No evidence was found for more than one form (Hebb, Stephens & Smith, 1972). To discover whether the goldfish is indeed another animal containing only a single choline acetyltransferase, and to compare preparations from different parts of the nervous system, we undertook an analysis of goldfish brain and muscle extracts by isoelectric focusing.

Low ionic strength extracts (KH_2PO_4, 10 mM, EDTA, 1 mM, pH 7.0) of goldfish muscle contain five major choline acetyltransferase activities, with isoelectric points of 7.4, 7.8, 8.3, 8.6 and 8.9. A minor component focused at pH 6.1. In contrast, the major activity in similar extracts of goldfish brain focused at pH 6.1. Relatively minor components focused at pHs between 7 and 9, coincident with the major activities in muscle.

Pooled fractions containing the acidic activity from brain, uncontaminated by the minor forms, refocused as a double peak activity at pHs of 5.8 and 6.05. No choline acetyltransferase was redistributed to pHs between 7 and 9. The focusing pattern of brain homogenates, however, reverted essentially to that of muscle on treatment with KCl (150 mM), either before or after the removal of membrane fragments by centrifugation. The increase in activity localized at pHs between 7 and 9, therefore, was entirely at the expense of the acidic activity, and was not the result of increased enzyme solubility at high ionic strengths.

Goldfish brain and muscle, therefore, contain identical choline acetyltransferase populations. Apparent differences in the concentrations of the individual components in the two tissues are likely to result from the binding of as many as three forms of the enzyme to one or more soluble components of brain, at low ionic strengths. That different parts of the goldfish nervous system contain identical populations and concentrations of choline acetyltransferase suggests the different forms do not originate from different cellular localizations. Different subcellular localizations remain a possibility.

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Regional distribution of glutamate and γ-aminobutyric acid and their associated enzymes in the frog central nervous system

J.F. MITCHELL, P.V. TABERNER & R.A. YATES*

Department of Pharmacology, University of Bristol, Bristol BS8 1TD

A preliminary study of the concentrations of some amino acids in the frog cerebral cortex and optic tectum has been reported previously (Mitchell & Yates, 1973). In the present study the concentrations, in other regions of the frog CNS, of the same amino acids, were determined as their [^{3}H]-dansyl derivatives (Roberts, Keen & Mitchell, 1973) and information on their associated enzymes was obtained.

Glycine was found to be concentrated in the thoracic cord (3.6 ± 0.3 μmol/g wet weight (mean + s.e.)) and brain stem (3.2 ± 0.2 μmol/g) relative to other regions, whilst γ-aminobutyric acid (GABA) was concentrated in the tectum (3.0 ± 0.3 μmol/g), cortex (3.5 ± 0.3 μmol/g) and mid brain (3.7 ± 0.5 μmol/g) relative to other regions. The distribution of glycine and GABA in the frog CNS therefore supports other evidence which suggests they may have an inhibitory transmitter function in the spinal cord and cerebral cortex respectively (Curtis, Hösli, Johnston & Johnston, 1968; Krnjević & Schwartz, 1968). There is less regional variation in the concentrations of aspartate and glutamate, and the highest concentration of these amino acids was found in the mid brain (0.8 ± 0.1 μmol/g and 6.2 ± 0.7 μmol/g respectively).

The distribution of L-glutamate-1-carboxy-lyase (GAD) was determined using the method of Balazs, Dahl & Harwood (1966).

The GAD activity in the tectum (24.4 ± 3.0 (μmol/h)/g wet weight) and mid brain (20.1 ± 1.4 (μmol/h)/g) was higher than that in other regions and frog GAD was shown to have a Km of 3.6 mM which is similar to the Km reported for the mammalian enzyme (Susz, Haber & Roberts, 1966) and, like the mammalian enzyme, was inhibited by aspartate. However, the frog enzyme had a pH optimum plateau of 6.7-8.0. Comparison of this range with the mammalian pH optimum of

6.4-7.0 illustrates that amphibian and mammalian GAD are not identical.

4-Aminobutyrate-2-ketoglutorate-aminotransferase (GABA-T) activity was determined by the method of Hall & Kravitz (1967). Its activity showed less regional variation than that of GAD but again the activity in the tectum (5.24 ± 0.5 (μmol/h)/g wet weight) and mid brain (4.5 ± 0.1 (μmol/h)/g) was higher than that of other regions.

It therefore appears that, in the frog brain, GABA is produced by the action of GAD on glutamic acid and is then transaminated by a GABA-T to succinic semialdehyde which is subsequently oxidized to succinic acid. This metabolic pathway closely resembles that which, in the mammalian system, has been designated the GABA shunt. The relatively high activity of GAD and GABA-T in the tectum and mid brain suggest that GABA shunt is particularly important in these regions.

The demonstration that GABA, together with its synthesizing and inactivating enzymes is relatively concentrated in the tectum and mid brain of the frog suggests a transmitter role for GABA in these tissues.

R.A. Yates is an M.R.C. Junior Research Fellow.

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Partial separation of retinal subcellular particles accumulating labelled γ-aminobutyric acid (GABA) at high and low concentrations

C.K. ATTERWILL* & M.J. NEAL

Department of Pharmacology, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX

The rabbit retina possesses both a high and a low affinity uptake process for GABA, the apparent Km values of the two processes being 10 μM and 250 μM respectively. The possibility that different cell types might possess only the high affinity, or only the low affinity uptake mechanism, was investigated by examining the distribution of

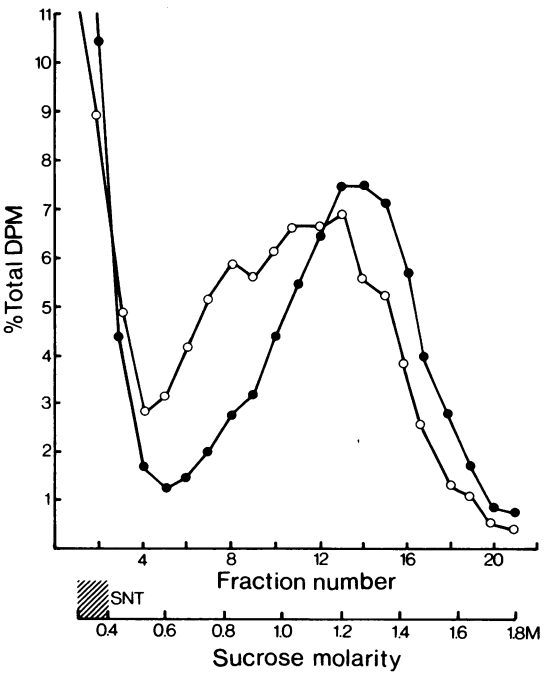

Fig. 1 Distribution of [^{14}C]-GABA (○), 5 mM, and [^{3}H]-GABA (●), 0.1 μM, on a linear continuous sucrose gradient (0.4-1.8 M). In both cases the results are expressed as a percentage of the total radioactivity (DPM) recovered in all fractions from the gradient.