Kinetic studies of coenzyme binding to *L. casei* dihydrofolate reductase

SUSAN M.J. DUNN & R.W. KING
(introduced by A.S.V. BURGEN)

National Institute for Medical Research, Mill Hill, London NW7 1AA

The kinetics of binding of substrates and substrate analogues to dihydrofolate reductase from a methotrexate-resistant strain of *L. casei* are currently being investigated by the stopped-flow technique. Complex formation can readily be monitored by measuring changes in protein or ligand fluorescence associated with binding. Reaction traces are collected by passing the amplitude photomultiplier signal into a 200 point signal averager for temporary storage prior to data transfer to the disc of an HP 3000 computer. Kinetic constants are determined by non-linear regression and both raw data and computed curves are plotted so that any deviation from, for example, a single exponential can be checked.

When NADPH is the substrate, under pseudo-first-order conditions the reaction curve, as monitored by dihydrofolate reductase fluorescence, shows a fast quench whose rate depends on NADPH concentration and a much slower first-order quench of rate approximately 0.03 s^{-1}. For the second order binding process a rate constant of 1.5×10^7 M^{-1} s^{-1} has been obtained. The amplitude of the fast phase as a percentage of the total signal change is not independent of ligand concentration and the results obtained suggest the existence of at least two interconvertible forms of dihydrofolate reductase, to one of which NADPH binds preferentially. The slow quench observed appears to be a reflection of the rate of interconversion of the enzymic forms.

S.M.J.D. is an MRC student.

Axotomy causes loss of synaptic contacts and loss of muscarinic receptors in the hypoglossal nucleus

N.J.M. BIRDSALL, A.S.V. BURGEN, P.M. FIELD, G. RAISMAN & A. ROTTER

National Institute for Medical Research, Mill Hill, London NW7 1AA

Tritiated propyl benzilylcholine mustard ([^{3}H]-PrBCM) is a potent, irreversible muscarinic antagonist whose binding satisfies the basic criteria for receptor-specific labelling (Burgen, Hiley & Young, 1974). We have used [^{3}H]-PrBCM to demonstrate autoradiographically the distribution of muscarinic receptors in the rat brain. In the brain stem the hypoglossal nuclei are heavily labelled.

Unilateral hypoglossal nerve axotomies (by ligation or by resection of a segment of nerve) were performed under anaesthesia in a series of adult (180–220 g) female Wistar rats. At various survival times after axotomy the animals for the autoradiographic study were sacrificed, coronal sections of unfixed brain stem (12 μm thick) cut in a cryostat, mounted on slides and briefly prefixed in cold 0.1% glutaraldehyde. Pre-incubation was carried out for 15 min in Krebs-Henseleit medium at 30°C with or without 10^{-6} M atropine followed by addition of cyclized [^{3}H]-PrBCM (40 Ci/mmol) to a final concentration of 5 nM for a further 15 minutes. The incubation was terminated by post-fixation in Carnoy's fluid followed by several washes in absolute alcohol. The slides were dipped in a 1:1 dilution of Ilford G5 nuclear emulsion, exposed at 6°C, developed in Ilford Phen-X and counter stained with cresyl fast violet. Specific binding is defined as the atropine-displaceable component of [^{3}H]-PrBCM uptake. The ratio of specific to non-specific binding was ca 3:1.

In a separate series of animals, the brains were prepared for light and electron microscopy after fixation by perfusion with a mixture of 1% formaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer and embedding in resin.

The hypoglossal nuclei were examined at progressively increasing times after axotomy; three short-term changes were detected.

(1) The specific binding of [^{3}H]-PrBCM was reduced by about half at 7 days.

(2) The neuronal nuclei showed indentations and perinuclear Nissl aggregations typical of chromatolysis. In the neuropil the dendritic profiles were much reduced in diameter; this correlates well with the previously described dendritic retraction and attenuation (Sumner & Watson, 1971).

(3) There was almost a 50% reduction in the number of synaptic contacts by 7 days. In place of the pre-synaptic terminals the former post-synaptic surfaces were covered with thin lamellae of glial cytoplasm; this agrees well with the findings of Sumner & Sutherland (1973).

At longer survivals, if the axon re-establishes functional contact with an appropriate target tissue, these changes are reversed. The hypoglossal motorneurones re-acquire a normal complement of afferent synapses (Sumner, 1975a, 1975b) and our observations show that the muscarinic receptor also reappears.

The parallel behaviour of the muscarinic receptor and the synaptic changes in the hypoglossal nucleus raises the possibility that there may be some causal