DEMONSTRATIONS

Methods for the Histopathological study of the drug-damaged guinea-pig organ of Corti

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Use of an *in vitro* hippocampal slice preparation in studies of the actions of the benzodiazepines

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Slices of tissue cut from various CNS regions and maintained *in vitro* exhibit many of the properties of those tissues *in vivo*, and are increasingly being used in pharmacological studies of CNS function. The structures within such slices can be visualised facilitating appropriate placement of recording and stimulating electrodes, and the ionic and pharmacological composition of the fluid environment of the tissue can be controlled more precisely than is possible *in vivo* (Phillis, 1978). In studies of drugs of the benzodiazepine group currently being undertaken in this laboratory an *in vitro* preparation of rat hippocampal slices similar to that described by Skrede and Westgaard (1971) is being used, since this structure contains a high density of benzodiazepine binding sites (e.g. Robertson, Martin and Candy, 1978) and also contains GABA-ergic inhibitory interneurones: a number of reports have demonstrated facilitation of GABA-ergic inhibition by benzodiazepines (e.g. Geller, Taylor and Hoffer, 1978).

Rats are killed by cervical fracture and the brain removed: a hippocampus is dissected out and cut into 300 μm slices with a McIlwain tissue chopper: the slices are kept in artificial cerebrospinal fluid (Skrede & Westgaard, 1971) bubbled with 95% O_2/5% CO_2 at 28°C until needed. Individual slices are then placed on a sintered glass disc in a perspex well and then continuously superfused with artificial cerebrospinal fluid delivered from a 19-gauge stainless steel tube situated immediately over the slice. The superfusing medium is heated to 34°C and is delivered at 0.5 ml min^{-1} by a Gilson Minipuls 2 peristaltic pump, which also removes excess fluid from the well through a second, chamfered, stainless steel tube, maintaining a steady level inside the well. The atmosphere over the slice is maintained by bubbling O_2/CO_2 through a heated water bath beneath the well, and channelling the gas over the slice. Field potentials are recorded with conventional glass micropipettes and evoked by negative square wave pulses (0.2 Hz, 0.05 msec, 1–5 v.) delivered through electrolytically etched 0.3 mm diameter tungsten wire positioned either in the alveus to elicit population spikes antidromically or in the radiatum layer to elicit population spikes or EPSP's. Under these conditions, stable potentials can be recorded for 4–6 h. The amplitude of the evoked potentials at peak are measured and plotted on a chart recorder. Drugs are made up in the superfusing fluid and then these solutions superfused over the slice. Our initial observations show that synaptic potentials are depressed by some benzodiazepines: thus flurazepam (5×10^{-4} M) superfused for 30 s reduces EPSP size by 50%, the effect outlasting the application by many minutes. However this effect is not exhibited by all benzodiazepines even at these high concentrations, and thus is unlikely to be associated with a specific action at benzodiazepine receptors. These effects, and interactions of GABA and benzodiazepines will be demonstrated at the meeting.

References

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