The effect of morphine on the choline acetyltransferase population of rat caudate nucleus

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The acetylcholine concentration in whole brain of rats increases after a single subcutaneous injection of morphine (Giarman & Pepeu, 1962), and returns to normal in rats treated daily (Large & Milton, 1970). In contrast, the choline acetyltransferase (EC. 2. 3. 1. 6.) activity in the caudate nucleus (Thal & Wajda, 1969) decreases by approximately 25-30% after a single dose of morphine and returns to normal within four days in chronically treated rats (Datta, Thal & Wajda, 1971). An explanation for the decrease in activity was sought in feedback inhibition by acetylcholine, and in changes in choline acetyltransferase conformation induced by morphine (Datta & Wajda, 1972). However, the coexistence of three discrete choline acetyltransferases in rat cerebral whole brain and caudate nucleus has recently been demonstrated, by isoelectric focusing (Malthe-Sørenssen & Fonnum, 1972). To discover whether morphine affects the structure, or the tissue concentration of these enzymes, therefore, we undertook a comparison of the caudate nucleus choline acetyltransferase populations of normal and morphine-treated rats.

Groups of male Wistar rats (150-200 g) were injected intraperitoneally with morphine sulphate (40 mg/kg) twice daily and killed by stunning 1 h after the final dose. Groups of six rats were treated for either one day or five days. The animals treated for one day showed the behavioural changes characteristically produced by morphine, those treated for five days developed tolerance. Caudate nucleus tissue from each group and from six control rats was homogenized separately in buffer (KH_2PO_4, 50 mM, pH 7.0; 8.0 ml/g, 0°C) and particle-free supernatants (100,000 *g*, 1 h) were analysed by isoelectric focusing.

The caudate nucleus of the control rats contained choline acetyltransferases that focused at pHs of 7.5, 7.8, and 8.3, a population similar to that reported previously for rat brain tissue. The concentrations of these enzymes in caudate nucleus extracts were in the ratio of 1 : 4 : 4 respectively. In contrast, the extracts from rats treated with morphine for one day contained the three forms of choline acetyltransferase with concentrations in the ratio of 4 : 4 : 0.5 respectively. In extracts from rats treated for a total of five days, however, the ratio of the three forms had reverted to normal.

Redistribution of the focused activities, a change in enzyme structure, or depression of the synthesis of the most basic choline acetyltransferase, accompanied by a compensatory increase in the most acidic form, are likely mechanisms for these actions of morphine. The relation of the changes in choline acetyltransferase population to the primary central actions of morphine remains to be clarified.

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The heterogeneity of goldfish brain and muscle choline acetyltransferase

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Several forms of choline acetyltransferase (EC. 2. 3. 1. 6), with different isoelectric points, have been demonstrated by electrofocusing. Guinea-pigs, pigeons, spider crabs and cockroaches each contain different, single forms of the enzyme. In contrast, rats, cats and squid each contain several discrete forms of choline acetyltransferase (Malthe-Sørenssen & Fonnum, 1972, Prince & Toates, 1973). High concentrations of choline acetyltransferase were found in axial muscle of the common goldfish (*Carassius auratus*) (Cohen, 1956), and the effect of temperature acclimatization on the brain enzyme has been investigated.

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

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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