Uptake and compartmentation of glutamic acid in sensory ganglia

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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, ^{3}H-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-U-^{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 1.1×10^{-3} M) and a high-affinity component (apparent K_m of 2.1×10^{-5} M), the latter being strongly sodium- and energy-dependent, similar to other high-affinity transport processes. Uptake at a glutamate concentration of 3.5×10^{-6} 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 (8.0×10^{-5} M), the pattern of labelling found was essentially similar to that for glutamate, whereas with U-^{14}C-pyruvate (4.5×10^{-4} 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 ^{3}H-GABA and ^{3}H-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

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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

suitable doses desensitizes or competitively reduces the size of evoked excitatory junction potentials (EJP).

The walking legs of *Eupagurus bernhardus* were removed at the autotomizing joint. The leg, with opener muscle and nerve bundles exposed, was placed in crab Ringer at room temperature, 18° C. The Ringer composition (mM) used was: NaCl, 490; KCl, 12.2; $CaCl_2$, 14.8; $MgCl_2$, 5.75; $NaHCO_3$, 1.79. The pH was 7.3. A constant volume (0.1 ml) of drug was added to the surface of the exposed muscle fibres.

The membrane potential changes and EJP were recorded from the dactyl abductor muscle of the leg using 3 M-KCl-filled glass electrodes. The EJP were evoked by stimulation of the excitatory nerve bundle with trains of pulses from a Devices Digitimer stimulator, via a suction electrode. The membrane potential and EJP were displayed on a Tektronix 502A oscilloscope and digital voltmeter and recorded on a Watanabe WTR-2C pen-recorder.

In most cases, concentrations of 1×10^{-4} M-L-glutamate or 5×10^{-4} M-L-aspartate were sufficient to depolarize the membrane and diminish EJP amplitude. When a solution of L-glutamate of sufficient concentration to give a submaximal response was combined with an equipotent solution of L-aspartate in the ratio 1 : 2 or 1 : 3 (0.3×10^{-4} M-L-glutamate + 3×10^{-4} M-L-aspartate), the depolarization of the membrane was greater than that produced by the original L-glutamate (1×10^{-4} M) or L-aspartate (5×10^{-4} M) concentration. This synergistic effect was also reflected in a reduction in amplitude of the evoked EJP.

These observations and others (Colton & Freeman, 1973), coupled with evidence that the concentration of L-aspartate in peripheral nerve of crab is five times that of L-glutamate (Evans, 1972) suggest that L-glutamate and L-aspartate may both play a role in transmission at the excitatory neuromuscular junction in crustacea.

References

COLTON, C. & FREEMAN, A.R. (1973). Evidence for a synaptic and non-synaptic response to L. Glutamate in Lobster. *The Physiologist*, **16**, 287.

EVANS, P.D. (1972). The free amino acid pool of the haemocytes of *Carcinus Maenas. J. Exp. Biol.*, **56**, 501-507.

FLOREY, E. (1967). Neurotransmitters and modulators in the animal kingdom. *Fed. Proc.*, **26**, 1164-1178.

KERKUT, G.A., LEAKE, L.D., SHAPIRO, A., COWAN, S. & WALKER, R.J. (1965). The presence of glutamate in nerve-muscle perfusates of *Helix, Carcinus,* and *Periplaneta. Comp. Biochem. Physiol.*, **15**, 485-502.

KRAVITZ, E.A., SLATER, C.R., TAKAHASHI, K., BOWNDS, M.D. & GROSSFIELD, R.M. (1970). Excitatory transmitters in invertebrates, glutamic acid as a potential neuromuscular transmitter. In, P. Anderson & J.K.S. Jansen (Eds.) *Excitatory Synaptic Mechanisms*, Universitetsforlaget, Oslo, pp. 85-93.

TAKEUCHI, A. & TAKEUCHI, N. (1963). Glutamate induced depolarisation in crustacean muscle. *Nature (Lond.)*, **198**, 490-491.

Pharmacological studies on single neurones in the substantia nigra of the rat

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γ-Aminobutyric acid (GABA) is the only substance for which there is good evidence for a neurotransmitter role in the substantia nigra. The substantia nigra contains high levels of GABA (Fahn & Côté, 1968; Okada, Nitsch-Hassler, Kim, Bak & Hassler, 1971). The inhibition of nigral neurones by electrical stimulation of the descending striato-nigral pathway is blocked by intravenously administered picrotoxin (Precht & Yoshida, 1971) and these nigral neurones are also inhibited by GABA (Feltz, 1971). Crossman, Walker & Woodruff (1974a) demonstrated that iontophoretically applied picrotoxin blocked the inhibition of nigral neurones both by stimulation of the caudate nucleus and by iontophoretically applied GABA.

Feltz (1971) reported that nigral neurones were inhibited only by GABA and not by glycine, acetylcholine (ACh) or dopamine. Gulley & Smithberg (1971), however, described four different types of synaptic terminals onto neurones in the substantia nigra and tentatively suggested that these might release GABA, ACh, noradrenaline and 5-hydroxytryptamine (5-HT). In the present study, therefore, we have examined the effects on neurones in the substantia nigra of iontophoretically applied GABA, imidazole acetic acid, glycine, ACh, noradrenaline and 5-HT.

Experiments were performed on 21 female