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THE UPTAKE OF L-GLUTAMATE BY THE PERIPHERAL NERVES OF THE CRAB, *CARCINUS MAENAS* (L)

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SUMMARY

A concentrative uptake mechanism with the following characteristics has been identified in the peripheral nerves of the crab *Carcinus maenas* for L-glutamate.

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

2. The Na^+-sensitive component showed the typical saturation kinetics of a carrier-mediated process. It had a V of $65.4 \cdot 10^{-6}$ μM/μl intracellular water per min and a K_m of 0.28 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 of chain length equal to or shorter than glutamate. The uptake was reduced in the presence of D-glucose (0.25 mM) and was temperature sensitive.

3. The Na^+-insensitive component was linearly related to the glutamate concentration of the medium, but at the crab blood plasma concentration of glutamate (0.01–0.05 mM) it represented less than 5% of the total uptake. The energy source for this component together with its significance to the crab are not clear.

4. The results are discussed in relation to active transport systems for amino acids in other tissues.

INTRODUCTION

A comparison of the free amino acid concentration in the blood plasma[1] with that of the peripheral nerves[1,2] of the shore crab, *Carcinus maenas*, reveals the true extent of their extreme concentration gradient across the neuronal membranes. For example, the tissue: plasma ratios for some of the amino acids were as follows: taurine, 390:1; aspartate, 18176:1; and glutamate, 933:1. These extreme gradients must be maintained either by very high metabolic rates of production, such that more amino acid is synthesized than leaks out, or by specific concentrative uptake mechanisms.

The latter possibility has been studied for glutamate in the present investigation. This amino acid was chosen because of its importance in the invertebrate nervous system. Besides its central metabolic role it helps to balance the extremely high intracellular K^+ concentration[3], and evidence is accumulating for its role as an excitatory synaptic transmitter at the invertebrate neuromuscular junction[4–8]. The accumulation of other amino acids by this tissue will be dealt with in later papers.

The concentrative uptake of amino acids by energy-dependent mechanisms in nervous tissue has been widely studied in vertebrate brain slices[9–14] and particulate fractions[9,10,15,16]. The few studies which have been performed on invertebrate nervous systems suggest interesting similarities and parallels between the various mechanisms involved. The presence of a selective uptake mechanism for glutamate was first noted in an invertebrate nerve preparation by Iversen and Kravitz[17], in their study of γ-aminobutyric acid in a lobster nerve–muscle preparation. They noted that the uptake was distinct from that of γ-aminobutyric acid and that it was Na^+ dependent. More recently, Baker and Potashner[18], have investigated the relation of glutamate uptake by the peripheral nerves of the spider crab, *Maia squinado*, to external Na^+ and K^+ concentrations. There are interesting parallels and differences between their findings and those of the present study on the peripheral nerves of the common shore crab *Carcinus maenas*. The influx of glumate into vertebrate peripheral nerve has also been investigated in the frog by Wheeler and Boyarsky[19,20] and in the rat by Yamaguchi *et al.*[21]. These studies again make an interesting comparison with the present investigation.

MATERIALS AND METHODS

Measurement of total uptake activity

Ligatured peripheral nerves, dissected from the walking legs of *Carcinus*, were incubated for various times in saline containing [U-^{14}C]glutamate (260 Ci/mole, Radiochemical Centre, Amersham) at a concentration of 0.01 mM and 2 μCi/ml. They were then washed for two 10-min periods in ice-cold saline to remove the extracellular radioactivity[18]. Controls showed that this procedure effectively removed all the radioactivity taken up by the nerve bundle in a brief 3-s exposure to the above labelled solution. The labelled glutamate was lost from the extracellular compartment with a half-time of 2.81 ± 0.19 min ($n = 12$) and the intracellular compartment still accounted for over 95% of its value at zero time after such a washing procedure.

They were then blotted and weighed prior to their solubilization 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 on a Packard Tri-Carb liquid scintillation spectrometer. Correction for quenching was made by reference to the external standard.

The influx 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 influx[11,18]. At this incubation time up to 90% of the radioactivity extractable in 60% aqueous ethanol, could be shown to be still present in the glutamate pool. The amino acids were extracted as described by Evans[2] and estimated on a Technicon automatic amino acid analyser. The influx was expressed in μM/μl intracellular water per min.

Estimation of extracellular and intracellular spaces

The extracellular space was estimated in this tissue by two methods. In the first the efflux of radioactivity into saline was plotted for a nerve which had been incubated in [U-^{14}C]sucrose (463 Ci/mole Radiochemical Centre, Amersham, 1 μCi/ml) for 15 or 30 min. The curves were subjected to a compartmental analysis

and the results were expressed as a percentage of the total water space of the tissue. The second method involved the exposure of the nerves to [^{14}C]inulin (13.7 Ci/mole, Radiochemical Centre, Amersham) for 20 min, the radioactivity taken up by the tissue in this time being estimated as described above. This method was used especially for the study of extracellular spaces of peripheral nerves in Na^+-free media containing various concentrations of glutamate up to 10 mM, since it has been reported that vertebrate nervous tissue[22] under these conditions, exhibits changes in the inulin space.

Rate of influx under various experimental conditions

The concentration of glutamate in the medium was varied and a dose–uptake curve constructed. A Lineweaver–Burk plot was used to obtain K_m and V estimates for this system. The effect of variation in the Na^+ levels of the medium has been studied, isotonicity being maintained by choline$^+$. The influence of two drugs known to influence ionic distributions have also been investigated *i.e.* ouabain (10^{-3} M) and amiloride (10^{-4} M).

The specificity of the glutamate uptake process has been examined in a series of competition experiments with various analogs. The effect of D-glucose, at its concentration in *Carcinus* blood (0.25 mM)[23] was also examined, as was a series of metabolic inhibitors. The temperature sensitivity of the uptake process was also investigated.

Composition of salines

The composition of the saline used in this study was as follows: Na^+, 494 mM; K^+, 11.3 mM; Ca^{2+}, 12.6 mM; Mg^{2+}, 18.3 mM; SO_4^{2-}, 18.2 mM; Cl^-, 532 mM and HCO_3^-, 2.5 mM. The pH of this saline was 7.1. The isotonicity was maintained in Na^+-free and reduced Na^+ solutions by substituting choline for Na^+.

RESULTS

Extracellular space measurements

The total nerve water accounted for $88.1 \pm 0.4\%$ ($\pm$S.E.) of the wet weight of the peripheral nerve bundle. An analysis of the efflux curves for labelled sucrose gave two exponentials, a fast and a slow one. The mean values of the compartments expressed as percentages of the total water space were 30.0% and 8.4% for a 15-min exposure and 35.0% and 15.0% for a 30-min exposure. The fast phase is taken to represent the extracellular compartment and the slow phase, which increases with exposure time, the intracellular compartment. This is a simplified interpretation of the data (see Solomon[24]) but since the rate of the fast phase was 500 times that of the slow it provides a very good approximation in this case. This analysis was used since the nerves accumulated sucrose intracellularly with time, as shown by the differences in the values of this component after 15 and 30 min exposures. The value for the extracellular space measured using inulin was $28.9 \pm 2.7\%$ ($n = 10$). Thus in all calculations an extracellular space of 30.0% has been assumed. This value is of the same order of 24.0–27.0% found by Lewis[3], and also agrees with Baker's[25] value of 30.0% for this space in the peripheral nerves of *Maia squinado*[25].

Uptake kinetics

The distribution ratio of radioactivity between the intracellular water space and the bathing medium was greater than unity within 10 min in nerves incubated in saline containing [^{14}C]glutamate (0.01 mM and 2 μCi/ml). Since the examination of efflux rates of glutamate from this tissue into a medium containing a high concentration of unlabelled glutamate (20 mM) showed no significant increase over control effluxes into saline, it is suggested that any exchange diffusion component present, would be so small that it could be ignored (Evans, P. D., unpublished). Thus the above distribution ratio may be indicative of a concentrative uptake mechanism for glutamate.

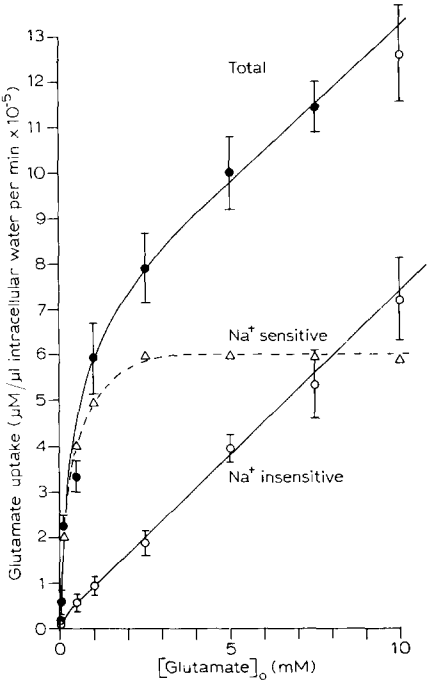

Fig. 1. The rate of uptake of glutamate is plotted against the concentration of glutamate in the bathing medium. The total uptake represents the uptake from normal saline (494 mM Na^+) and the Na^+-insensitive uptake represents the influx from a Na^+-free (choline) medium. The linear portions of the 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 $n=10$.

Fig. 1 shows that the influx of L-glutamate can be divided into two components, since in the absence of Na^+ from the bathing medium there is still an appreciable influx. This latter component, Na^+ insensitive, was linearly related to the glutamate concentration of the medium between 0.01 and 10 mM. The second component, the Na^+-sensitive one, was obtained by deducting the Na^+-insensitive fraction from the total uptake measured in normal saline (494 mM Na^+) at the various experimental points. The Na^+-sensitive uptake showed the typical saturation kinetics of a carrier-mediated process. It is to be noted that the range of *Carcinus* blood plasma concentrations found for glutamate, by Evans[1] *i.e.* 0.01 – 0.05 mM, lay on

the steepest portion of the curve. A Lineweaver–Burk analysis of the Na^+-sensitive data gave a V of $65.4 \cdot 10^{-6}$ μM/μl intracellular water per min and a K_m of 0.28 mM.

Na^+ dependence of glutamate uptake

The effect of reduction in the external Na^+ level upon the Na^+-sensitive influx of L-glutamate is shown in Fig. 2. Plots of $1/V$ against $1/[Na^+]_0$, where V is the initial rate of influx and $[Na^+]_0$ the external Na^+ concentration 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.

This can be interpreted as indicating that the Na^+-sensitive uptake mechanism is activated by a single sodium atom. The addition of 10^{-4} M amiloride to the medium (which is thought to prevent passive Na^+ movements across membranes) did not reduce the rate of glutamate influx, whilst the addition of 10^{-3} M only reduced the rate of influx significantly after a period of preincubation. This latter finding is paralleled in frog sciatic nerve[20].

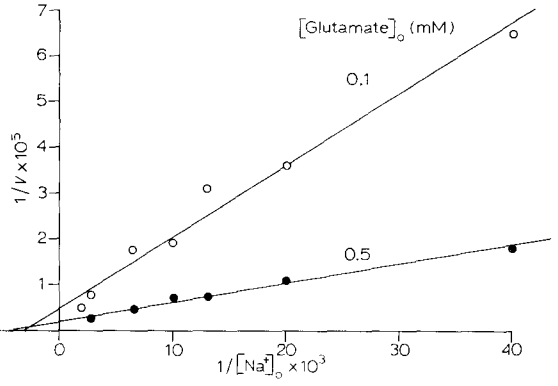

Fig. 2. The variation in the rate of the Na^+-sensitive component of the glutamate influx, V, with the variation in the Na^+ concentration of the bathing medium, $[Na^+]_0$, at two concentrations of glutamate, 0.1 and 0.5 mM. Isotonicity was maintained using choline. The results are shown as a reciprocal plot. The best-fit straight lines were obtained by a linear regression analysis of the data.

Specificity of uptake system

The most potent competitors of glutamate influx were other dicarboxylic amino acids such as L-cysteic acid, L-aspartic acid, DL-hydroxyaspartic acid, D-glutamic acid and L-hydroxyglutamic acid (Table I). The presence of another dicarboxylic amino acid, L-2-amino-adipic acid, which has an extra carbon in the chain compared to glutamic acid caused only a small reduction in the glutamate influx. Closely related analogues of L-glutamate such as glutamine and γ-aminobutyric acid were not effective competitors, whilst arginine caused a significant stimulation in the influx. The Na^+-insensitive component was more difficult to inhibit even by the use of other dicarboxylic amino acids mentioned above.

The effect of inhibitors

It can be seen from Table II that the influx from normal saline containing 494 mM Na^+, was not significantly impaired by the presence of most of the inhibitors

TABLE I

THE EFFECT OF OTHER AMINO ACIDS ON INFLUX

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 0.1 mM and 1 μCi/ml in the normal Ringer, and 2.0 mM and 5 μCi/ml in the Na^+-free (choline) Ringer. 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.

Competitors	*% Influx ± S.E.*	
	Normal Ringer	*Na^+-free (choline) Ringer*
L-Cysteic acid	21.7 ± 2.0	88.2 ± 6.8
L-Aspartic acid	26.2 ± 5.5	—
DL-*threo*-β-Hydroxyaspartic acid	46.5 ± 6.5	73.1 ± 12.8
D-Glutamic acid	58.4 ± 4.9	—
Glycine	62.2 ± 5.2	—
L-*allo*-γ-Hydroxyglutamic acid	63.4 ± 5.8	105.0 ± 8.6
L-Leucine	65.5 ± 11.0	—
D-Leucine	67.7 ± 11.0	—
L-2-Aminoadipic acid	73.6 ± 10.0	—
DL-Leucine	80.2 ± 18.0	117.5 ± 6.3
L-Phenylalanine	97.6 ± 22.9	97.2 ± 2.8
Taurine	98.8 ± 9.9	—
L-Glutamine	104.0 ± 6.5	—
γ-Aminobutyric acid	107.3 ± 5.6	—
L-Arginine	161.8 ± 18.2	98.0 ± 12.0

at the concentrations used, either singly of in various combinations. However, the use of a 20-min preincubation in a non-radioactive medium in the presence of the inhibitors, prior to the radioactive incubation did reveal apparent reductions in the levels of radioactivity accumulated in certain cases. The preincubation seemed to have little effect on the presence of azide and the system was insensitive to 2 mM cyanide.

It was possible that the Na^+-insensitive component could be energised by a direct coupling to energy rich molecules such as ATP, as has been suggested for this component in other tissues[26,27]. This hypothesis was tested by incubating nerves in labelled Na^+-free saline *plus* a number of metabolic inhibitors. The results are also shown in Table II. It can be seen that a combination of certain inhibitors did significantly reduce the influx, but that a considerable percentage of the Na^+-insensitive component still remained after the 20-min radioactive incubation and a 20-min preincubation in non-radioactive saline *plus* inhibitors.

D-Glucose (0.25 mM) also appeared to cause a significant inhibition of glutamate influx after a 20-min preincubation.

Effect of temperature

The effect of temperature on both components of the uptake system is shown in Fig. 3. It can be seen that as expected reducing the temperature from 25 °C to

TABLE II

EFFECT OF INHIBITORS ON GLUTAMATE INFLUX

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 a 20-min period and were preceeded where indicated by 20-min preincubations in non-radioactive salines in the presence of the various inhibitors. The concentrations of the inhibitors used were cyanide, 2 mM; iodoacetate, 1 mM; 2,4-dinitrophenol, 0.2 mM; azide, 1 mM; and fluoride, 1 mM. The compositions of the solutions used were as described in Table I. Each result is the mean of six determinations and is expressed ±S.E.

	% Influx of glutamate		
	Na^+-free (choline) Ringer (after pre-incubation)	*Normal Ringer*	*Normal Ringer (after pre-incubation)*
+CN^-	92.3 ± 6.0	109.6 ± 5.6	111.5 ± 20.6
+Iodoacetic acid	—	83.9 ± 22.9	78.6 ± 7.7
+2,4-Dinitrophenol	—	99.8 ± 12.3	68.0 ± 2.9
+Azide	—	71.5 ± 12.1	75.5 ± 15.8
+Fluoride	—	109.7 ± 14.7	97.0 ± 6.8
+(CN^- + iodoacetic acid)	67.7 ± 4.7	114.4 ± 12.7	—
+(CN^- + 2,4-dinitrophenol)	76.3 ± 7.6	143.9 ± 33.9	—
+D-Glucose (0.25 mM)	—	—	35.1 ± 10.7

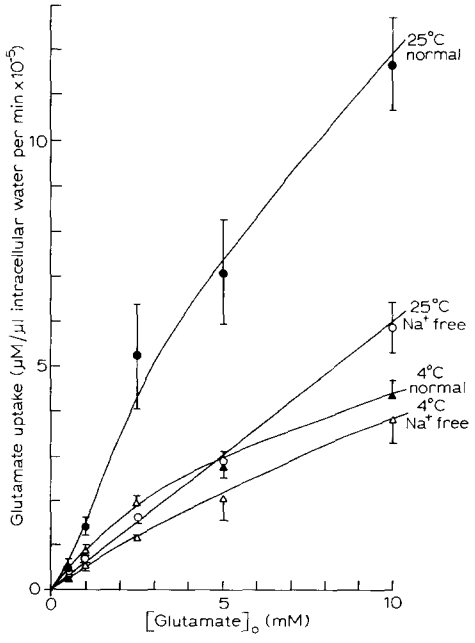

Fig. 3. The effect of temperature on the relation of the uptake of glutamate to its concentration in the bathing medium. Normal represents the uptake from normal saline (494 mM Na^+) and Na^+-free the uptake from Na^+-free saline, isotonicity being maintained by choline. The bars represent 2 S.E. and $n=6$.

4 °C reduced the uptake levels in normal saline to a level just below that of the Na^+-insensitive component at 25 °C. This is consistent with the idea of the Na^+-sensitive component being a carrier-mediated energy-requiring process.

It is also to be noted that reducing the temperature of the Na^+-free medium only reduced the Na^+-insensitive uptake by 40%. This suggests that only a part of the Na^+-insensitive system is an energy-dependent carrier-mediated process. The bulk of the component, however, seemed to be temperature insensitive.

DISCUSSION

The present study has shown that, as for amino acid accumulation processes in many other tissues[15,18,28–32], the influx of L-glutamate into the peripheral nerves of *Carcinus* can be divided into Na^+-sensitive and Na^+-insensitive components.

Na^+-sensitive glutamate influx

The V measured for this component was $65.4 \cdot 10^{-6}$ μM/μl intracellular water per min and the K_m was 0.28 mM. This compares with a K_m of 0.035 mM found for L-glutamate in frog peripheral nerve[19]. It has been suggested for mammalian tissues that the rate of amino acid transport may reflect its concentration in the tissue[21,33]. Thus it is interesting to note that the maximum uptake rate for L-glutamate is 16 times in *Carcinus* compared to the frog peripheral nerve[19] whilst the glutamate concentrations are 56.2 mM and 0.84 mM, respectively.

The dependence of the saturable component of the glutamate influx upon the external Na^+ concentration, $[Na^+]_0$, in the present study is in agreement with the findings for glutamate uptake in other tissues[18,20,21,34]. However, the present study suggests that the kinetic dependence on Na^+ is proportional to the first power of $[Na^+]_0$ in *Carcinus* peripheral nerve, whereas Baker and Potashner[18] found that their glutamate uptake into *Maia squinado* peripheral nerve was proportional to $[Na^+]^2_0$. It can be seen from Table III that glycine uptake into pigeon red cells is the only other system besides that of Baker and Potashner to show a second order dependence. Also that apart from this glycine system there does not appear to be any proven relationship between kinetic dependence and observed coupling ratios. Thus to what extent the kinetic order dependence of $[Na^+]_0$ and the coupling coefficient describe the composition of the Na^+–amino acid transport site complex is not clear and must await further investigation.

The results of the present specificity study for the L-glutamate carrier are in close agreement to those found for the analogous systems in frog peripheral nerve[19] and in *Maia* peripheral nerve[18]. In all cases the most significant competitors were other dicarboxylic amino acids of the same or shorter chain length. The following conclusions can be drawn concerning the binding of glutamate to the carrier site. (1) The terminal carboxyl group is required since glutamine, in which an amide group is substituted for the terminal carboxyl group does not compete well. (2) The carboxyl group adjacent to the amino group is essential since γ-aminobutyric acid in which a hydrogen is substituted for the α-carboxyl group, does not compete well with L-glutamic acid. (3) The presence of the α-amino group is necessary since pentanediotic acid and α-ketoglutarate were not effectively taken up[19]. (4) Spatial arrangements are important since the D-form of glutamate is not as effectively taken

TABLE III

ORDER OF Na^+ DEPENDENCE OF AMINO ACID UPTAKE PROCESSES

Amino acid transport system	*Kinetic dependence on* $[Na^+]_o$		*Coupling coefficient*
	1st order	*2nd order*	
L-Glutamate into *Carcinus* peripheral nerve (present study)	Yes	—	—
L-Aspartate into *Carcinus* peripheral nerve (Evans, P. D., unpublished)	Yes	—	—
L-Glutamate and L-aspartate into rat brain (high affinity system)[38]	Yes	—	—
L-Glutamate and L-aspartate across brush border of rabbit ileum[34]	Yes	—	1.1 ± 0.4
L-Glutamate into *Maia squinado* peripheral nerve[18]	—	Yes	—
Glycine influx into pigeon red blood cells[28, 35]	—	Yes	1.8[28]
	—	—	1.53 ± 0.45[39]
Alanine influx into pigeon red blood cells[36, 37]	Yes	—	2.5
β-Alanine influx into pigeon red blood cells	—	(Higher than first order)	~ 1.0
Proline influx into pigeon red blood cells	Yes	—	< 0.5
Asparagine influx into pigeon red blood cells	Yes	—	1.7
Hydroxyproline influx into pigeon red blood cells	Yes	—	3.0
Serine influx into pigeon red blood cells	Yes	—	4.0
Threonine influx into pigeon red blood cells	Yes	—	4.5

up as the L-form. (5) The length of the molecule is important since the smaller chained cysteic and aspartic acids act as very effective competitors whereas the longer chained L-2-aminoadipic acid does not.

The above information is suggestive of a three point attachment of the substrate to the glutamate carrier molecule and would seem to suggest that acidic amino acids may all be handled by the same transport system in *Carcinus* peripheral nerve. A more detailed kinetic study of the interaction of L-glutamate and L-aspartate transport processes in this tissue will be reported in a separate communication.

The inhibition of the glutamate influx in the presence of D-glucose has also been found recently for frog peripheral nerve[20]. A similar effect has also been reported in the dog intestine[40]. The interaction of Na^+-dependent membrane processes is discussed by Schultz and Curran[36]. In contrast to the above findings, however, it has been noted that the presence of glucose appears to have a stimulatory effect on glutamate uptake in the central nervous tissues of vertebrates[21, 14]. At present the mechanism of the glucose effect is not clear.

The dependence of this saturable component of glutamate influx upon external Na^+ could be due to the Na^+ gradient providing the energy source for this fraction and/or to some activating role of Na^+ on an enzyme process concerned with the uptake mechanism. The apparent sensitivity of this component of glutamate influx to the presence of certain metabolic inhibitors, after a period of preincubation, is suggestive of a role for energy-dependent processes in this system. This is in agreement with the findings on frog peripheral nerve[20], as was the temperature sensitivity of

this component. In the latter case it was suggested that this implied the utilisation of metabolic energy by the uptake mechanism. This again suggests that some part of the Na^+-sensitive uptake mechanism for glutamate in *Carcinus* peripheral nerve could also be directly linked to metabolic energy. It seems unlikely that this effect could be due to inhibition of the Na^+ pump maintaining the Na^+ gradient across the cell membranes as direct inhibition of this system with ouabain only showed effects after a prolonged incubation period was used, giving time for the Na^+ gradient to run down. It is possible, however, that at 4 °C the permeability of the nerve and/or glial membranes was altered so that the ion gradient was abolished in a much shorter time.

Thus on the basis of the concentrative uptake process against a concentration gradient, which shows saturation kinetics and which is specific for L-glutamate and is Na^+-dependent, it is suggested that the peripheral nerves of *Carcinus* possess 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 *plus* metabolic processes.

Na^+-insensitive uptake component

This non-saturable component of the glutamate uptake was found to be linearly related to the glutamate concentration of the bathing medium. This is in agreement with the findings of Baker and Potashner[18]. The presence of metabolic inhibitors such as cyanide, iodoacetate and dinitrophenol at best only inhibited 30% of the glutamate influx by this mechanism. This suggests that at least a part of this process could be directly linked to metabolic energy, in a similar fashion to Na^+-independent amino acid fluxes in other tissues[26,27]. In the present study ATP levels were not actually measured so that there remains the possibility that the inhibitors used, at the concentrations specified, did not reduce the ATP levels effectively, and that there was still enough present to energise the remaining fraction of the Na^+-insensitive influx of glutamate.

Reducing the temperature of the incubation from 25 °C to 4 °C only reduced this component by 40%. This is a somewhat greater reduction than would be expected for a free diffusion of glutamate into the intracellular compartment. The specificity of this influx has again been investigated using competition experiments and although in both the present study and that of Baker and Potashner[18] this component was more difficult to inhibit than the Na^+-sensitive component, even by the most potent inhibitors of the latter system, it can be seen that some compounds proved to be better inhibitors than others. This again suggests that this component is not wholly a free diffusion of glutamate but that at least some of it could be a carrier-mediated system with its own separate receptor sites to those of the Na^+-sensitive system.

It has been suggested in other tissues (*e.g.* rat brain[14,41]) that this Na^+-insensitive component could be an artifact due to the swelling induced in the tissues by the added concentration of glutamate. The swelling results in an increase in non-inulin space which appears to be proportional to the glutamate concentration[22]. An increase in the glutamate concentration up to 10 mM in Na^+-free media did not produce any significant change in the intracellular (or non-inulin) space of peripheral nerve from *Carcinus* (see Fig. 4). Thus it seems unlikely that this component could be attributed to the above artifact in this tissue. The energising source for this component of glutamate uptake in *Carcinus* peripheral nerve is thus not clear.

Further investigation is required to establish what, if any, importance it has to the functioning of crab peripheral nerve.

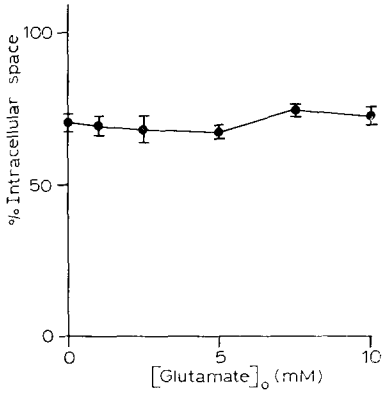

Fig. 4. Plot of intracellular space of nerve against glutamate concentration in a Na^+-free medium, where isotonicity was maintained using choline. The intracellular spaces were measured by deducting the calculated hydroxymethyl-[^{14}C]inulin spaces from the total water space of the tissue. The bars represent 2 S.E. and $n=10$.

Such uptake processes as described above would be of importance to a transmitter molecule such as glutamate which needs to be kept at low levels in synaptic regions. It could also provide an alternative replenishment mechanism to that of synthesis from the metabolic pool for the maintenance of the high levels of glutamate found in the peripheral nerves of the crab. However, due to the cellular heterogeneity of this tissue it has not been established whether all cell types, neuronal and glial, take up the amino acid or whether it is a property of restricted to one or other of these compartments. A further communication from this laboratory will deal with an autoradiographical study, at the light and electron microscope levels, carried out to elucidate this problem (Evans, P. D., unpublished).

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REFERENCES

1 Evans, P. D. (1972) *J. Exp. Biol.* 56, 501–507
2 Evans, P. D. (1973) *J. Neurochem.*, in the press
3 Lewis, P. R. (1952) *Biochem. J.* 52, 330–338
4 Kravitz, E. A., Slater, C. R., Takahashi, K., Bownds, M. D. and Grossfeld, R. M. (1970) in *Excitatory Synaptic Mechanisms* (Andersen, P. and Jansen, J. K. S., eds), pp. 85–93, Universitetsforlaget, Oslo
5 Takeuchi, A. and Takeuchi, N. (1964) *J. Physiol. London* 170, 296–317

6 Usherwood, P. N. R. and Machili, P. (1968) *J. Exp. Biol.* 49, 341–361
7 Usherwood, P. N. R., Machili, P. and Leaf, G. (1968) *Nature* 219, 1169–1172
8 Pitman, R. M. (1971) *Comp. Gen. Pharmacol.* 2, 347–371
9 Iversen, L. L. and Johnston, G. A. R. (1971) *J. Neurochem.* 18, 1939–1950
10 Johnston, G. A. R. and Iversen, L. L. (1971) *J. Neurochem.* 18, 1951–1961
11 Smith, S. E. (1967) *J. Neurochem.* 14, 291–300
12 Margolis, R. K. and Lajtha, A. (1968) *Biochim. Biophys. Acta* 163, 374–385
13 Neame, K. D. (1962) *J. Physiol. London* 162, 1–12
14 Yamaguchi, T., Yamaguchi, M. and Lajtha, A. (1972) *Biochim. Biophys. Acta* 266, 422–435
15 Peterson, N. A. and Raghupathy, E. (1972) *J. Neurochem.* 19, 1423–1438
16 Navon, S. and Lajtha, A. (1969) *Biochim. Biophys. Acta* 173, 518–531
17 Iversen, L. L. and Kravitz, E. A. (1968) *J. Neurochem.* 15, 609–620
18 Baker, P. F. and Potashner, S. J. (1971) *Biochim. Biophys. Acta* 249, 616–622
19 Wheeler, D. D. and Boyarsky, L. L. (1968) *J. Neurochem.* 15, 1019–1031
20 Wheeler, D. D. and Boyarsky, L. L. (1971) *J. Neurobiol.* 2, 181–190
21 Yamaguchi, M., Yanos, T., Yamaguchi, T. and Lajtha, A. (1970) *J. Neurobiol.* 1, 419–433
22 Van Harreveld, A. and Fifikova, E. (1971) *Exp. Mol. Pathol.* 15, 61–81
23 Binns, R. (1969) *J. Exp. Biol.* 51, 29–39
24 Solomon, A. K. (1960) in *Mineral Metabolism* (Comar, C. L. and Broner, F., eds), Vol. 1A, Chapter V, Academic Press, London
25 Baker, P. F. (1965) *J. Physiol. London* 180, 439–447
26 Lin, K. T. and Johnstone, R. M. (1971) *Biochim. Biophys. Acta* 244, 144–158
27 Potashner, S. J. and Johnstone, R. M. (1971) *Biochim. Biophys. Acta* 233, 91–103
28 Vidaver, G. A. (1964) *Biochemistry* 3, 795–799
29 Vidaver, G. A. (1971) *Biochim. Biophys. Acta* 233, 231–234
30 Fox, M., Their, S., Rosenberg, L. and Segal, S. (1964) *Biochim. Biophys. Acta* 79, 167–176
31 Rosenberg, L. E., Blair, A. and Segal, S. (1961) *Biochim. Biophys. Acta* 54, 479–488
32 Munck, B. G. and Schultz, S. G. (1969) *J. Gen. Physiol.* 53, 157–182
33 Lajtha A. (1968) in *Brain Barrier System, Progress in Brain Research* (Lajtha, A. and Ford, D., eds), Vol. 29, pp. 201–216, Elsevier, Amsterdam
34 Schultz, S. G., Yu-Tu, L., Alvarez, O. O. and Curran, P. F. (1970) *J. Gen. Physiol.* 56, 621–639
35 Wheeler, K. B., Inui, Y., Hollenberg, P. F., Eavenson, E. and Christensen, H. N. (1965) *Biochim. Biophys. Acta* 109, 620–622
36 Schultz, S. G. and Curran, P. F. (1970) *Physiol. Rev.* 50, 637–718
37 Koser, B. and Christensen, H. N. (1968) *Fed. Proc.* 27, 643
38 Balear, V. J. and Johnston, G. A. R. (1972) *J. Neurochem.* 19, 2657–2666
39 Wheeler, K. P. and Christensen, H. N. (1967) *J. Biol. Chem.* 242, 3782–3788
40 Annegers, J. H. (1964) *Proc. Soc. Exp. Biol. Med.* 116, 9333–938
41 Neame, K. D. and Smith, S. E. (1965) *J. Neurochem.* 12, 87–91