could be, at least partially, dependent upon metabolic energy. This is similar to the findings of Evans (1973*a*) on *Carcinus* peripheral nerve and also to those of Baker & Potashner (1973) on *Maia* peripheral nerve and on squid giant axon. The relative importance of metabolic energy and ion gradients in energizing Na^+-dependent amino acid transport processes is at present unclear and a matter of some controversy (cf. Schultz & Curran, 1970; Lin & Johnstone, 1971; Potashner & Johnstone, 1971; Baker & Potashner, 1973; Geck, Heinz & Pfeiffer, 1974; Heinz & Geck, 1974). It also seems likely that at least a part of the Na^+-independent component is also directly linked to metabolic energy in a similar fashion to Na^+-independent amino acid fluxes in other tissues (cf. Evans, 1973*a*).

The concentrative uptake (which shows saturation kinetics, is specific for L-glutamate and is Na^+-dependent) suggests that the abdominal nerve cord of *Periplaneta* possesses an active uptake process for glutamate, which is apparently dependent on the energy provided by a combination of the Na^+ gradient across the cell membranes and of metabolic energy. The relationship between the transport and its possible energy sources awaits further investigation.

It is of interest to speculate upon the localization of this uptake process in the abdominal nerve cord of the cockroach in view of the presence of the blood–brain barrier which is known to restrict the intercellular movement of ions into this tissue. In *Carcinus* peripheral nerve a similar glutamate uptake system has been shown to be preferentially located in the glial cell component (Evans, 1974). Thus it seems possible

that this uptake of glutamate by the cockroach abdominal nerve cord could be located in the glial cells of the perineurium, which by virtue of their extended and tortuous perineurial clefts, would present a relatively large outwardly directed perineurial surface for uptake (cf. Treherne & Pichon, 1972). The existence of a glial-mediated transport system for carbohydrates has been suggested by Wigglesworth (1960), who demonstrated in a histochemical study a sequential deposition of glycogen, through the nervous system of starved cockroaches, following the injection of glucose into the haemolymph. The glial cytoplasm was found to transfer the material to the neuronal cell bodies via the trophospongeum of Holmgren. Whether or not charged amino acids transverse the same pathway across the perineurium is as yet unknown. It is hoped that a pulse-chase autoradiographical study at present in progress will help to elucidate the localization of the amino acid uptake and its subsequent translocation and metabolism.

I am indebted to Dr J. E. Treherne for helpful discussions during the course of this work and to Dr J. G. Hildebrand and Dr W. Woodward for their comments on the manuscript.

REFERENCES

BAKER, P. F. & POTASHNER, S. J. (1971). The dependence of glutamate uptake by crab nerve on external Na^+ and K^+. *Biochim. biophys. Acta* **249**, 616–22.

BAKER, P. F. & POTASHNER, S. J. (1973). The role of metabolic energy in the transport of glutamate by invertebrate nerve. *Biochim. biophys. Acta* **318**, 123–39.

DOWD, J. E. & RIGGS, D. S. (1965). A comparison of estimates of Michaelis Menten kinetic constants from various linear transformations. *J. biol. Chem.* **240**, 863–9.

EVANS, P. D. (1973*a*). The uptake of L-glutamate by the peripheral nerves of the crab, *Carcinus maenas* (L.). *Biochim. biophys. Acta* **311**, 302–13.

EVANS, P. D. (1973*b*). Amino acid distribution in the nervous system of the crab, *Carcinus maenas* (L.). *J. Neurochem.* **21**, 11–17.

EVANS, P. D. (1974). An autoradiographical study of the localization of the uptake of glutamate by the peripheral nerves of the crab, *Carcinus maenas* (L.). *J. Cell Sci.* **14**, 351–67.

EVANS, P. D. & CROSSLEY, A. C. (1974). Free amino acids in the haemocytes and plasma of the larvae of the blowfly *Calliphora vicina*. *J. exp. Biol.* **61**, 463–72.

FAEDER, I. R. & SALPETER, M. M. (1970). Glutamate uptake by a stimulated insect nerve muscle preparation. *J. Cell Biol.* **46**, 300–7.

GECK, P., HEINZ, E. & PFEIFFER, B. (1974). Evidence against a direct coupling between amino acid transport and ATP hydrolysis. *Biochim. biophys. Acta* **339**, 419–24.

GERSCHENFELD, H. M. (1973). Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.* **53**, 1–119.

HEINZ, E. & GECK, P. (1974). The efficiency of energetic coupling between Na^+ flow and amino acid transport in Ehrlich cells – a revised assessment. *Biochim. biophys. Acta* **339**, 426–30.

HOLDEN, J. S. (1973). Free amino acid levels in the cockroach, *Periplaneta americana*. *J. Physiol., Lond.* **232**, 61*P*–62*P*.

KRAVITZ, E. A., SLATER, C. R., TAKAHASHI, K., BOWNDS, M. D. & GROSSFELD, R. M. (1970). Excitatory transmission in invertebrates – glutamate as a potential neuromuscular transmitter compound. In *Excitatory Synaptic Mechanisms* (ed. P. Andersen & J. K. S. Jansen), pp. 85–93. Oslo: Universitets Forlaget.

LANE, N. J. & TREHERNE, J. E. (1970). Uptake of peroxidase by the cockroach central nervous system. *Tissue & Cell* **2**, 413–25.

LANE, N. J. & TREHERNE, J. E. (1972). Studies on perineurial junctional complexes and the sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. *Tissue & Cell* **4**, 427–36.

LIN, K. T. & JOHNSTONE, R. M. (1971). Active transport of glycine by mouse pancreas. Evidence against Na^+ gradient hypothesis. *Biochem. biophys. Acta* **249**, 144–58.

MADDRELL, S. H. P. & TREHERNE, J. E. (1967). The ultrastructure of the perineurium in two insect species (*Carausius morosus* and *Periplaneta americana*). *J. Cell Sci.* **2**, 119–28.

MILLER, R., LEAF, G. & USHERWOOD, P. N. R. (1973). Blood glutamate in Arthropods. *Comp. Biochem. Physiol.* **44A**, 991–6.

KEAME, K. D. & RICHARDS, T. G. (1972). *Elementary Kinetics of Membrane Carrier Transport*. London: Blackwell.

PICHON, Y. (1970). Ionic content of haemolymph in the cockroach, *Periplaneta americana*. A critical analysis. *J. exp. Biol.* **53**, 195–209.

PICHON, Y. & TREHERNE, J. E. (1970). Extraneuronal potentials and potassium depolarization in cockroach giant axon. *J. exp. Biol.* **53**, 485–93.

PICHON, Y. & TREHERNE, J. E. (1971). Mise en evidence d'une barrier hematoencephalique chez un invertebre, la blatte, *Periplaneta americana* L. *J. Physiol., Paris* **63**, 141–2.

PICHON, Y., MORETON, R. B. & TREHERNE, J. E. (1971). A quantitative study of the ionic basis of extraneuronal potential changes in the central nervous system of the cockroach (*Periplaneta americana* L.). *J. exp. Biol.* **54**, 757–77.

POTASHNER, S. J. & JOHNSTONE, R. M. (1971). Cation gradients, ATP and amino acid accumulation in Ehrlich ascites cells. *Biochim. biophys Acta* **233**, 91–103.

RAY, J. W. (1964). The free amino acid pool of the cockroach (*Periplaneta americana*) central nervous system and the effect of insecticides. *J. Insect Physiol.* **10**, 587–97.

SCHULTZ, S. G. & CURRAN, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637–718.

TREHERNE, J. E. (1960). The nutrition of the central nervous system in the cockroach, *Periplaneta americana* L. The exchange and metabolism of sugars. *J. exp. Biol.* **37**, 513–33.

TREHERNE, J. E. (1961*a*). The exchange and metabolism of sugars in the central nervous system of *Periplaneta americana* L. *Proc.XIth Int. Cong. Ent.* vol. 1, 632–5.

TREHERNE, J. E. (1961*b*). Sodium and potassium fluxes in the abdominal nerve cord of the cockroach, *Periplaneta americana*. *J. exp. Biol.* **38**, 315–22.

TREHERNE, J. E. (1961*c*). The movements of sodium ions in the isolated abdominal nerve cord of the cockroach, *Periplaneta americana* L. *J. exp. Biol.* **38**, 629–36.

TREHERNE, J. E. (1962). The distribution and exchange of some ions and molecules in the central nervous system of *Periplaneta americana*. *J. exp. Biol.* **39**, 163–217.

TREHERNE, J. E. & PICHON, Y. (1972). The insect blood–brain barrier. *Adv. Insect Physiol.* **9**, 257–313.

TREHERNE, J. E., SCHOFIELD, P. K. & LANE, N. J. (1973). Experimental disruption of the blood–brain barrier system in an insect (*Periplaneta americana*). *J. exp. Biol.* **59**, 711–23.

TREHERNE, J. E., LANE, N. J., MORETON, R. B. & PICHON, Y. (1970). A quantitative study of potassium movements in the central nervous tissue of *Periplaneta americana*. *J. exp. Biol.* **53**, 109–36.

TUCKER, L. E. & PICHON, Y. (1972). Sodium efflux from the central nervous connectives of the cockroach. *J. exp. Biol.* **56**, 441–57.

WAREHAM, A. C., DUNCAN, C. J. & BOWLER, K. (1973). Bicarbonate ions and the resting potential of cockroach muscle implications for the development of suitable saline media. *Comp. Biochem. Physiol.* **45A**, 239–46.

WHEELER, D. D. & BOYARSKY, L. L. (1968). Influx of glutamic acid in peripheral nerve-characteristics of influx. *J. Neurochem.* **15**, 1019–31.

WHEELER, D. D. & BOYARSKY, L. L. (1971). Influx of glutamic acid in peripheral nerve, energy, ionic and pH dependence. *J. Neurobiol.* **2**, 181–90.

WIGGLESWORTH, V. B. (1960). The nutrition of the central nervous system of the cockroach, *Periplaneta americana*. The mobilization of reserves. *J. exp. Biol.* **37**, 500–12.

YAMAGUCHI, M., YAMOS, T., YAMAGUCHI, T. & LAJTHA, A. (1970). Amino acid uptake in the peripheral nerve of the rat. *J. Neurobiol.* **1**, 419–33.

YAMAGUCHI, T., YAMAGUCHI, M. & LAJTHA, A. (1972). Selective inhibition by heat of amino acid transport in slices of mouse brain. *Biochim. biophys. Acta* **266**, 422–35.

YAMASAKI, T. & NARAHASHI, T. (1957). Studies on the mechanism of action of insecticides (XIV). Intracellular microelectrode recordings of resting and action potentials from the insect axon and the effects of DDT on the action potential. *Botyu-kagaku* **22**, 305–13.

YAMASAKI, T. & NARAHASHI, T. (1959). The effects of potassium and sodium ions on the resting and action potentials of the cockroach giant axon. *J. Insect Physiol.* **3**, 146–58.