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γ-AMINOBUTYRIC ACID AND INHIBITION IN THE SEPTAL NUCLEI OF THE RAT

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SUMMARY

1. The electrophoretic application of γ-aminobutyrate (GABA) and glycine to septal neurones inhibited their discharge, but the currents required to cause equivalent degrees of inhibition were always smaller for GABA than for glycine.

2. The actions of GABA but not those of glycine were antagonized by bicuculline and not by strychnine and vice versa.

3. The recurrent collateral inhibition of lateral septal neurones and the direct inhibition of medial cells evoked by stimulation of the fimbria were blocked by bicuculline but not by strychnine.

4. The bursting patterns of discharge of laterally situated medial septal neurones was disrupted by bicuculline, but required a longer application of the drug than was needed to block the direct fimbrial inhibition. It is suggested that this disruption is due to diffusion of the drug to affect the inhibitory interneurones of the lateral septum.

5. It is concluded that the inhibitory processes in the septum involve GABA as the synaptic transmitter.

INTRODUCTION

Stimulation of the hippocampal fimbria has been shown to elicit prolonged inhibitory effects on neurones in the medial and lateral regions of the septum in the rat (McLennan & Miller, 1974). Two different mechanisms have been described as mediating these responses, both of which involve identified inhibitory interneurones: (1) a direct inhibition of medial septal neurones and (2) a recurrent collateral inhibition of neurones in the lateral septum. The whole system has been postulated as a feed-back circuit from the hippocampus which underlies the development of synchronized theta activity in that region. The aim of this study was to identify the inhibitory transmitters involved in these inhibitory processes.

There is considerable evidence to suggest major roles for γ-amino butyrate (GABA) and for glycine as transmitters of post-synaptic inhibition in various regions of the mammalian central nervous system (Krnjević & Schwartz, 1967; Curtis, Hösli & Johnston, 1968; Werman, Davidoff & Aprison, 1968; Obata, Takeda & Shinozaki, 1970; Curtis, Duggan, Felix, Johnston & McLennan, 1971). Furthermore, it is known that bicuculline administered either systemically or electrophoretically causes a selective antagonism of GABA-induced and synaptically evoked inhibitions at a number of sites (Curtis, Duggan, Felix & Johnston, 1971; Curtis *et al.* 1971; Duggan & McLennan, 1971; Curtis & Tebēcis, 1972) while the depressant action of glycine is unaffected by this drug. On the other hand the administration of strychnine has been shown to block the actions of glycine without affecting those produced by GABA (Curtis *et al.* 1968; Curtis, Duggan & Johnston, 1971).

In view of the fact that in other areas GABA and glycine are involved in post-synaptic inhibitory processes, the effects of these putative transmitters on neurones in the medial and lateral septum and of the effects of bicuculline and strychnine on GABA or glycine-induced and synaptic inhibitions have been examined.

METHODS

Experiments were performed on twenty-one male Wistar rats acutely prepared under urethane anaesthesia (1·5 g/kg I.P.) and maintained at a body temperature of 36–38° C. Details of the surgical procedures have been described in the preceding paper (McLennan & Miller, 1974). Concentric bipolar stimulating electrodes were stereotaxically lowered into the fimbrial hippocampus according to the co-ordinates of König & Klippel (1963). Single square-wave pulses of 0·1–0·3 msec duration and 0·1–0·4 mA intensity were used for stimulation.

Extracellular unit activity was recorded through the central barrel, containing 4 M-NaCl, of 7-barrel micropipettes which had an over-all tip diameter of 4–8 μm. The other barrels contained aqueous solutions of the following drugs: GABA (0·5 M; pH 3·0; HCl), glycine (0·5 M; pH 3·0; HCl), L-glutamate (0·5 M; pH 8·0; NaOH,) strychnine sulphate (10 mM in 165 mM-NaCl) and bicuculline (5 mM in 165 mM-NaCl; pH 3·5; HCl). Bicuculline methochloride (kindly provided by Professor D. R. Curtis) was used in some experiments in a solution of 50 mM in 100 mM-NaCl (pH 3·5; HCl). The drugs were ejected electrophoretically using appropriate anionic or cationic currents, upon neurones in the lateral and medial septal nuclei which were identified by their characteristic patterns of response to fimbrial stimulation (McLennan & Miller, 1974). The effects of intravenous injections of bicuculline in doses of 0·05–0·5 mg/kg were examined on septal neurones at the termination of nine experiments.

The extracellular activity of septal cells was amplified and displayed on an oscilloscope, and records of the integrated rates of firing and of post-stimulus histograms were obtained as described in the previous paper. The sites of electrode placement were also confirmed histologically as earlier described.

RESULTS

Extracellular records were obtained from a total of 108 neurones identified according to the patterns evoked by single volleys delivered to the fimbria. Lateral septal cells exhibited an activation-inhibition sequence in response to stimulation, while neurones in the medial septum displayed only an inhibitory reaction. Since the spontaneous firing frequency of many neurones was relatively low it was necessary in such cases to enhance the discharge rate by a continuous application of glutamate with currents of 2–20 nA. Relatively constant firing rates could be maintained during the administration of other drugs by adjusting the levels of the glutamate ejecting current. No differences were observed between the responses of neurones excited by glutamate and those having a sufficiently high spontaneous firing frequency.

Electrophoretic application of GABA with currents not exceeding 30 nA reduced or completely inhibited the discharge of all neurones in both the lateral and medial septal regions. The onset of inhibition usually began within 3 sec of the start of application of GABA and the firing frequency returned to control levels 4–10 sec after termination of the current. In some cases there was a rebound excitation following the GABA-induced inhibition. Bicuculline ejected with currents of 40–125 nA reduced or abolished the effect of GABA on eighty-one of the ninety-four septal neurones upon which it was examined. This action was observed 2–3 min following the onset of the bicuculline current, and persisted for a similar time period after its termination. Complete recovery was usually observed within 5–10 min. The action of GABA on twelve neurones was unaltered by bicuculline even when currents in excess of 125 nA were used.

In order to determine the specificity of the GABA-bicuculline interaction, the effects of glycine and strychnine were also tested on the same neurones. The administration of glycine depressed the firing of all cells in a manner similar to that of GABA except that higher currents (25–75 nA) were required to produce an equivalent inhibition (Fig. 1*A*, *B*). During the administration of bicuculline the inhibitory effect of GABA was completely blocked while that produced by glycine remained unaltered (Fig. 1*A*); by contrast strychnine ejected with currents of 20–80 nA blocked the depression produced by glycine without affecting that of GABA (Fig. 1*B*).

A comparison of the effects of bicuculline on GABA-induced and synaptically evoked inhibitions in a lateral septal neurone is shown in Fig. 2*A–F*. The histogram of Fig. 2*D* illustrates the activation-inhibition sequence evoked by stimulation of the fimbria, the period of inhibition

in this instance lasting for 530 msec. Electrophoretic application of GABA with a current of 5 nA completely abolished the firing of this cell (Fig. 2*A*) and the administration of bicuculline both eliminated the GABA-induced inhibition (Fig. 2*B*) and shortened that elicited by stimulation (Fig. 2*E*). The activation of the cell which preceded the inhibition was not affected. In most cases slightly higher bicuculline-ejecting currents were required to produce a decrease in synaptic inhibitions compared to those

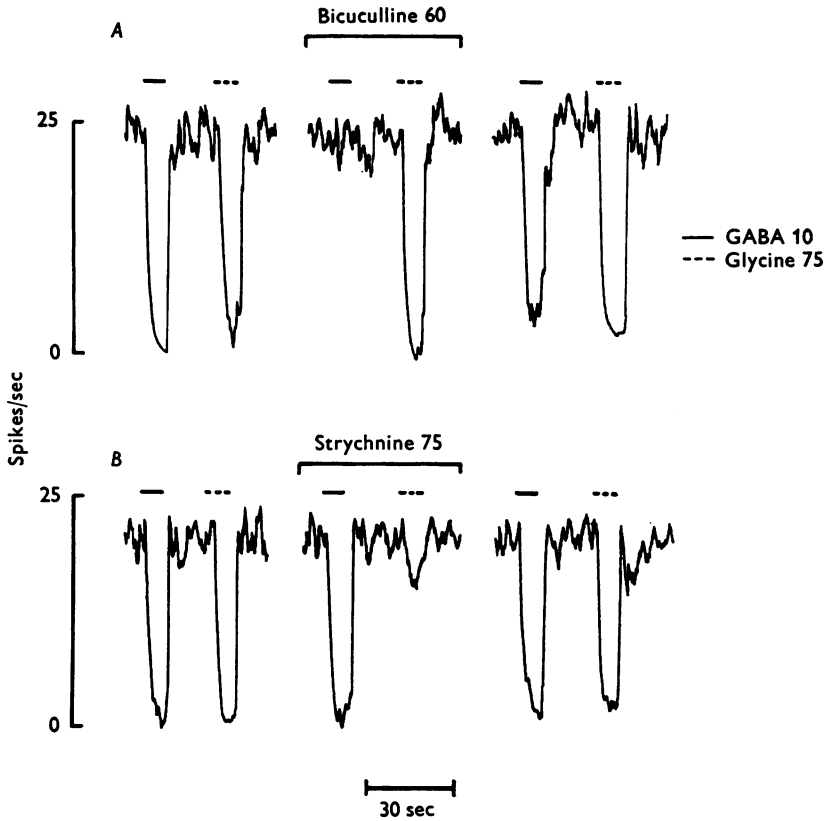

Fig. 1. The effects of bicuculline (*A*) and strychnine (*B*) on inhibition of the spontaneous firing of a lateral septal neurone by GABA (10 nA) and glycine (75 nA). The periods of administration of the depressant amino acids are indicated by horizontal continuous and interrupted lines. *A*, before, during and 3 min after the administration of bicuculline (60 nA, 2 min). *B*, before, during and 5 min after the administration of strychnine (75 nA, 4 min).

required to block the actions of GABA. Complete recovery of the evoked inhibitions was observed 8–10 min following termination of the bicuculline current (Fig. 2*F*). No change in the duration of the inhibition produced

by fimbrial stimulation was observed to follow the application of strychnine with currents which were completely effective in blocking the action of glycine.

In the previous study (McLennan & Miller, 1974) fimbrial stimulation was shown to evoke either a lengthy inhibition of medial septal neurones when they were firing in an irregular pattern, or a comparatively short

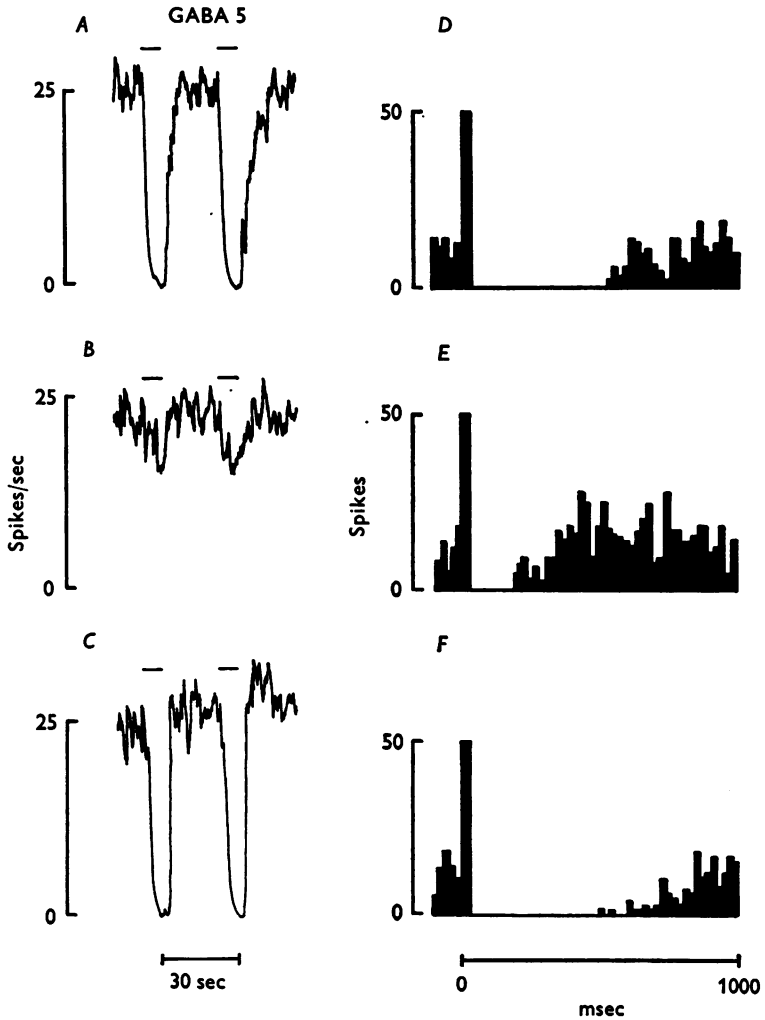

Fig. 2. The effect of bicuculline on the inhibition elicited in a neurone of the lateral septum by the electrophoretic application of GABA (5 nA; *A*, *B*, *C*) and by stimulation of the fimbria (*D*, *E*, *F*). The histograms show the summed responses to fifty stimuli. *A* and *D*, controls; *B* and *E*, 5 min after a current of 80 nA began to eject bicuculline; *C* and *F*, 10 min after this current was terminated.

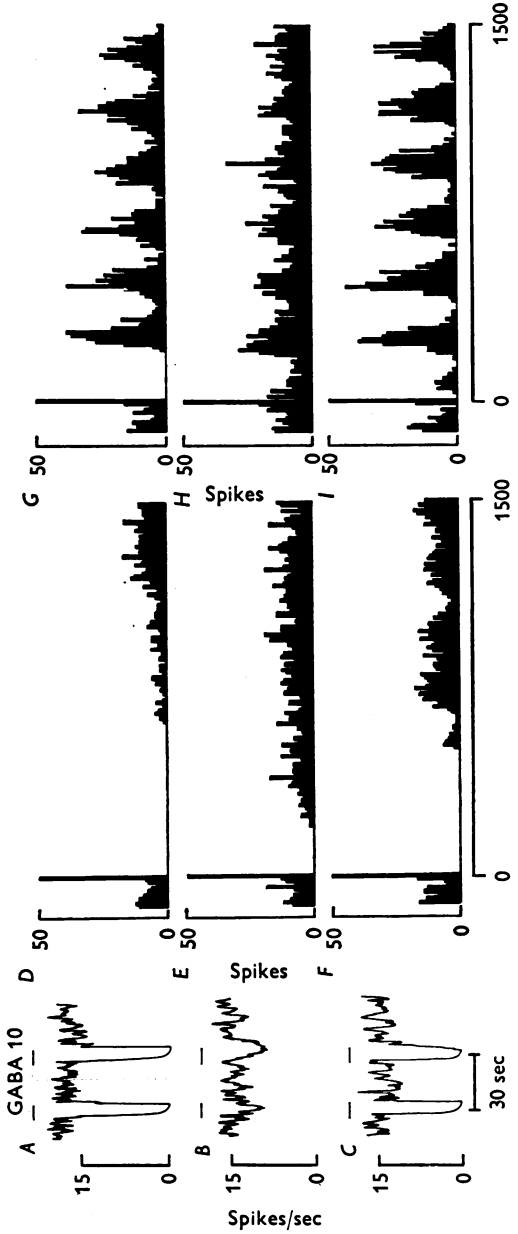

Fig. 3. The effect of bicuculline upon a medial septal neurone exhibiting two different discharge patterns. The administration of bicuculline (75 nA) reduced the inhibitory effect of GABA (10 nA) on this cell (*A*, *B*, *C*), decreased the period of inhibition evoked by stimulation of the fimbria when the cell was firing irregularly (*D*, *E*, *F*); and abolished the stimulus-locked bursting and short inhibition elicited from the fimbria when the background firing was in bursts (*G*, *H*, *I*). The histograms show the summed responses to 50 stimuli. *A*, *D* and *G*, controls; *B*, 2 min, *E*, 4 min and *H*, 9 min after the start of the bicuculline-ejecting current; *C*, *F*, and *I*, 12–17 min after cessation of that current.

inhibition followed by bursts of firing which were temporally locked to the stimulus. The effects of bicuculline on neurones exhibiting both of these types of behaviour were examined. Examples of records obtained from a medial septal neurone are shown in Fig. 3, and the period of recording in this instance was sufficiently long that an analysis could be made of the effects of bicuculline when the cell was firing both irregularly and in bursts. The application of sufficient bicuculline to eliminate the effect of GABA (Fig. 3*B*) resulted in a reduction of the evoked inhibition when the neurone was firing irregularly (Fig. 3*E*). The bursting pattern of discharge was also eliminated by a similar dose of bicuculline and was replaced by an irregular firing of the cell (Fig. 3*H*); however, under these circumstances the short inhibitory period which followed fimbrial stimulation often was not markedly reduced. It was regularly noted that for an effect on the bursting discharge to be obtained bicuculline had to be applied for a longer time (4–6 min) than was required for reduction of the direct fimbrial inhibition observed when the cell was firing irregularly (< 3 min). All effects were fully reversible within a few minutes of cessation of the bicuculline-ejecting current (Fig. 3*F*, *I*). On two medial septal neurones where bicuculline was shown to block both the long inhibition and the bursting discharge, strychnine was without effect when applied with a current sufficient to abolish the action of glycine upon the cells.

The action of bicuculline administered systemically was also examined on the synaptically evoked inhibitions produced in both lateral and medial septal neurones. In six of the eleven cells examined, bicuculline in doses of 0·2 mg/kg decreased the inhibition with only a minimal increase in spontaneous discharge rate. The onset of the antagonism occurred within 3–4 min of the injection and a return to control levels was obtained within 20–60 min. Lower doses (0·05–0·1 mg/kg) were ineffective on three neurones.

DISCUSSION

The results of the present investigation indicate that GABA and glycine are potent inhibitors of septal neurones as is true at many other sites in the nervous system. Since the ejecting currents necessary to obtain the same degree of inhibition were invariably lower for GABA than for glycine, the former compound appears to be a more potent depressant in the septal area. The antagonism of the action of GABA by bicuculline and of those of glycine by strychnine are also similar to those reported in other studies (Curtis *et al.* 1971).

The synaptic inhibition of septal neurones produced by fimbrial stimulation was reduced or abolished by the electrophoretic application of bicuculline with currents which also blocked the inhibitory effects of

GABA, but was unaffected by strychnine. This observation, together with the fact that GABA appears to be a more potent depressant of septal neurones, indicates that this amino acid in all likelihood is the transmitter liberated at the inhibitory synapses impinging upon septal cells.

Electrophysiological results (McLennan & Miller, 1974) have demonstrated that inhibitory interneurones are present within the septum which in the case of the medial region are excited directly from the fimbria and in the lateral area are involved in a recurrent collateral feed-back system, and interference with their operation can explain most of the results here described. Only one effect requires special comment, that of bicuculline in abolishing the bursting discharge of medial neurones, for this might imply that the inhibitory periods between the bursts are the result of an active inhibitory mechanism presumably mediated by GABA. In the model presented in the preceding paper it was proposed that the recurrent collateral system in the lateral septum was the 'rate-limiting' mechanism involved in the production of the bursting discharge pattern. Since bicuculline applied in the vicinity of medial cells changed this pattern of discharge it is necessary to assume that the interneurones involved in the collateral system are situated relatively close to the medial septal neurones and thus could be affected by bicuculline applied to the latter. Several pieces of evidence indicate that this may be true: (1) a majority of the inhibitory interneurones involved in the lateral septal system are located in the most medial part of the lateral region; (2) bicuculline applied to medial neurones lying at a distance from the border zone between medial and lateral areas had no effect on the bursting type of discharge; and (3) the time required for an effect of bicuculline on the bursts to be produced was always longer than that for reduction of inhibition, suggesting that a diffusional spread of the drug to some more remote point was required for the former action to become apparent. It is therefore reasonable to conclude that the inhibition of both medial and lateral septal neurones is caused by interneurones situated in the septum and that the transmitter liberated from the terminals of these neurones is GABA.

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REFERENCES

CURTIS, D. R., DUGGAN, A. W., FELIX, D. & JOHNSTON, G. A. R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. *Brain Res.* **32**, 69–96.

CURTIS, D. R., DUGGAN, A. W., FELIX, D., JOHNSTON, G. A. R. & MCLENNAN, H. (1971). Antagonism between bicuculline and GABA in the cat brain. *Brain Res.* **33**, 57–73.

CURTIS, D. R., DUGGAN, A. W. & JOHNSTON, G. A. R. (1971). The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. *Expl Brain Res.* **12**, 547–565.

CURTIS, D. R., HÖSLI, L. & JOHNSTON, G. A. R. (1968). A pharmacological study of the depression of spinal cord neurones by glycine and related amino acids. *Expl Brain Res.* **6**, 1–18.

CURTIS, D. R. & TEBĒCIS, A. K. (1972). Bicuculline and thalamic inhibition. *Expl Brain Res.* **16**, 210–218.

DUGGAN, A. W. & MCLENNAN, H. (1971). Bicuculline and inhibition in the thalamus. *Brain Res.* **25**, 188–191.

KÖNIG, F. C. & KLIPPEL, R. A. (1963). *The Rat Brain.* Baltimore: Williams and Wilkins.

KRNJEVIĆ, K. & SCHWARTZ, S. (1967). The action of γ-aminobutyric acid on cortical neurones. *Expl Brain Res.* **3**, 320–336.

MCLENNAN, H. & MILLER, J. J. (1974). The hippocampal control of neuronal discharges in the septum of the rat. *J. Physiol.* **237**, 607–624.

OBATA, K., TAKEDA, K. & SHINOZAKI, H. (1970). Further study on pharmacological properties of the cerebellar-induced inhibition of Deiters neurones. *Expl Brain Res.* **11**, 327–342.

WERMAN, R., DAVIDOFF, R. A. & APRISON, M. H. (1968). Inhibitory action of glycine on spinal neurons in the cat. *J. Neurophysiol.* **31**, 81–95.