Short communications

GABA and hippocampal inhibition

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Bicuculline, a specific GABA antagonist, diminishes basket cell inhibition of hippocampal pyramidal neurones, an inhibition which is not affected by strychnine.

The inhibitory transmitter released by basket cells is thus probably GABA.

The recent demonstration that bicuculline blocks the inhibition of cortical, cerebellar, thalamic and Deiters neurones by both electrophoretically administered gamma-aminobutyric acid (GABA), and impulses in appropriate inhibitory pathways (Curtis, Duggan, Felix & Johnston, 1970; Curtis, Duggan & Felix, 1970; Duggan & McLennan, 1970) provides valuable confirmatory evidence for the probable function of this amino-acid as a supraspinal inhibitory transmitter. The particular inhibitory processes affected by bicuculline are insensitive to strychnine (Eccles, 1969).

Within the hippocampal cortex, the activity of both GABA-transaminase-dehydrogenase (van Gelder, 1965) and glutamic acid decarboxylase (Fonnum & Storm-Mathisen, 1969) is high in the vicinity of pyramidal cell bodies. Furthermore, decarboxylase levels persist after lesions of the fimbria, indicating that this GABA-synthesizing enzyme is probably associated with the inhibitory basket cells (Storm-Mathisen & Fonnum, 1969). Electrophoretically administered GABA depresses the activity of hippocampal neurones (Stefanis, 1964; Biscoe & Straughan, 1966; Steiner & Ruf, 1967) and basket cell inhibition of pyramidal cells is not altered by intravenous strychnine (Andersen, Eccles, Løyning & Voorhoeve, 1963). The present communication is concerned with the effects of bicuculline on synaptic and chemical inhibition of hippocampal neurones.

Methods.—Cats used in these experiments were anaesthetized with sodium pentobarbitone. The dorsal hippocampus was exposed by removal of the overlying cortex, and coaxial stimulating electrodes were placed on the surface of the contralateral hippocampus, the fimbria and the alveus close to the site of recording in regions CA1 or CA2. The tissue was irrigated continuously with a carbogenated Ringer solution, and the surface was stabilized with a small 'pressure' plate.

Extracellular action potentials of single neurones were recorded by the central 4M NaCl-containing barrel of seven barrel micropipettes of overall tip diameter 4–6 micrometres. The other barrels contained aqueous solutions of the following compounds, from which the active ions were ejected electrophoretically: DL-homocysteate (DLH, 0·2M, pH 7·5, NaOH); glycine (0·5M, pH 3, HCl); GABA (0·5M, pH 3, HCl); β-alanine (0·5M, pH 3, HCl); strychnine (10 mM hydrochloride in 165 mM NaCl) and bicuculline (Fluka, 5 mM hydrochloride in 165 mM NaCl, pH 3).

Hippocampal pyramidal cells were identified by depth beneath the cortical surface (0·2–0·6 mm), antidromic response to stimulation of the fimbria, and the characteristic inhibitory 'pause' which followed fimbrial, contralateral or 'local' cortical stimulation (Andersen, Eccles & Løyning, 1964).

Results and Discussion.—The majority of pyramidal cells from which satisfactory records were obtained (seventeen cells, six cats) were firing spontaneously, and continuously ejected DLH (1–3nA) was used to maintain firing rates of the order of thirty–sixty spikes per second. Elevation of the firing rate beyond these levels often resulted in the recording of slow synchronized positive-negative waves which interrupted the firing of the cell under investigation (see Biscoe and Straughan, 1966).

Glycine, β-alanine and GABA readily depressed the firing of hippocampal cells: on the basis of electrophoretic currents GABA was more effective than glycine or β-alanine. During the administration of bicuculline (80–200nA) the frequency of cell firing was usually enhanced, often with the appearance of slow waves, and the inhibitory effects of GABA and β-alanine were reduced. The tracings of Fig. 1A, C and E illustrate the reversible effect of bicuculline on the sensitivity of a hippo-

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campal neurone to depressant amino-acids. The action of glycine was relatively insensitive to bicuculline, although when the alkaloid was ejected with very high currents (250–300nA) some reduction was observed in depression induced by this amino-acid. Such currents did not influence the activity of the neurones. In contrast, electrophoretically administered strychnine (10–30 nA) reduced the firing rate of hippocampal cells and the effects of all three depressant amino-acids, with no obvious selectivity towards glycine.

Synaptic inhibition of the firing of

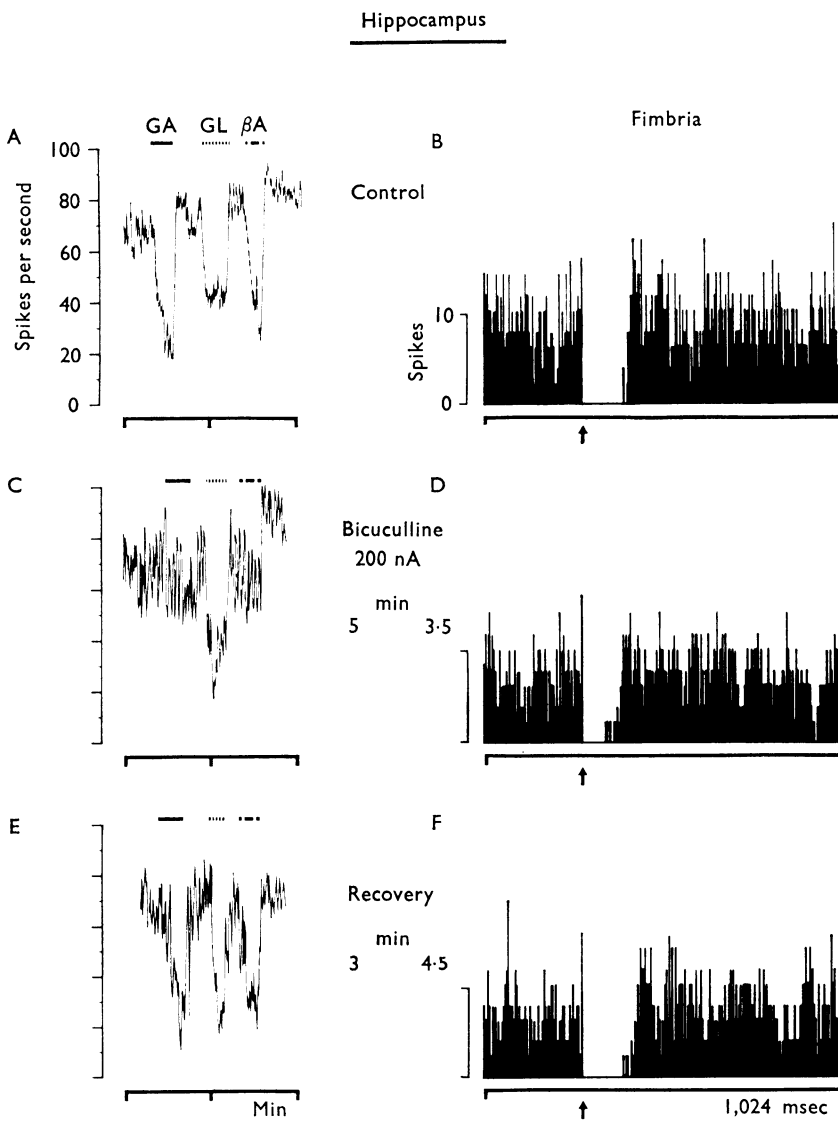

FIG. 1.—Effects of bicuculline on the inhibition of a hippocampal pyramidal cell. A, C, E, Inhibition by electrophoretic administration of depressant amino-acids, indicated above the tracings: glycine (GL, dotted line, 40 nA), β-alanine (βA, broken line, 40 nA), GABA (GA, solid line, 20 nA). The rate of cell firing was maintained with DLH. B, D, F, Inhibition of firing by electrical stimulation of the fimbria (3 volt pulse, 0·1 ms duration, frequency 0·5 Hz, marked with an arrow). The number of action potentials (ordinate) occurring in 256 intervals, each of 4 ms duration (abscissae, 1,024 ms period of analysis), was analysed by a computer for a total of thirty sweeps. A, B, control records. C, D, five and 3·5 min respectively after a current of 200 nA began to eject bicuculline into the vicinity of the neurone for a total period of 6 minutes. E, F, three and 4·5 min after this current was terminated.

neurones was assessed from pre- and post-stimulus histograms constructed on-line from data obtained from thirty–forty sweeps. Bicuculline consistently and reversibly reduced the period of inhibition which followed stimulation of the fimbria, the contralateral hippocampus or the surface of the alveus close to the recording site; care was taken to maintain constant firing rates before, during and after the administration of the alkaloid by appropriate adjustment of the DLH-ejecting current.

The records of Fig. 1, B, D and F illustrate the effects of bicuculline (200 nA) on inhibition produced by stimulation of the fimbria: the inhibitory 'pause' was reversibly shortened from 112 to 60 milliseconds. Similar effects were observed with other cells using smaller and higher 'doses' of bicuculline, in the absence of an increase in the firing rate of the neurone. Bicuculline occasionally reduced the increased firing (rebound) which followed the inhibitory 'pause', as has been observed in the thalamus (Duggan & McLennan, 1970). Although strychnine also shortened the period of inhibition of some neurones, the effect was not consistent, and cannot be related to specific suppression of inhibition by a particular amino-acid.

The reduction by bicuculline of the inhibition of hippocampal cells resulting from both the activation of basket cells and electrophoretically administered GABA thus suggests that GABA could be the basket cell inhibitory transmitter. This proposal is consistent with the neurochemical evidence cited above.

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