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BICUCULLINE AND THE FROG SPINAL CORD

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1 The effects of bicuculline on dorsal and ventral root activity and upon the depressant effect of γ-aminobutyric acid (GABA) and glycine on ventral root responses have been studied on the isolated spinal cord of the frog.

2 In the absence of stimulation, the alkaloid induced a variety of activity of which the most notable was phasic simultaneous slow wave depolarization in the dorsal and ventral roots which could be reduced or suppressed by magnesium.

3 With low concentrations of bicuculline, the adjacent dorsal root response evoked by a single stimulus was depressed maximally before an increase in the ventral root response could be discerned.

4 The bicuculline-induced dorsal root activity (in the absence of stimulation) was still apparent at times when the evoked dorsal root response was reduced.

5 Bicuculline did not differentiate between the depressant effects of GABA and glycine on the evoked ventral root responses.

6 The excitant effects of bicuculline reported here did not appear to be attributable to specific antagonism of the postsynaptic depressant action of GABA.

Introduction

The alkaloid bicuculline induces convulsions in the frog and rabbit (Welch & Henderson, 1934) and in the cat (Godfraind, Krnjević & Pumain, 1970). It has been reported to antagonize selectively the depressant action of γ-aminobutyric acid (GABA) in various parts of the cat brain (Curtis, Duggan, Felix & Johnston, 1970; Curtis, Duggan, Felix, Johnston & McLennan, 1971; Curtis & Felix, 1971) and upon the cat spinal Renshaw cell (Curtis *et al.*, 1970).

The purpose of the investigation was two-fold, namely to examine the electrical characteristics of the action of bicuculline in the isolated spinal cord of the frog and to assess to what extent these characteristics can be attributed to selective antagonism of the postsynaptic depressant action of GABA on spinal neurones.

The effect of bicuculline upon ventral and adjacent dorsal root activity in the absence of stimulation and upon the root responses evoked by single stimuli has been studied on the frog isolated spinal cord and evidence was sought of the ability of the alkaloid to antagonize selectively the depressant effect of GABA on the ventral root response using the depressant action of glycine as a reference.

It is assumed on theoretical grounds that the decrease in presynaptic inhibition produced by bicuculline in the frog spinal cord (Davidoff, 1972) cannot be a factor influencing either the spinal root activity in the absence of stimulation or the evoked ventral root response to a single stimulus. A preliminary report of some aspects of this work has been communicated to the British Pharmacological Society (Pixner, 1973).

Methods

After removal from the frog (*Rana temporaria*), the spinal cord was mounted on its side in the centre compartment of a perspex bath in cooled (15° C) oxygenated (95% O_2 and 5% CO_2) frog Ringer solution (mM: NaCl 74.5, KCl 2.5, Na_2HPO_4 2.5, NaH_2PO_4 0.45, $CaCl_2$ 1.9, $NaHCO_3$ 35.0; glucose 2.0 g/litre). A lumbar ventral root was drawn through a hole in a partition into one side chamber and the corresponding dorsal root and the dorsal root adjacent to it were drawn in a similar manner into the other side chamber. Both side chambers were filled with mineral oil to prevent desiccation of the roots. The dorsal root was mounted upon a pair of platinum wire stimulating electrodes delivering (when required) a supramaximal rectangular pulse of 40 μs duration. The ventral root and adjacent dorsal root were each mounted upon platinum wire recording electrodes and the signals led to DC amplifiers prior to oscillographic display.

Bicuculline (5×10^{-6} to 5×10^{-5}M) was added

to the bathing medium and changes in adjacent dorsal and in ventral root activity observed for about 90 min ($n = 7$).

For the investigation of the selective antagonism of GABA, control depressions of the evoked ventral root response by GABA and glycine were first obtained. Following application, the amino acid action was monitored at 15 or 30 s intervals for 3 min to allow for maximum effect, at which point the drug was removed with several changes of bath fluid. The depressant effects of consecutive control applications of the selected concentrations (range 10^{-3} to 10^{-2} M) of each amino acid were consistent. Bicuculline (7×10^{-5} to 2.5×10^{-4} M) was then applied until suitable enhancement of the evoked ventral root response was obtained. Then the test dose of GABA or glycine was applied and the interaction monitored at 15 or 30s intervals for 3 minutes. In some experiments two different amino acid test doses, one low and one high were selected so that the effect of bicuculline on two grades of depression could be compared to ensure that the depression was submaximal. The influence of bicuculline on GABA depressant action was investigated in 10 preparations and in 7 of these the interaction with glycine was also examined. In 4 experiments the preparations were pre-treated with magnesium (2 to 3.5 mM $MgCl_2$) in an attempt to confine amino acid action to motoneurones by depressing interneuronal circuits, on the assumption that magnesium, which acts by reducing transmitter release, would have a depressant effect increasing in proportion to the number of synapses in the pathway.

Bicuculline (Pierce Chemical Co.) was recrystallized by my colleague, Dr M. Pringle and used as the hydrochloride salt. GABA and glycine were obtained from BDH Chemicals Ltd.

Results

The effects of bicuculline upon spinal root activity in the absence of electrical stimulation

Depending on the concentration, exposure to bicuculline was followed (after 5-40 min) by the simultaneous occurrence in ventral and adjacent dorsal roots of distinctive electrical activity. This activity varied both qualitatively and quantitatively from preparation to preparation and even from moment to moment in the same preparation.

Bicuculline (1×10^{-4} M) exposure was followed by the appearance of discharges in the ventral root unassociated with detectable slow depolarization (synaptic potential). In spontaneously active preparations there was an increase in the frequency of discharge. In addition bicuculline could induce waves of slow depolarization reflecting synaptic potentials (Barron & Matthews, 1938; Eccles, 1946) associated towards their peak with an asynchronous motoneurone discharge, in the ventral root (Fig. 1b, lower trace). Alternation of these types of activity was observed.

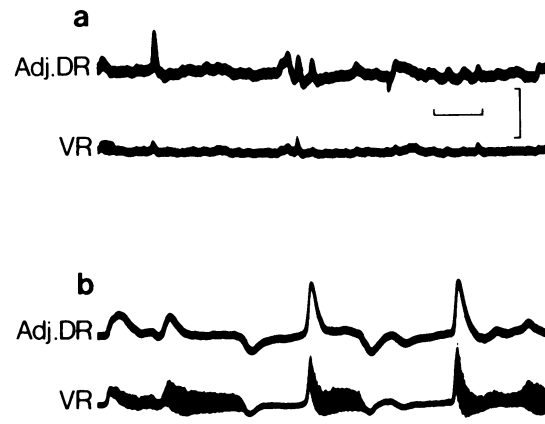

Fig. 1 The level of activity in the adjacent dorsal (upper trace of each pair) and ventral (lower trace) roots of the frog spinal cord in the absence of electrical stimulation. (a) control; (b) induced activity during exposure to bicuculline (7×10^{-5}M). Calibrations: (a) 200 μV for upper and lower traces; (b) 400 μV for both traces, time bar 5 s.

Bicuculline also promoted slow wave activity in the adjacent dorsal root (Fig. 1b, upper trace). Although these dorsal root slow waves usually occurred at the same time as the ventral root waves, they occasionally appeared in the absence of any change in the ventral root. As in the ventral root, discharges occurred in the dorsal root which were not associated with slow wave depolarization in either dorsal or ventral roots.

All bicuculline-induced root activity could be abolished by magnesium (10 mM $MgCl_2$) in concentrations insufficient to suppress completely the electrically evoked slow waves in dorsal and ventral roots, the latter indicating that the motoneurones were still responsive.

The effects of bicuculline on evoked dorsal and ventral root responses

Figure 2b-e shows that, during prolonged exposure to low (5×10^{-5}M) concentrations of bicuculline, there was a progressive decrease in the amplitude of the adjacent dorsal root response which became maximal (at about 26 min) before any increase in the ventral root response appeared. Although

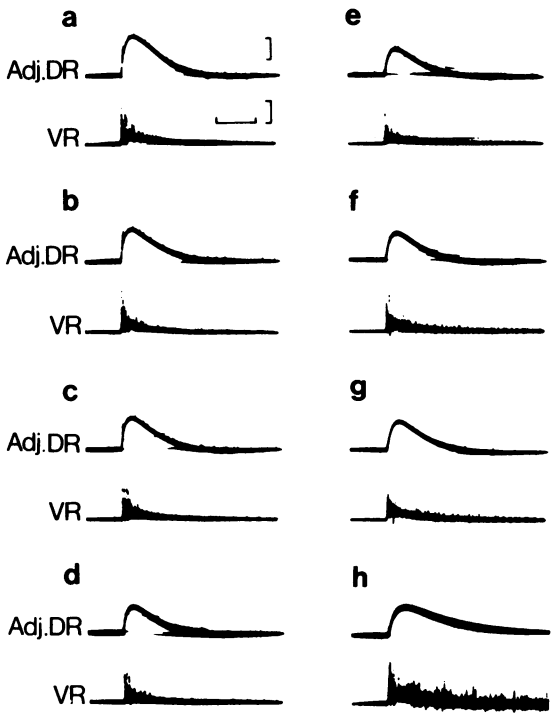

Fig. 2 Evoked responses in the adjacent dorsal (upper traces) and ventral (lower traces) roots of frog spinal cord. (a) control; (b-e) show progressive depression of the dorsal root response which precedes enhancement of the ventral root response; (f and g) during the action of bicuculline (5×10^{-5}M). Duration of exposure: (b) 4.5 min; (c) 9 min; (d) 18 min; (e) 26 min; (f) 43 min; (g) 58 min; (h) 34 min after raising the concentration of bicuculline to 7×10^{-5}M (117 min after initial exposure to the lower concentration of bicuculline) the ventral root response was still further enhanced but there was no further depression of the dorsal root response. Calibrations: upper trace, 500 μV; lower trace, 1 mV, 400 ms.

continued exposure to this concentration of bicuculline produced no further reduction of dorsal root potential amplitude, raising the concentration to 7×10^{-5}M increased the ventral root response (at 117 min) but did not add to the depression of the dorsal root response. Figure 2 illustrates the typical temporal and concentration-sensitive separation of bicuculline-induced changes in the dorsal and ventral root responses.

The effects of bicuculline on amino acid depression of evoked ventral root responses

GABA (10^{-3} to 10^{-2}M) greatly reduced the control evoked ventral root response (Figure 3b).

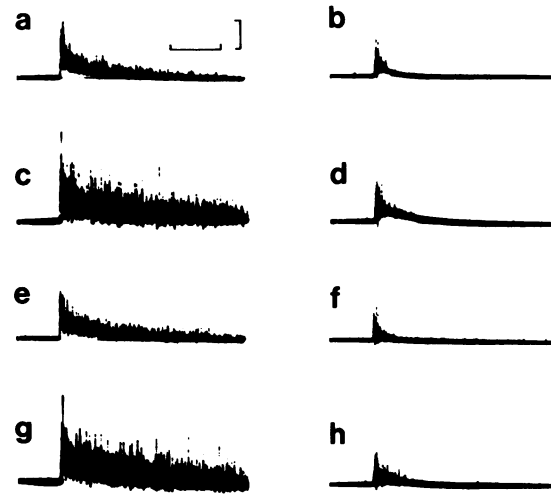

Fig. 3 The depressant effects of γ-aminobutyric acid (GABA) (a-d) and glycine (e-h) on the evoked ventral root response of frog spinal cord before and after exposure to bicuculline (6×10^{-5}M). (a) control; (b) maximum depression produced by 3 min exposure to GABA (10^{-2}M). GABA was removed and the preparation allowed to recover. (c) 35 min after initial exposure to bicuculline; (d) maximum depression produced by 3 min exposure to the test dose of GABA during the action of bicuculline. (e) control; (f) maximum depression produced by 3 min exposure to glycine (2×10^{-2}M). The glycine was removed and the preparation allowed to recover. (g) 65 min after initial exposure to bicuculline; (h) maximum depression produced by 3 min exposure to the test dose of glycine during the action of bicuculline. Calibrations for all records: 500 μV, 400 ms.

When the cord was exposed to bicuculline, the control response was substantially augmented (Fig. 3c) but was nevertheless still reduced by the same dose of GABA (Figure 3d).

Glycine too (10^{-4} to 10^{-2}M) depressed both control (Fig. 3f) and bicuculline augmented (Fig. 3h) responses.

Similar results were obtained in experiments in which a low and a high dose of amino acid (from within the above ranges) were used. Two grades of amino acid depression were employed to indicate that the concentration of amino acid was not supramaximal. Further evidence of submaximal depression was provided by the persistence of a residual discharge.

Results obtained from preparations pre-treated with magnesium (Pixner, 1973) were similar to those in untreated preparations.

Each amino acid produced the same degree of depression of the bicuculline augmented response, indicating that bicuculline had no selectivity for

either. Further, the amino acids produced the same degree of depression of both control and bicuculline augmented responses.

Discussion

Bicuculline action on the frog spinal cord consisted of changes in root activity in the absence of stimulation and in the evoked root responses. These changes consisted of the appearance of (in quiescent preparations) or an increase in (spontaneously active preparations) phasic activity which occurred simultaneously in dorsal and ventral roots. The evoked ventral root response was enhanced whilst the evoked dorsal root response was depressed in amplitude.

The occurrence of root potentials in the absence of stimulation may throw some light on the site of bicuculline action within the cord but in itself provides no indication of whether or not antagonism of the depressant action of GABA is the mechanism.

Both the occurrence of root potentials in the absence of stimulation and enhancement of the evoked ventral root response are electrical manifestations of convulsant activity. It seems probable, then, that spinal neurones participating in one aspect of this convulsant activity also participate in the other and that cells active in the absence of stimulation are also contributing to the enhancement of the evoked ventral root response.

Where GABA and glycine were used to produce submaximal depression of the evoked ventral root response, bicuculline did not selectively antagonize the action of GABA with respect to glycine. Even the possibility that bicuculline antagonized the depressant action of both amino acids is not supported by the results since the depressant action of either amino acid was little altered in the presence of bicuculline whereas the control responses were considerably enhanced by bicuculline. Whilst differences in drug access may provide a reason why bicuculline did not antagonize the depressant effect of applied GABA, there seems to be no immediate explanation here, where bicuculline is so obviously exerting a convulsant action and yet the submaximally depressant action of GABA is barely altered.

Assuming that there are no differences between applied and endogenously released GABA, the failure of bicuculline to antagonize GABA-induced depression of the evoked ventral root potential does not support the possibility that the observed spinal convulsant action of bicuculline (in the absence of stimulation) is attributable to antagonism of GABA in the frog cord.

The absence, reported here, of any selective antagonism of GABA by bicuculline in the frog is at variance with the findings of Curtis and co-workers in the cat (1970, 1971a, b) but inconsistent antagonism has been reported in the cerebral cortex of the cat (Godfraind *et al.*, 1970; Hill, Simmonds & Straughan, 1971).

The temporal and concentration-sensitive separation between bicuculline-induced depression of the evoked dorsal root response and enhancement of the evoked ventral root response suggests that the phenomena are not directly related. The greater sensitivity of the dorsal root potential, however, suggests that presynaptic inhibition is more sensitive to depression by bicuculline than the mechanism (obtaining here) by which the ventral root response to a single shock is enhanced. Since the conditioning-test shock technique was not used here, it is not possible to estimate this difference in sensitivity. The depression of the evoked dorsal root potential by bicuculline is compatible with antagonism by this alkaloid of the depolarizing action of endogenous GABA on dorsal root terminals (Davidoff, 1972). A similar situation has been reported to obtain with picrotoxin in the toad spinal cord in which picrotoxin depressed the evoked dorsal root potential and the depolarizing action of applied GABA on dorsal root terminals but did not antagonize the depressant action of GABA on the evoked ventral root response (Tēbecis & Phillis, 1969). Tēbecis & Phillis also reported a lack of consistency between changes in the evoked dorsal and ventral root responses during the action of picrotoxin.

Enhancement of the evoked ventral root response and prolongation of the after-discharge indicates an action of bicuculline at an interneuronal level in the frog spinal cord. An action of bicuculline on activity in interneurones in connection with both afferent terminals and motoneurones would readily explain the observed simultaneous occurrence of dorsal and ventral root activity in the absence of stimulation and is supported by the depression of this activity by magnesium. The predominantly phasic activity seen with bicuculline in the absence of stimulation does not suggest a direct excitatory action which would be characterized by tonic activity (unaffected by magnesium) as is the case with the excitant amino acids. It is possible that bicuculline antagonizes a post synaptic inhibitory system which normally masks a phasic background activity within the cord. However, the evidence gained from this study does not indicate what the inhibitory transmitter might be.

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