Specific *in vivo* autoradiographic localization of [^{3}H]-β-alanine uptake sites in macro- as opposed to microglial cells

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Recently Schon & Kelly (1974) showed the GABA analogue [^{3}H]-β-alanine to be a substrate for the high affinity GABA uptake system of glial cells in the peripheral nervous system. In autoradiographic studies [^{3}H]-β-alanine also proved to be a selective marker for glial cells in slices of cerebral cortex (Schon & Kelly, 1975), but not for the nerve terminals associated with [^{3}H]-GABA uptake. We have, therefore, examined the possibility that [^{3}H]-β-alanine might be used *in vivo* as a specific marker for glial cells throughout the CNS. Small volumes of [^{3}H]-β-alanine (1 µl containing 0.41 nmol and 15 µCi) were microinjected under pressure through a fine glass microelectrode directly into the cerebellar and cerebral cortices and into the cuneate and facial nuclei. In order to prevent the catabolism of [^{3}H]-β-alanine by GABA: glutamate transaminase the animals were pretreated with amino-oxyacetic acid (20 mg/kg). The animals were killed within 15 min by perfusion fixation with glutaraldehyde 5%. Electron microscope autoradiographs showed high densities of silver grains evoked by [^{3}H]-β-alanine over both oligodendrocytes and astrocytes. No silver grains lay over adjacent nerve terminals, neuronal cell bodies or pericytes and endothelial cells lying within the basement membrane of blood vessels.

Following the proliferation of macroglia and microglia caused by either anterograde or retrograde lesions of afferent or efferent pathways, the 'reactive' astrocytes and oligodendrocytes were densely labelled by [^{3}H]-β-alanine. However, no silver grains were found over microglia or transitional cells lying in the vicinity of labelled macroglia. The failure of the microglial transitional cells and pericytes to accumulate [^{3}H]-β-alanine may be in keeping with the view that these cells are of mesodermal origin.

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Potentiation of γ-aminobutyric acid (GABA) action by inhibiting neuroglial uptake

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Specific transport systems for the inhibitory neurotransmitter GABA exist in nerve terminals and in neuroglial cells (Iverson & Kelly, 1975). It seems reasonable to suppose that these play some role in the clearance of extracellular GABA. However, since tracer studies have not established the appropriate degree of net uptake at low substrate concentrations, the clearance function of the carrier has been questioned (Levi & Raiteri, 1974).

Sympathetic neurones are depolarized by GABA and the surrounding neuroglial cells possess a GABA carrier (see Bowery, Brown, Collins, Galvan, Marsh & Yamini, 1976). This juxtaposition has allowed a fairly direct assessment of the clearance capacity of this carrier, by observing whether the depolarizing action of GABA is indeed affected when inward carrier-mediated transport by the glial cells is inhibited.

The experiments were performed on isolated, desheathed rat superior cervical ganglia, superfused with Krebs' solution at 25°C as described previously (Bowery *et al.*, 1976). Depolarizing responses to GABA and 3-aminopropanesulphonic acid (3-APS—a potent GABA receptor agonist with very low affinity for the carrier) were recorded. Inward transport of GABA was inhibited by reducing external [Na^+] or by adding 1 mM (±)-nipecotic acid (Krogsgaard-Larsen & Johnston, 1975) or 1 mM β-aminobutyric acid (Bowery *et al.*, 1976) as false substrates.

These procedures clearly enhanced the depolarizing responses to low ($\leqslant$10 µM) concentrations of GABA but did not materially affect the equivalent response to 3-APS. For example, the depolarization produced by 3 µM GABA was increased 2–3 times during carrier-inhibition: the rate of uptake of 3 µM ^{3}H-GABA was reduced 35–90% under these conditions. From dose-response curves in normal and low Na^+ media, it was estimated that glial transport reduced the apparent interstitial GABA concentration from 3 µM to 0.6 µM or from 10 µM to 3 µM.

Thus the glial transport system in the ganglion is capable of substantial net clearance of extracellular GABA at a concentration within the range of the

'high-affinity' transport system ($K_T \sim 7\ \mu M$). Further, Curtis, Game & Lodge (1976) have recently obtained electrophysiological evidence for potentiation of iontophoretically-applied GABA in the central nervous system by inhibiting both neuronal and glial carriers. This does not of course imply that carrier-mediated transport affects the action of synaptically-released GABA.

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Inhibition of substance P release from the isolated rat substantia nigra by GABA

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The undecapeptide substance P is widely distributed within the rat central nervous system and highest levels are found in the substantia nigra (Kanazawa & Jessell, 1976). In addition biochemical and immunohistochemical studies have indicated the presence of substance P containing fibres in striato-nigral and pallido-nigral pathways in the rat brain (Kanazawa, Emson & Cuello, 1976). Using a sensitive radioimmunoassay we have previously demonstrated the potassium-evoked and calcium-sensitive release of endogenous substance P from superfused slices of rat hypothalamus (Jessell, Iversen & Kanazawa, 1976), and in the present study we have refined this technique to investigate the release of substance P from the isolated rat substantia nigra.

Substantia nigra tissue from two rats (10–12 mg) was dissected from 0.8 mm thick coronal sections of the mesencephalon and chopped at 0.2 mm intervals in two directions. Nigral slices were superfused at 37°C with Krebs bicarbonate containing 0.5% albumin at a rate of 375 μl/min. Superfusate samples were collected at 1 min intervals and substance-P like immunoreactivity in each sample, and in the nigral tissue recovered after superfusion was determined by radioimmunoassay. After 5 min of superfusion the spontaneous efflux of substance P remained constant (8.40 ± 0.31 fmol mg^{-1} min^{-1}, mean ± s.e. mean $n=4$) and represented approximately 0.5% of tissue stores released per minute. Raising the potassium concentration in the superfusing medium to 47 mM for 2 min evoked 39.03 ± 4.04 fmol/mg (mean ± s.e. mean $n=4$) increase in substance P release. Furthermore, the potassium-evoked release of substance P from the rat substantia nigra was calcium-dependent and increased as a function of the Ca^{2+} concentration over the range of 0.1 to 3.0 mM Ca^{2+}.

In addition to the substance P pathway described, there is also strong evidence for a descending GABA mediated projection from the corpus striatum to the substantia nigra, although the synaptic connections of GABA-releasing neurones within the substantia nigra are unknown (Dray & Straughan, 1976). Superfusion of substantia nigra slices with Krebs bicarbonate containing GABA (5×10^{-5} M) inhibited the potassium-evoked release of substance P by $77.6 \pm 12.0\%$ (mean ± s.e. mean $n=8$). The inhibitory effect of GABA could be reversed by the addition to the superfusion medium of picrotoxin (5×10^{-5} M), a GABA receptor antagonist. Superfusion with Krebs bicarbonate containing picrotoxin (5×10^{-5} M) in the absence of GABA did not affect the spontaneous or potassium-evoked release of substance P. It is likely, therefore, that GABA-containing neurones exert an inhibitory effect on substance P terminals within the substantia nigra, although the mechanism of this inhibition remains to be clarified.

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