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.

References

DATTA, K., THAL, L. & WAJDA, I.J. (1971). Effects of morphine on choline acetyltransferase levels in the caudate nucleus of the rat. *Br. J. Pharmac.*, **41**, 84.

DATTA, K. & WAJDA, I.J. (1972). Morphine-induced kinetic alterations of choline acetyltransferase of the rat caudate nucleus. *Br. J. Pharmac.*, **44**, 732.

GIARMAN, N.J. & PEPEU, G. (1962). Drug induced changes in brain acetylcholine. *Br. J. Pharmac. Chemother*, **19**, 226.

LARGE, W.A. & MILTON, A.S. (1970). The effect of acute and chronic morphine administration on brain acetylcholine levels in the rat. *Br. J. Pharmac.*, **38**, 451.

MALTHE-SØRENSSEN, D. & FONNUM, F. (1972). Multiple forms of choline acetyltransferase in several species demonstrated by isoelectric focusing. *Biochem. J.*, **127**, 229.

THAL, L. & WAJDA, I.J. (1969). The effects of morphine on choline acetyltransferase levels in the caudate nucleus of the rat. *Fedn. Proc. (USA)*, **28**, 261.

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.