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STUDIES ON CONVULSANTS IN THE ISOLATED FROG SPINAL CORD. II. EFFECTS ON ROOT POTENTIALS

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SUMMARY

1. In the isolated frog spinal cord picrotoxin, bicuculline, and strychnine were evaluated for their effects on synaptically induced root potentials recorded by the sucrose gap technique.

2. Picrotoxin ($> 10^{-4}$ M) completely blocked the dorsal root potential (DRP) elicited by stimulating the ventral root of the same segment (VR-DRP). Although picrotoxin antagonized the DRP elicited by stimulation of either an adjacent dorsal root (DR-DRP) or the lateral column (LC-DRP), a slower component to these potentials appeared and increased in size as the concentration of picrotoxin was increased. Thus picrotoxin brings out a later, picrotoxin resistant component to the DR-DRP and LC-DRP.

3. Strychnine (10^{-8}–10^{-5} M) reduced and abolished the VR-DRP without prolongation and progressively increased and prolonged the DR-DRP (and LC-DRP) and the DR-VRP. Strychnine in higher concentrations ($> 10^{-4}$ M) also reduced the amplitude and prolonged the duration of the compound action potential of afferent fibres.

4. These results combined with those presented in the preceding paper (Barker, Nicoll & Padjen, 1975) suggest that (1) a GABA-like transmitter mediates the final step in the DR-DRP and LC-DRP pathways and that (2) either taurine or β-alanine may mediate the last step in the VR-DRP pathway.

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INTRODUCTION

γ-Aminobutyric acid or a closely related substance has been proposed as the putative transmitter mediating the synaptically evoked depolarizations of primary afferent fibres (i.e. dorsal root potentials). The original proposal was based mainly on the evidence that picrotoxin, an antagonist of GABA effects, antagonizes DRPs (Eccles, Schmidt & Willis, 1963). Since that observation a large number of studies appeared on the involvement of GABA in primary afferent depolarization (for a review, cf. Curtis & Johnston, 1974). It has been emphasized that qualitative studies are not sufficient in experiments utilizing antagonists for the identification of transmitter substances (Diamond, Roper & Yasargil, 1973). Ideally, one should show that quantitatively the antagonist is equally effective against both the synaptic potential and the action of the putative transmitter. The isolated, hemisected frog spinal cord represents a preparation of choice for such a study. In the preceding paper (Barker *et al.* 1975) the antagonistic action of convulsants on amino acid responses of primary afferent fibres was described. In this paper, quantitative data are presented on the interaction between the same convulsants and the synaptically elicited root potentials. Finally, an attempt is made to compare quantitatively the action of antagonists on amino acid and synaptic responses. A preliminary account of some of the results has appeared (Nicoll & Barker, 1973).

METHODS

Preparation, solutions, recording and general comments on methods (see Barker *et al.* 1975).

Fig. 1 represents a schematic diagram of the arrangement for simultaneous recording of synaptically evoked potentials in dorsal and ventral roots. The 8th dorsal root (DR8) was led from the chamber containing the spinal cord (R_1) through a Vaseline-lined slit into and through the sucrose compartment (S) and then through another Vaseline coated slit into a pool of Ringer solution (R_2). In a similar way the 9th ventral root (VR9) was led to another pool of Ringer solution (R_3). Pools R_2 and R_3 were connected to separate compartments (not presented on the diagram) via Ringer-agar bridges. The caudal portion of the cord was twisted (arrow) to achieve a close contact of the ventral root exit from the spinal cord with the Ringer-sucrose interface. On the opposite side of R_1 the 8th ventral (VR8) and 9th dorsal (DR9) roots were placed on platinum wires for stimulation in the compartment containing paraffin oil (P). A Vaseline coated cover was tightly placed over the chamber to maintain the separation of the pools.

Stimulation. Stimulation of the dorsal and ventral roots was accomplished through platinum wires. In some preparations a low resistance micro-electrode filled with 2 M-NaCl was used to stimulate the lateral columns and thus generate a dorsal root potential (LC–DRP).

Antagonism of synaptic and amino acid responses. After control recording of the root potentials, increasing concentrations of amino acid antagonists (picrotoxin, bicuculline, or strychnine) were added to the perfusing solution and permitted to

produce their maximal effect (requiring about 20–40 min). When seizure activity had developed, care was taken to elicit DRPs from a quiet base line, since superposition of a DRP on a depolarizing seizure wave resulted in erroneously small potentials. All responses were recorded with DC amplifiers, since root potentials, in the presence of convulsants, often lasted for many minutes.

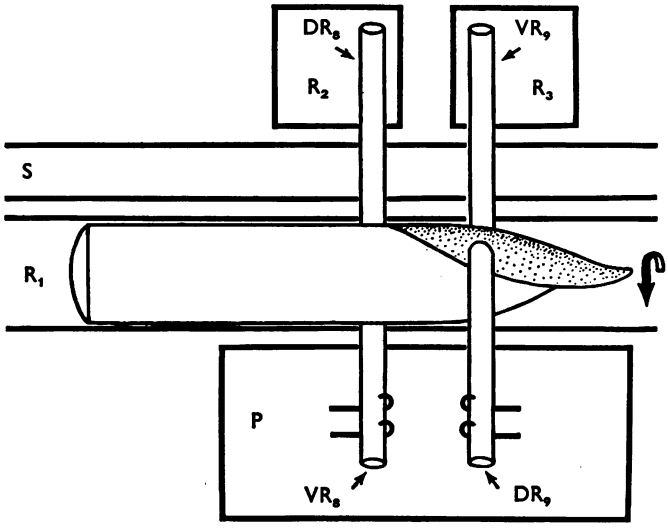

Fig. 1. Schematic diagram of the arrangement used for sucrose gap recording of root potentials. Further description in text.

RESULTS

Action of convulsants on root potentials

Synaptic potentials recorded with the sucrose gap technique were considerably larger than those recorded in the conventional manner (differential recording, using Ag-AgCl wires as electrodes, between a dorsal root in paraffin pool and cord in the bath or between two points on a dorsal root in paraffin pool). The synaptic potentials discussed in this paper will be abbreviated by listing the root stimulated first, followed by the root from which the potential was recorded. Using conventional recording techniques, DR-DRPs ranged in size from 1 to 4 mV and VR-DRPs from 0·1 to 1·5 mV (Barker & Nicoll, 1972, 1973); however, the use of sucrose gap techniques increased the size of the DR-DRPs recorded to 8–12 mV and VR-DRPs to 2–5 mV (e.g. Fig. 2).

As has been reported with conventional recording techniques, picrotoxin antagonized the DR-DRP and abolished the VR-DRP, while markedly augmenting the DR-VRP (Fig. 2*A*). Its action on the DR-DRP was, however, quite complex, as originally noted by Tebecis & Phillis (1969).

Fig. 2*B* shows superimposed records of the DR-DRP obtained at 5 min intervals after beginning perfusion with a Ringer solution containing 5×10^{-5} M picrotoxin. The early phase of the DR-DRP was progressively reduced by the picrotoxin even to 40% of control, during which time a second phase began to appear, eventually becoming larger than the earlier, picrotoxin-sensitive component. In high concentrations of picrotoxin

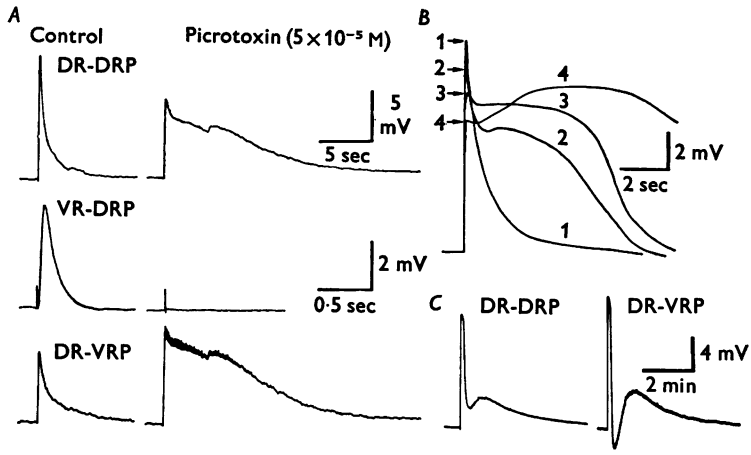

Fig. 2. Effect of picrotoxin on dorsal and ventral root potentials. *A*: specimen records of controls and 20 min after start of picrotoxin (5×10^{-5} M). *B*: superimposed records of DR-DRPs obtained at 5 min intervals after 5×10^{-5} M picrotoxin (added between records 1 and 2). Note the decrease in the size of the early component of the DRP (numbers with arrows) and increasing size of the late component. *C*: Dorsal and ventral root potentials after one hour bathing in 10^{-3} M picrotoxin. Note the increased duration and appearance of another later depolarizing component on the DR-DRP. The DR-VRP is now even more complex with the prolonged depolarizing phase (associated with intense motoneurone discharge) followed by a hyperpolarizing phase and then another long lasting depolarizing component.

($> 5 \times 10^{-4}$ M) an even later depolarizing component appears on both the DR-DRP and DR-VRP, often lasting for 5 min (Fig. 2*C*). Any root potential elicited during this sustained depolarization, which would not be recorded with an AC amplifier, is considerably reduced in amplitude. The DR-VRP potential regularly showed a transient hyperpolarization following the intense discharge of the motoneurones.

A more detailed comparison of the action of picrotoxin on the various root potentials is illustrated in Fig. 3 where the size of the potential (based on the measurement of the largest component) has been plotted as a function of the picrotoxin concentration. The VR-DRP was much more

sensitive to picrotoxin, being totally abolished at 10^{-4} M picrotoxin. The duration of this potential was unaffected by picrotoxin. On the other hand, the DR-DRP was reduced at relatively high concentrations of picrotoxin (10^{-5}–5×10^{-5} M) and then tended to recover to a normal amplitude at even higher concentrations. This recovery was due to the increased expression of the late picrotoxin-resistant component (cf. Fig. 2*B*). The amplitude was measured after the DR-DRP had stabilized in a particular picrotoxin concentration which required 20–40 min. The LC-DRP (not illustrated) behaved in a manner similar to the DR-DRP. The DR-VRP

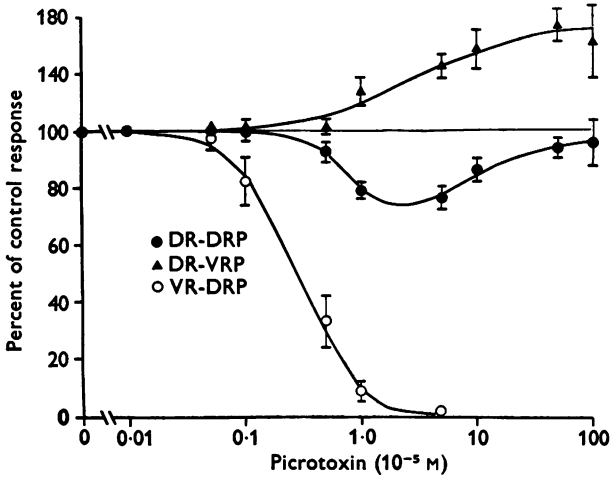

Fig. 3. Picrotoxin antagonism of the root potentials. The peak of the largest component of the synaptic potential is plotted as a function of increasing picrotoxin concentration for the DR-DRP (filled circles), the VR-DRP (open circles) and the DR-VRP (triangles) and expressed as a percentage of control (average of five to eight experiments ± S.E.).

was progressively increased in size with increasing concentrations of picrotoxin. Bicuculline (examined in three preparations, but not illustrated) produced results similar to those obtained with picrotoxin except that bicuculline was approximately 5–10 times more potent.

The effect of another convulsant, strychnine, on the DR-DRP and VR-DRP is illustrated in Fig. 4. The DR-DRP (and LC-DRP, not illustrated) tended to increase in size and became markedly prolonged (about 20–40 times control durations; note change in time scale between *b* and *c*) as the concentration of strychnine increased. On the other hand, the VR-DRP gradually decreased in size and was abolished at 10^{-5} M strychnine. The VR-DRP did not become prolonged, during its reduction,

suggesting that there was little increase in excitability of the interneurones involved in this pathway.

The curve for the DR-VRP, although not included in the graph, was similar to the curve obtained for the DR-DRP.

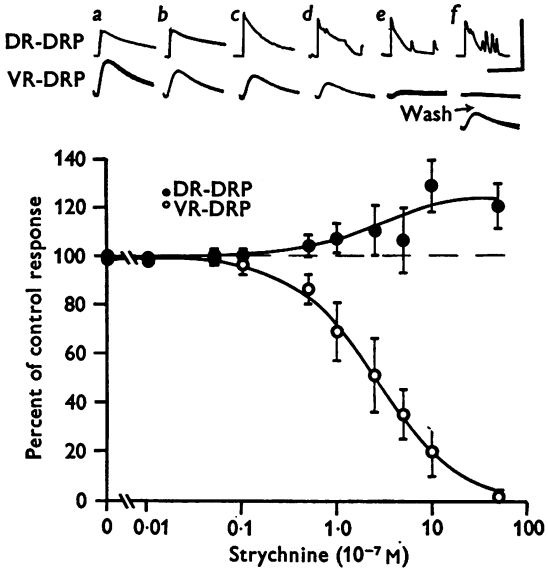

Fig. 4. Strychnine antagonism of the VR-DRP. The peak of the largest synaptic potential is graphed as a function of increasing strychnine concentration for the DR-DRP (filled circles) and VR-DRP (open circles) and expressed as a percent of control (average of four to six experiments ±S.E.). The VR-DRP is antagonized while the DR-DRP is increased in amplitude and markedly prolonged. Specimen records are illustrated above the graph. The lower case letters indicate the concentration of antagonist at which the record was obtained: a = control; $b = 5 \times 10^{-8}$; $c = 10^{-7}$; $d = 5 \times 10^{-7}$; $e = 10^{-6}$; $f = 5 \times 10^{-6}$; wash = VR-DRP 90 min after washing in strychnine-free Ringer. Calibration: 500 msec in a and b and 5 sec in c–f and 10 mV for the DR-DRP and 5 mV for the VR-DRP. The VR-DRP is composed of three to five superimposed traces.

Associated with the picrotoxin, bicuculline, and strychnine actions on the root potentials, there was a gradual depolarization of approximately 2 mV of both primary afferent and motoneurone membranes. Since both roots are depolarized to a similar extent the selective antagonism of the dorsal root potentials (compared to the ventral root potentials) means that the antagonism is unlikely to result from the depolarization *per se*. The addition of magnesium ions to the Ringer solution entirely blocked the depolarizations caused by the convulsants. Furthermore, in the range of concentration used to block synaptic responses convulsants did not show

any depolarizing action on the isolated primary afferents (see below). All these findings suggest that the observed depolarizing action of convulsants was caused by increased synaptic bombardment resulting from the convulsive state.

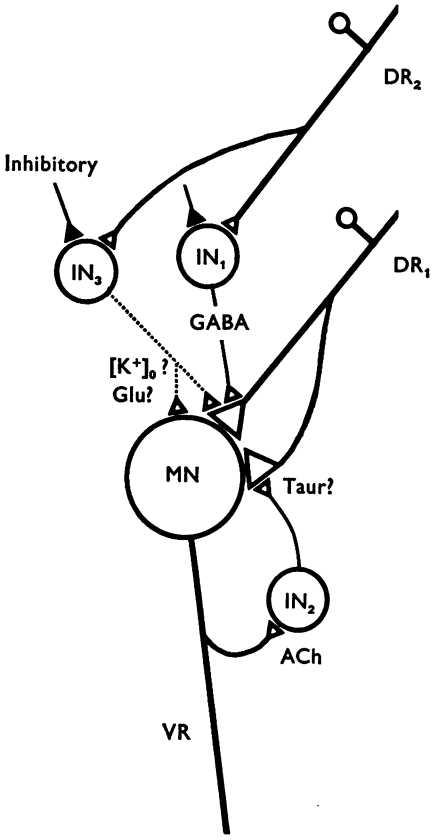

Fig. 5. Schematic diagram of proposed DR-DRP and VR-DRP pathways: DR_1 and DR_2 are two adjacent dorsal roots. IN_1, IN_2, and IN_3 are three interneurones presumed to be involved in the pathways. MN is a motoneurone. VR is the ventral root. Detailed description in the text.

Action of convulsants on the compound action potential of primary afferents

In addition to amino acid antagonism, the convulsants used in this study, particularly strychnine, are known to have a number of other actions on excitable membranes, including alterations in the action potential of peripheral nerves (Maruhashi, Otani, Takahashi & Yamada, 1956; Freeman, 1973), decreased excitability of primary afferent terminals

(Wall, McCulloch, Lettvin & Pitts, 1955; Curtis & Ryall, 1966) and decreases in active Na and K currents of invertebrate neurones and axons (Freeman, 1973; Klee, Faber & Heiss, 1973). Therefore, the action of convulsants was examined on the compound action potential of the primary afferent fibres. A reduction in size and a marked prolongation of the action potential occurred in the presence of strychnine. These changes were evident at a concentration of at least 10^{-4} M strychnine (at 18° C). The effect of picrotoxin was similar, but required higher concentrations, especially in regard to the prolongation of the action potential. These changes in the compound action potential were not associated with any significant change in the membrane potential of the primary afferents. It seems unlikely that the above effects, which required relatively high concentrations, are related to the specific amino acid antagonisms observed on the primary afferent terminals.

DISCUSSION

Effect of convulsants on root potentials

The root potentials examined in this study demonstrated differential sensitivity to the convulsants. The VR-DRP was antagonized by both picrotoxin (and bicuculline) and strychnine. The DR-DRP and LC-DRP were resistant to the action of strychnine and sensitive to the action of picrotoxin and bicuculline. The DR-VRP was not antagonized by any of the convulsants. These results suggest that, on a pharmacological basis, these three synaptic potentials are generated by pathways that at some point have different synaptic transmitters. Moreover, the observation that both picrotoxin and strychnine can block the VR-DRP raises the possibility that they might both antagonize the same agonist.

In addition to the picrotoxin-sensitive component of the DR-DRP and LC-DRP, there was also a later component which was markedly augmented by, and resistant to the action of picrotoxin. Thus at high concentrations of picrotoxin the amplitude of the resistant component of the DR-DRP (and LC-DRP) returns toward the value obtained in normal Ringer solution. To what extent this picrotoxin resistant component contributes to the normal DR-DRP is unclear, but it is possible that up to 40% of the DR-DRP is due to this component (calculated from the maximum reduction observed with picrotoxin). A number of factors could contribute to this picrotoxin-resistant potential. An acidic amino acid (such as glutamate) which is not blocked by picrotoxin might be released from adjacent synapses and activate the glutamate receptors on the primary afferent terminals. The possibility that transmitters released from a synapse might affect immediately adjacent primary afferent terminals

was originally proposed by Fatt (1954). Another possibility is that elevated extracellular K which occurs in the spinal cord following afferent volleys (Vyklicky, Sykova, Kriz & Ujec, 1972; Krnjević & Morris, 1972; Liebl, Lux and Ten Bruggencate, 1972; Kriz, Sykova, Ujec & Vyklicky, 1974; Somjen & Lothman, 1974) could depolarize the afferent terminals, and this depolarization would be resistant to picrotoxin, since picrotoxin has no effect on potassium depolarization of primary afferent fibres (see below).

That strychnine increased the size of the DR-DRP (and LC-DRP) while blocking the VR-DRP confirms previous reports (Eccles & Malcolm, 1946; Schmidt, 1963; Grinnell, 1970). The increase in the DR-DRP size presumably results from the release of post-synaptic inhibition on the interneurones involved in generating DR-DRP (Schmidt, 1963).

Comparison of the action of convulsants on amino acid and synaptic responses

Based on the sensitivity of the root potentials and amino acid responses to the action of picrotoxin, bicuculline and strychnine three distinct categories of responses are evident (cf. Barker *et al.* 1975): (1) those sensitive to all the convulsants (β-alanine, taurine and VR-DRP); (2) those sensitive only to picrotoxin and bicuculline (GABA and DR-DRP); and (3) those sensitive to none of the convulsants (glutamate and DR-VRP) (Table 1). Glycine responses on the primary afferents are also resistant to all of the convulsants but the low depolarizing potency of this amino acid makes it an unlikely transmitter candidate for depolarizing synaptic potentials. A comparison of the effects of picrotoxin on GABA responses and on the DR-DRP revealed that both phenomena were reduced at similar picrotoxin concentrations. However, the synaptic potential was less reduced due to the appearance of a resistant component of the DR-DRP, as discussed above. Furthermore, strychnine did not block either the GABA response or the DR-DRP.

The interaction of picrotoxin with β-alanine and the VR-DRP is quantitatively very similar. However, antagonism of β-alanine responses required about ten times the concentration of strychnine than did the VR-DRP. It is possible that if taurine had been used instead of β-alanine for this quantitation, a better agreement would have been obtained. Another possibility is that strychnine might also affect other steps in this pathway. For instance, the postulated cholinergic step (Kiraly & Phillis, 1961; Grinnell, 1966; Koketsu *et al.* 1969) might be affected by the curare-like action of strychnine (e.g. Alving, 1961; Landau, 1967).

Although the use of picrotoxin and bicuculline antagonism has limitations because it is not entirely specific for GABA, other biochemical and pharmacological data (Miyata & Otsuka, 1972; Bell & Anderson, 1972;

Roberts & Mitchell, 1972; Collins, 1974) also support the notion that GABA is a spinal cord transmitter. The specificity of strychnine for β-alanine and taurine does suggest that either of these compounds could be the transmitter in the VR-DRP. However, the greater sensitivity of the VR-DRP *vis-a-vis* β-alanine raises the possibility that strychnine may not be acting only at the final step in this pathway. Of all the amino acids tested in the frog spinal cord, taurine is present in the highest concentration (Collins, 1974) which makes taurine a more likely transmitter candidate.

TABLE 1. Categorization of convulsant responses from root potential sensitivity and amino acid response

Root potential	Antagonism: Picro	Antagonism: Stryc	Amino acid response
DR-DRP	+ +	0	GABA
VR-DRP	+ + +	+ + +	Taurine β-alanine
DR-VRP	0	0	Glutamate

In Fig. 5 an attempt is made to relate the present findings to those reported previously in the literature. Volleys in DR_2 are proposed to activate interneurones (IN_1) (there may be more than one in series) which in turn liberate GABA at axo-axonic synapses on to the terminal of DR_1. The terminal depolarization of DR_1 would then reduce the action potential in DR_1's terminal and thus the post-synaptic excitatory potentials recorded in the motoneurone (MN) (i.e. pre-synaptic inhibition). This process has been reported to occur in afferents which end both on interneurones (Grinnell, 1966; Davidoff, 1972) and also on motoneurones (Meij & Holemans, 1969) (shown in Fig. 5).

Stimulation of the VR will excite interneurones (IN_2) via recurrent collaterals through a cholinergic synapse. Although considerable pharmacological evidence supports such a pathway, a Renshaw-type interneurone has not been described physiologically in the frog spinal cord. These interneurones are shown as ending directly on primary afferents, as suggested by Meij & Holemans (1969), but the long latency of the VR-DRP suggests that more than one interneurone may be involved. It is proposed that either β-alanine or taurine may be liberated at the axo-axonic contact formed by these interneurones. The strychnine sensitivity of this 'recurrent' pathway parallels the Renshaw-type system in the mammal, although the inhibitory mechanism has shifted from a presynaptic (frog) to a post-synaptic (mammal) process. However, the picrotoxin and bicuculline sensitivity of recurrent inhibition in the frog, but not in the mammal, indicate that differences exist between these two systems.

Whether the difference is due to different sensitivities of the depolarizing (presynaptic) and hyperpolarizing (post-synaptic) amino acid receptors to picrotoxin and bicuculline or to the liberation of different transmitters remains to be elucidated.

In addition to the synaptic potentials described above which are blocked by the convulsants, there is also a component of the DR-DRP which is resistant to, and markedly augmented by the convulsants. This resistant component is shown by IN_3 which is proposed to be disinhibited by the convulsants (block of black synapse). The manner in which this pathway exerts its effect on DR_1 is unsettled but might result from increased extracellular potassium or possibly by a picrotoxin-resistant transmitter, such as glutamate. If the resistant component were due only to elevated extracellular K, a separate interneuronal system would not be necessary and the increased K could arise from activity in the interneurones which release GABA.

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