amplitude when elicited at frequencies of 0.033 Hz; at 0.1 Hz the amplitude of second and subsequent responses declined by 25–30%. The amplitude of the 5-HT depolarization was augmented by hyperpolarization and decreased by depolarization of the soma membrane of the ganglion cell.

Iontophoretic currents were not exciting nerve terminals and releasing acetylcholine because (a) iontophoretic responses were unaffected by hexamethonium (5×10^{-4} M), whereas synaptic potentials were depressed by 70%; (b) responses were reduced in amplitude by cyproheptadine (5×10^{-5} M) by about 50%; (c) responses were reduced in amplitude by superfusing the ganglion with 10^{-6} M 5-HT and nearly abolished on superfusing with 5×10^{-5} M 5-HT, although these concentrations of 5-HT did not affect the synaptic potentials.

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References

WALLIS, D.I. & WOODWARD, B. (1974). The facilitatory actions of 5-hydroxytryptamine and bradykinin in the superior cervical ganglion of the rabbit. *Br. J. Pharmac.*, **51**, 521–532.

WALLIS, D.I. & WOODWARD, B. (1975). Membrane potential changes induced by 5-hydroxytryptamine in the rabbit superior cervical ganglion. *Br. J. Pharmac.*, **55**, 199–212.

The uptake of ^{45}calcium into slices of rat cerebral cortex: effect of depolarizing stimuli

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Previous studies have shown that neurotransmitter uptake is markedly dependent on slice size (Levi & Raiteri, 1973) and it has been postulated that varying slice size can be used to examine uptake into neuronal and glial cells (Riddall, Leach & Davison, 1976; Leach, Riddall & Winkley, 1976). The intracellular concentration of calcium may be increased by various depolarizing stimuli and the effects of these stimuli on ^{45}calcium uptake into slices of rat cerebral cortex has been examined.

Two sizes of slice were employed ($0.1 \times 0.1 \times 2$ mm and $0.4 \times 0.4 \times 2$ mm) and they were prepared in the manner described by Iversen & Neal (1968) except that pH 7.4 Tyrode medium was used. Slices were pre-incubated at 37°C for 15 min and portions (equivalent to 20 mg wet wt.) of this suspension were transferred to Tyrode (containing the depolarizing stimuli) or to potassium stimulation media both of which contained $^{45}Ca^{2+}$ (0.1 μCi ml, 1.78 mM). Flasks were incubated for varying times and uptake was terminated by the addition of 1 ml of quench medium (24 mM EGTA-tris, 72 μM ruthenium red in Tyrode). The suspension was immediately filtered under vacuum, washed, and the resulting tissue sample was digested and counted by the method of Dent & Johnson (1974).

The basal flux of $^{45}Ca^{2+}$, i.e. 'exchange', was greater in 0.4 mm slices than in 0.1 mm slices giving asymptotic 'uptake' values of 5.64 ± 0.26 and 1.94 ± 0.11 μmol Ca^{2+}/g wet wt. ($P < 0.01$) respectively. In the presence of ouabain (0.1 mM) the corresponding asymptotic values for $^{45}Ca^{2+}$ uptake into 0.4 mm and 0.1 mm slices were increased to 12.27 ± 0.38 and 3.44 ± 0.23 μmol Ca^{2+}/g wet wt. ($P < 0.01$). Pre-treatment with the calcium ionophore, A23187 (200 μg/ml) increased $^{45}Ca^{2+}$ uptake into both slice sizes at 10 and 60 min of incubation. Glutamate (5 mM) had no effect on $^{45}Ca^{2+}$ uptake into either slice size.

Potassium exhibited different effects on the uptake of $^{45}Ca^{2+}$ into the two slice sizes. K^+ (49 mM) stimulation of $^{45}Ca^{2+}$ uptake into 0.1 mm slices was characterized by a rapid initial phase which lasted for approximately one minute. This rapid initial phase was followed by a slower phase which was similar in magnitude to the uptake found in the absence of elevated K^+. In contrast K^+ (49 mM) had no significant effect on $^{45}Ca^{2+}$ uptake into 0.4 mm slices.

Uptake mechanisms for putative neurotransmitters into small slices ($0.1 \times 0.1 \times 2$ mm) of rat cerebral cortex are well documented. Conversely, there are little, if any, data relating to the release mechanisms operating in these slices. The results presented above indicate a prominent role for glial cells in the regulation of Ca^{2+} flux. This role is demonstrated by the effects of slice size and depolarizing stimuli on $^{45}Ca^{2+}$ uptake into rat cerebral cortex.

References

DENT, J.G. & JOHNSON, P. (1974). *Solubilisation of mammalian tissue for scintillation counting in Liquid Scintillation Counting.* Vol. 3, eds. Crook, M.R. & Johnson, P. Heydén: London.

IVERSEN, L.L. & NEAL, M.J. (1968). The uptake of [^{3}H]GABA by slices of rat cerebral cortex. *J. Neurochem.*, **15**, 1141–1149.

LEACH, M.J., RIDDALL, D.R. & WINKLEY, C.M. (1976). Uptake of L-2,4-diamino[4-^{3}H]butyric acid into slices of rat cerebral cortex: Effect of slice size. *J. Neurochem.*, **27**, 1281–1282.

LEVI, G. & RAITERI, M. (1973). Detectability of high and low affinity uptake systems for GABA and glutamate in rat brain slices and synaptosomes. *Life Sci.*, **12**, 81–88.

RIDDALL, D.R., LEACH, M.J. & DAVISON, A.N. (1976). Neurotransmitter uptake into slices of rat cerebral cortex *in vitro:* Effect of slice size. *J. Neurochem.*, **27**, 835–839.

Prolongation of post-synaptic inhibition by barbiturates

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When slices of guinea-pig olfactory cortex are incubated *in vitro* and the lateral olfactory tract (LOT) is stimulated the evoked excitatory post-synaptic potential (EPSP) recorded within the mono-synaptically-innervated neurones is followed by an inhibitory post-synaptic potential (IPSP). This IPSP is manifest by a large conductance increase and a small depolarization at the normally-recorded resting membrane potential of −76 mV (Scholfield, 1976). The present report concerns a unique and substantial prolongation of this IPSP by barbiturates.

Surface slices of guinea-pig olfactory cortex were maintained in a superfusing stream of Krebs solution at 25°C bubbled with 95% oxygen/5% carbon dioxide, and neurones in the prepyriform cortex were impaled with glass microelectrodes filled with K acetate as described previously (Scholfield, 1976). Extracellular surface potentials were monitored with a saline-filled microelectrode. The LOT input was stimulated at < 1 min intervals.

Sodium pentobarbitone (100 μM) increased the time to half-decay of the post-synaptic inhibitory conductance from 0.17 ± 0.04 to 1.68 ± 0.22 s (mean ± s.e. mean, 5 slices). The apparent reversal potential for the IPSP (initially −70 to −69 mV) shifted some 10 mV in the depolarizing direction during the course of the individual barbiturate-prolonged IPSP. Spikes were not generated during this prolonged depolarization.

The initial EPSP, spike potential and resting input resistance and time constant were unchanged below 200 μM pentobarbitone. At higher concentrations input resistance fell substantially, the membrane depolarized towards −60 mV and all synaptic potentials were depressed.

During a train of LOT stimuli the second EPSP is shunted by the IPSP and fails to generate a spike, but subsequently recovery normally ensues such that repetitive EPSP-spike combinations result (Scholfield, 1976). In contrast, pentobarbitone not only prevented recovery but intensified EPSP depression during repetitive LOT stimulation. This accords with previous observations made with extracellular electrodes (Scholfield & Harvey, 1975).

Effects of pentobarbitone within the range 20 μM–1 mM could be replicated by phenobarbitone (0.2–5 mM). In contrast procaine (2–10 mM) simply attenuated the spike and depressed all post-synaptic potentials. This confirms the previously-drawn (Scholfield & Harvey, 1975) distinction between the actions of barbiturates and local anaesthetics on synaptic processes in this preparation.

The present results accord with observations in other parts of the CNS *in vivo*, such as those of Nicholl, Eccles, Oshima & Rubia (1975). Since selective potentiation of the IPSP occurs at concentrations within or below the range expected during anaesthesia, such an effect may contribute to the actions of barbiturates *in vivo*.

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References

NICOLL, R.A., ECCLES, J.C., OSHIMA, T. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. *Nature, Lond.*, **258**, 625–627.

SCHOLFIELD, C.N. (1976). A depolarizing inhibitory post-synaptic potential in mammalian brain slices. *J. Physiol., Lond.* (in press).

SCHOLFIELD, C.N. & HARVEY, J.A. (1975). Local anaesthetics and barbiturates: effects on evoked potentials in isolated mammalian cortex. *J. Pharmac. exp. Ther.*, **195**, 522–531.