

Uptake and compartmentation of glutamic acid in sensory ganglia

P. KEEN & P.J. ROBERTS*

Departments of Pharmacology, University of Bristol, Bristol BS8 1TD & The Middlesex Hospital Medical School, London W1P 7PN

Although isolated nerve-ending fractions will accumulate glutamate by a high-affinity transport process (Logan & Snyder, 1972), the major uptake site for terminating the action of synaptically released amino acid is likely to be the glial cell (Van den Berg & Garfinkel, 1971). Following uptake into rat dorsal root ganglia, ^3H -glutamate becomes localized exclusively in the satellite glial cells (Schon & Kelly, 1974).

In an attempt to delineate biochemically the glutamate uptake-compartment, we have examined the uptake of ^{14}C -glutamate into fresh dorsal root ganglia, and into ganglia cultured under conditions which favour glial proliferation; similarly, a comparative study has been made of the metabolism of ^{14}C -pyruvate and ^{14}C -acetate which serve different (although as yet unidentified) compartments (Van den Berg, 1973).

Rat dorsal root ganglia were isolated, desheathed and either incubated immediately with L- ^{14}C -glutamate, or following organ culture (Trowell, 1959) for two days in modified medium 199.

The uptake was mediated by two distinct systems: a low-affinity component (apparent K_m of $1.1 \times 10^{-3}\text{ M}$) and a high-affinity component (apparent K_m of $2.1 \times 10^{-5}\text{ M}$), the latter being strongly sodium- and energy-dependent, similar to other high-affinity transport processes. Uptake at a glutamate concentration of $3.5 \times 10^{-6}\text{ M}$ was approximately linear over a 40 min period and, after incubation for 30 min, a tissue: medium ratio of 9:1 was attained. Following culture, a

2.21 times increase in the rate of glutamate uptake was observed.

Analysis of the fate of ^{14}C label derived from glutamate, indicated that in fresh ganglia over 70% of the accumulated radioactivity was located in glutamine and that the remainder was largely in glutamate itself. In cultured ganglia, glutamine synthesis was apparently suppressed as evidenced by the decreased specific radioactivity of glutamine in the tissue (relative to glutamate) from 3.2 to 1.25. When ganglia were incubated with U- ^{14}C -acetate ($8.0 \times 10^{-5}\text{ M}$), the pattern of labelling found was essentially similar to that for glutamate, whereas with U- ^{14}C -pyruvate ($4.5 \times 10^{-4}\text{ M}$), the ratio of incorporated radioactivity in fresh ganglia was approximately 1.0. The net effect observed for cultured ganglia however, was an overall decreased labelling of each amino acid derived from pyruvate.

These results will be discussed in relation to current knowledge of the compartmentation of glutamate metabolism in nervous tissue.

References

- LOGAN, W.J. & SNYDER, S.H. (1972). High affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous system. *Brain Research*, **42**, 413-431.
- SCHON, F. & KELLY, J.S. (1974). Autoradiographic localization of ^3H -GABA and ^3H -glutamate uptake over satellite glial cells. *Brain Research*, **66**, 275-28.
- TROWELL, O.A. (1959). The culture of mature organs in a synthetic medium. *Expl. Cell Res.*, **16**, 118-127.
- VAN DEN BERG, C.J. (1973). A model of compartmentation in mouse brain, based on glucose and acetate metabolism. In: *Metabolic Compartmentation in the Brain* (R. Balázs & J.E. Cremer, Eds.), Macmillan, London, pp 137-165.
- VAN DEN BERG, C.J. & GARFINKEL, D. (1971). A simulation study of brain compartments—metabolism of glutamate and related substances in mouse brain. *Biochem. J.*, **123**, 211-218.

The excitatory effects of aspartate and glutamate on the crustacean neuromuscular junction

G.A. KERKUT & H.V. WHEAL*

Department of Physiology and Biochemistry, University of Southampton

There is good evidence that the excitatory transmitter at the crustacean neuromuscular junction is

the amino acid L-glutamate (Takeuchi & Takeuchi, 1963; Kerkut, Leake, Shapiro, Cowan & Walker, 1965; Florey, 1967; Kravitz, Slater, Takahashi, Bownds & Grossfield, 1970).

The neuromuscular junction of the dactyl opener muscle of the walking leg of the hermit crab, *Eupagurus bernhardus*, is depolarized by iontophoretic or bath application of L-glutamate. The preparation is from two to ten times more sensitive to L-glutamate than to L-aspartate. Bath application of L-glutamate or L-aspartate in