The subcellular distribution of ^{14}C-γ-aminobutyric acid GABA and ^{3}H-dopamine in the rabbit retina

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Specific uptake mechanisms for GABA and dopamine are present in the retina, and autoradiographic studies suggest that, while dopamine is accumulated by neurones (Kramer, Potts & Mangnall, 1971), GABA is taken up largely by the glial Müller cells (Iversen & Neal, 1972). The present study was undertaken to determine whether the subcellular distribution of ^{14}C-GABA and ^{3}H-dopamine could be related to the sites of uptake as suggested by autoradiography.

Albino rabbits were killed by cervical transection and both eyes were enucleated. The retinae were rapidly dissected in ice cold Krebs bicarbonate medium and then incubated at 37° C for 30 min with ^{14}C-GABA and ^{3}H-dopamine. The tissue was then homogenized in 0·32 M sucrose and a crude mitochondrial pellet (P_2) was prepared as described by Whittaker (1965). The resuspended P_2 pellet was subjected to density gradient centrifugation on either continuous (0·4–1·6 M), or discontinuous four-step sucrose gradients. After centrifugation at 100,000 *g* for 90 min, the gradients were fractionated and the fractions obtained were assayed for ^{14}C-GABA and ^{3}H-dopamine by liquid scintillation counting.

On continuous gradients ^{14}C-GABA appeared as a single peak. This peak was reduced by subjecting the P_2 pellet to hypo-osmotic shock before layering onto the gradient, suggesting that the GABA was present in a particulate fraction. The particles which accumulated ^{14}C-GABA had a median equilibrium density equivalent to 1·3 M sucrose and could be partially separated from ^{3}H-dopamine containing particles which had a median equilibraum density equivalent to 0·9–1·0 M sucrose (Fig. 1).

On discontinuous gradients, the activity of glutamate decarboxylase (GAD) was loca-

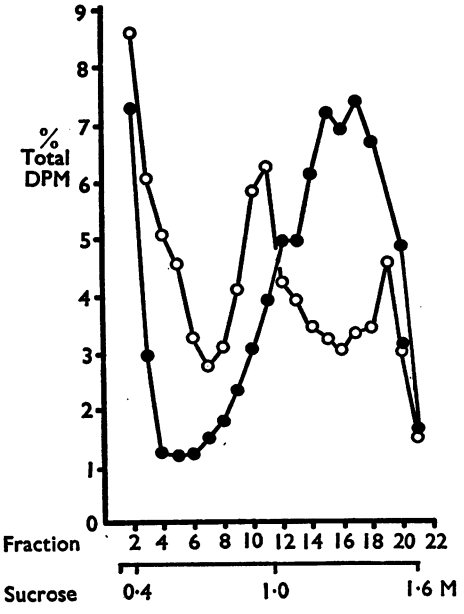

FIG. 1. Distribution of ^{14}C-GABA (●) and ^{3}H-dopamine (○) on a linear continuous sucrose gradient (0·4–1·6 M). In each case the results are expressed as a percentage of the total radioactivity recovered in all fractions from the gradient.

lized in bands between 1·0 M and 1·4 M sucrose. However, GABA-T and MAO were predominantly localized in fractions characteristic of mitochondria.

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Application of the dansyl technique to an investigation into the identity of the primary sensory transmitter in the spinal cord

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The identity of the primary sensory transmitter in the spinal cord is still unknown, although glutamate has been suggested as a candidate (Johnson, 1972). We have now developed a fully quantitative detection technique, based on the method described by Osborne, Briel & Neuhoff (1971), which has allowed us to measure, with a sensitivity in the picomole range, amino acids, amines and other possible transmitters in the form of their fluorescent ^{3}H-dansyl (1-dimethylaminonapthalene-5-sulphonyl chloride) derivatives, in rat dorsal sensory neurones.

It is widely accepted that a substance is likely to be uniquely, or disproportionately concentrated in those presynaptic cell bodies and nerve terminals where it has a transmitter function. We have therefore made a quantitative comparison of dorsal and ventral root chemical components. Of the 32 major substances detected, three amino acids were found in significantly higher concentrations in the dorsal roots: glutamate (1·27 times ventral root) and, in much lower total amounts, threonine (1·40 times ventral root) and arginine (1·27 times ventral root). The marked disparity between dorsal and ventral root levels of glutamate confirms the findings of other workers (Duggan & Johnston, 1970; Johnson & Aprison, 1970). This evidence suggests that the higher dorsal root levels of glutamate might result from synthesis in the cell body of the neurone, and subsequent anterograde transport towards the nerve terminals in a manner analogous to that found for other transmitters (Evans & Saunders, 1967; Dahlström, 1971).

We have tested this possibility in two ways: after an 8 h ligation of dorsal roots *in vivo*, analysis of 0·5 cm sections of roots indicated that a number of dansyl-reactive substances accumulated on both sides of the ligature, suggesting that accumulation was partly due to inflammatory and other non-specific changes. However, when compared with the central section there appeared to have been a selective accumulation of glutamate, alanine and glycine peripheral to the ligature. The second method employed was to inject the dorsal root ganglia with ^{14}C-glucose (2·5 μCi) in a manner similar to that described by Lasek (1968), in the expectation that label might be rapidly incorporated into the neurotransmitter, and then transported centrally along the axon.

Although there was a slow exponential passage of radioactivity into the nerve over a 6 h period, no rapid transport of radioactivity along the axon was detected, and radioactivity was not found to accumulate on the cell body side of a crush made at the point of entry of the dorsal root into the cord. However, this latter finding is not conclusive because the rapid turnover of labelled material could have prevented the detection of a rapid transport process.

These experiments did not establish the unique existence of any substance in the dorsal root, or the rapid transport of a substance along the dorsal root, but they do provide additional evidence for the role of glutamate as the sensory transmitter.

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