solution would usually pass current and eject dye up to two weeks after their manufacture but fresh solutions and electrodes were generally prepared once per week. Pipettes filled with the dye were capable of passing direct currents and square wave pulses of either polarity to magnitudes greater than 200 nA. Dye was ejected by anodal current. When a pipette was inserted into a muscle fibre a suitable rate of dye injection was effected by currents of 20-80 nA. Direct currents of 40 nA were normally used. Larger currents often caused blockage of the pipette due to coagulation of dye at the tip. A noticeable 'mark' could be seen in the muscle fibre within twenty seconds. Rapid fixation was necessary since the dye appears to diffuse from the point of injection within 5-10 minutes. For histological examination, cold Calcium Formol fixation (4°C for 4 h) results in low background fluorescence and the marked fibre is easily detected under a Fluorescence microscope (excitation 380 nm, emission 540 nm). For electron microscopic examination, the usual glutaraldehyde/osmium fixative is unsuitable as the former induces intense background fluorescence and the latter quenches all fluorescence completely. Of other fixatives tested, one comprising 0.8% glutaraldehyde 5% paraformaldehyde buffered to pH 7.2 has been used with some success.

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The use of halothane-induced sleeping time as an index of central nervous system excitability

M.J. TURNBULL & J.W. WATKINS

Department of Pharmacology and Therapeutics, Ninewells Hospital Medical School, Dundee, DD1 9SY, Scotland

Sleeping time following an i.p. injection of a central nervous system (CNS) depressant drug e.g. hexobarbitone or pentobarbitone has often been used as an index of CNS excitability, particularly in drug tolerance studies. However, we have reported previously that a change in the rate of drug metabolism tends to mask any change in sensitivity of the CNS (Stevenson & Turnbull, 1970) and more useful information on the sensitivity of the brain to barbiturate may be obtained by determining the duration of anaesthesia following an intracerebroventricular injection of pentobarbitone (Stevenson & Turnbull, 1974). However, this latter method is unsuited to the study of change in CNS sensitivity occurring with time. We have therefore explored the usefulness of the measurement of the duration of anaesthesia produced by carefully controlled exposure to halothane.

The gas flow from an anaesthetic apparatus, incorporating a Fluotec Mark 2 vaporizer, was passed through a perspex box of dimensions 35 x 15 x 10 cm in which up to six 200 g rats could be placed. Oxygen containing 5% halothane was passed through the box at a flow rate of 2 litres min^{-1} for 3 min and the rats were exposed to this mixture for a further 5 min after switching off the gas flow. The animals were then removed from the box, placed on cotton wool and the time taken to regain the righting reflex was determined for each animal using a stopwatch. A sleeping time of between 3 min and 7 min, depending on the time of day at which animals were exposed to halothane, was obtained.

Pretreatment of animals with a CNS stimulant (amphetamine 1 mg kg^{-1} for 10 min) or depressant (sodium pentobarbitone 5 mg kg^{-1} for 5 min) shortened (2.58 ±0.18 (3) (± s.e.m.) min) or lengthened (9.18 ± 0.10 (3) min) the sleeping time respectively compared with saline pretreated rats (6.85 ± 0.17 (4) minutes).

Induction or inhibition of hepatic drug-metabolizing enzyme activity had no effect on the halothane-induced sleeping time (phenobarbitone pretreated 5.38 ± 0.20 (4); SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate HCl) pretreated 5.67 ± 0.26 (4); control 5.25 ± 0.10 (5) minutes).

Halothane-induced sleeping time could be repeatedly determined in the same animals with no evidence of tolerance, although a marked diurnal variation was observed. We have made repeated halothane sleeping time determinations in rats pretreated both acutely and chronically with a number of CNS depressant drugs and have found that drug exposure is followed by a cross-tolerance to halothane. This tolerance is frequently followed by a post-tolerance hypersensitivity to halothane and brain halothane concentrations determined on awakening confirm that the tolerance and hypersensitivity are due to changes in the sensitivity of the CNS to the anaesthetic.

Thus it is concluded that determination of halothane-induced sleeping time appears to be a sensitive index of the excitability of the CNS and has the advantage that repeated measurements can be made over a relatively short period of time.

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Selective autoradiographic markers for GABA-releasing interneurones and nerve terminals

D. CHAPMAN & FABIENNE DICK (introduced by J.S. KELLY)

M.R.C. Neurochemical Pharmacology Unit, Department of Pharmacology, Medical School, Hills Road, Cambridge CB2 2QD

In this light and electron microscopic autoradiographic study the cerebellum was used as a test model since only in this region of the brain has ultrastructure been fully correlated with function (Eccles, Ito & Szentagothai, 1967). In addition, neuropharmacological data is available to allow us to suggest that γ-aminobutyric acid (GABA) is the sole transmitter of the inhibitory interneurones of the cerebellum.

Either [^{3}H]-DABA (DL-2,4-diaminobutyric acid) or [^{3}H]-GABA dissolved in 2 μl of artificial CSF were microinjected under pressure into the centre of a single cerebellar folium of anaesthetized cats or rats at a depth of about 1 mm using a fine glass microelectrode (tip diameter <20 μm). Twenty minutes later the animals were killed by perfusion fixation with 5% glutaraldehyde. Subsequently 300 μm thick coronal slices of the lobule were cut on an Oxford 'vibratome' and prepared for electron microscopic autoradiography as described previously (Schon & Kelly, 1974).

Light and electron microscopic autoradiographs from the cerebellar folia following the microinjection of [^{3}H]-DABA (50 μCi) and [^{3}H]-GABA (25 μCi) provided similar results and were characterized by intensely and discretely labelled cell bodies in all layers of the cerebellum. At the electron microscopic level the labelled cells were identified as stellate cells in the molecular layer, as basket or Golgi cells in the Purkinje cell and upper granular layers. The Purkinje cells themselves, however, were virtually devoid of silver grains and their somas were silhouetted against a dense rim of radioactivity which became broader towards their rounded ends. Electron microscopic observations showed this dense rim to be composed of dense cluster of sliver grains located over unmyelinated axons and nerve terminals in synaptic contact with the Purkinje cell soma. These axons and terminals were tentatively identified to be basket cell axons or Purkinje cell recurrent collaterals.

Clusters of silver grains were also found in association with the cerebellar glomeruli; the silver grains were located exclusively over Golgi axon terminals in close contact with the granule cell dendrites. Neither the granule cell bodies or their dendrites nor the mossy fibre terminals were labelled.

In this study we have shown that light and electron microscopic autoradiographic techniques are now available to identify and map the distribution of the neurones in the mammalian CNS which use GABA as their transmitter. This technique is based on the hypothesis that such neurones possess a unique high affinity uptake site for the amino acids that they employ as their transmitter.

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