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DEPOLARIZATION OF FELINE PRIMARY AFFERENT FIBRES BY ACIDIC AMINO ACIDS

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SUMMARY

1. When administered micro-electrophoretically into the spinal grey matter of cats anaesthetized with pentobarbitone, acidic amino acids known to be neuronal excitants lower the threshold of electrically stimulated muscle and cutaneous primary afferent fibres and terminations. This depolarizing effect was not observed with fibres stimulated in the white matter.

2. Depolarization by micro-electrophoretic potassium and excitant amino acids appeared not be be associated with an alteration in terminal membrane conductance since there was no change in synaptically evoked primary afferent depolarization.

3. Excitant amino acid depolarization was not blocked by the γ-aminobutyric acid antagonist bicuculline methochloride, but was reduced by selective excitant amino acid antagonists.

4. The results are discussed in relation to the probable absence of specific excitant amino acid receptors on afferent terminals, the depolarizing effect of the amino acids on myelinated fibres and non-myelinated terminals being more likely a consequence of changes in the extracellular medium associated with the depolarization and firing of neurones.

INTRODUCTION

Under both *in vitro* and *in vivo* conditions amino acids which excite neurones also depolarize the intraspinal portion of vertebrate primary afferent fibres (Phillis, 1960; Schmidt, 1963; Curtis & Ryall, 1966; Barker, Nicoll & Padjen, 1975; Curtis, Lodge & Brand, 1977; Evans, 1978). In the case of the superfused amphibian spinal cord, such depolarization has been proposed (Evans, 1980; see also Barker *et al.* 1975) to result not from a direct interaction with excitant amino acid receptors, such as those which occur on neurones, but indirectly as a consequence of the elevated extracellular potassium ion concentration resulting from neuronal depolarization and firing (Kudo & Fukuda, 1976; Sonnhof & Buhrle, 1980). In contrast, differences in the depolarization by L-glutamate of various types of afferent fibre in the amphibian isolated spinal cord have led to the proposal that an important factor is electrical coupling to depolarized neurones, primary afferent fibres having synapses upon motoneurones being more

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depolarized by L-glutamate than fibres of muscle and cutaneous origin which established only polysynaptic connexions with these cells (Shapovalov, Shiriaev & Tamarova, 1983).

In *in vitro* studies of this type, however, in which amino acids are added to the solution bathing the preparation, and responses are recorded from one or many dorsal root fibres, definitive evidence is unlikely to be obtained regarding the site of amino acid depolarization. The insensitivity to excitant amino acids of *extra*spinal dorsal root fibres has led to the assumption that only the *terminations* within the cord are depolarized (Evans, 1980). Yet, as with potassium, micro-electrophoretically administered L-glutamate lowers the electrical threshold, and thus presumably depolarizes, both the terminal and non-terminal portions of group I a afferent fibres in the grey matter of the feline spinal cord *in vivo* (Curtis & Lodge, 1982; D. R. Curtis, P. M. Headley & D. Lodge, unpublished observations). This contrasts with the depolarizing action of micro-electrophoretic γ-aminobutyric acid (GABA) and some related amino acids, which is restricted to the terminal regions of these and other afferent fibres, and has been related to the function of depolarizing bicuculline-sensitive axo-axonic synapses where GABA is the transmitter (see Curtis & Lodge, 1982; Curtis, Lodge, Bornstein, Peet & Leah, 1982). Such synapses are presumably associated with bicuculline-sensitive depolarization of primary afferent fibres and terminals (primary afferent depolarization, p.a.d.) produced by impulses in nerve fibres of segmental and descending origin, a depolarization accompanied by a reduction in the electrical threshold of primary afferent fibres and their central terminals. When administered micro-electrophoretically, however, many amino acids, including GABA, structurally related compounds which inhibit the firing of neurones, and others which have no effect on neuronal firing, *increase* the electrical threshold of non-terminal myelinated portions of I a afferent fibres stimulated within the spinal grey matter (Curtis *et al.* 1982). Such an effect seems unlikely to be related to any synaptic process.

This paper reports in more detail an investigation of the effects of excitant amino acids upon muscle and cutaneous afferent fibres in the cat spinal cord. The results support the proposal that the depolarizing effects are indirect, being the consequence of the excitation of neurones, and are not restricted to the terminations of afferent fibres. This depolarization, unlike that mediated by GABA, may thus be insignificant as a process controlling the input of afferent information to the mammalian central nervous system.

METHODS

The experiments were carried out on primary afferent fibres within the L_7 and S_1 segments of spinal cats (T_{12} section) anaesthetized with sodium pentobarbitone (35 mg kg^{-1} intraperitoneally initially, supplemented intravenously as necessary). The terminations of group I a afferent fibres of the plantaris, flexor digitorum longus and gastrocnemius muscles were stimulated within the respective motonuclei of the ventral horn and also in the intermediate nucleus (Rexed lamina VI) close to interneurones fired monosynaptically by group I a or group I b volleys. These afferents were also stimulated in the dorsal horn and in the dorsal columns. The terminations of cutaneous (sural, superficial peroneal and tibial) afferent fibres (Aα and Aδ) were studied in the dorsal horn, and the faster conducting fibres also within the dorsal column. When fibres or terminations were stimulated in the dorsal horn or column, sites of stimulation were subsequently checked histologically in relation to either dye marks (Acid Fast Green) or micromanipulator coordinates of cord surface features.

As in previous investigations (Curtis & Lodge, 1982), fibres and terminations were stimulated at 10 Hz using 0·3 ms negative pulses of less than 2 μA amplitude passed through the central 3·6 M-NaCl-containing barrel (4–7 MΩ) of seven-barrel micropipettes, the other barrels containing aqueous solutions of other substances used in this study. Antidromically conducted impulses were recorded monophasically from the central end of transected peripheral nerves, and the amplitude of the threshold pulse was monitored continuously using a feed-back device which maintained the firing index of a particular fibre at 50 % (Curtis, 1979). The multiplying factor in the feed-back system was set at 1·00, and percentage changes in the amplitude of the threshold pulse, indicated by a continuous plot of the multiplying factor, were used as a measure of changes in threshold (ΔT). Terminations (boutons, pre- and inter-bouton non-myelinated fibres) were identified on the basis of location near neurones fired monosynaptically by impulses in fibres of that type (determined on the basis of conduction velocity), anodal blocking factors (see Curtis & Lodge, 1982) less than 4, depolarization by micro-electrophoretic GABA or piperidine-4-sulphonate (P4S) using currents of 20–40 nA, and the reduction of primary afferent depolarization (p.a.d.) by micro-electrophoretic bicuculline methochloride (20–40 nA). For convenience in the text, the term p.a.d. will be used to refer to the percentage reduction in threshold of fibres and terminals by appropriately timed volleys in other segmental afferent fibres. The p.a.d. of Ia extensor afferent terminations was generated by repetitive stimulation (four volleys at 320 Hz) of low threshold posterior biceps–semitendinosus (p.b.s.t.) afferent fibres 30 ms prior to each threshold testing pulse for 6–8 s. Similar tetanic stimulation of low threshold cutaneous afferent fibres lowered the threshold of terminations of cutaneous fibres.

Amino acids and other substances were ejected electrophoretically with currents not exceeding 100 nA from aqueous solutions within the outer barrels of the pipettes, all currents were balanced by passing either Na^+ or Cl^- ions from a barrel containing NaCl. The following substances were used: NaCl (1·8 M); Acid Fast Green FCF (saturated in 2 M-NaCl); KCl (200 mM); GABA (200 mM, pH 3, HCl); piperidine-4-sulphonate (P4S, 20 or 50 mM in 150 mM-NaCl, pH 3·2); bicuculline methochloride (BMC, 10 mM in 150 mM-NaCl); L-glutamate (200 mM, pH 7·5, NaOH); L-aspartate (200 mM, pH 7·5, NaOH); DL-homocysteate (DLH, 200 mM, pH 7·5, NaOH); D-homocysteate (100 mM, pH 7·5, NaOH); *N*-methyl-D-aspartate (NMDA, 50 mM in 150 mM-NaCl, pH 7·5, NaOH); kainate (5 mM in 150 mM-NaCl, pH 7·5, NaOH); quisqualate (5 mM in 150 mM-NaCl, pH 7·5); D-α-aminoadipate (0·2 M, pH 8, NaOH); ($\pm$)-2-aminophosphonovalerate (2APV, 50 mM in 150 mM-NaCl, pH 7·1, NaOH).

In this investigation, P4S was generally used, rather than GABA, as a potent bicuculline-sensitive GABAmimetic poorly taken up by spinal tissue, and hence less likely to produce a biphasic decrease and increase in the electrical threshold of afferent fibre terminations (see Curtis *et al.* 1982).

RESULTS

Site of excitant amino acid actions

The effects of excitant amino acids were studied on primary afferent fibres at three different sites in the lumbar cord: in the dorsal column and at non-terminal and terminal regions within the grey matter (Fig. 1).

In the dorsal columns, the thresholds of eighteen group Ia muscle (ten discussed by Curtis & Lodge, 1982) and twenty cutaneous fibres were reduced by potassium, reductions in threshold of 5–15 % being produced with ejection currents of 40–80 nA. No consistent changes occurred with GABA, P4S, L-glutamate or DLH ejected with similar currents, although DLH frequently reduced the threshold of deeper fibres close to the border of the dorsal horn. In the dorsal column bicuculline methochloride (20–80 nA) had no effect on p.a.d. of either muscle or cutaneous afferent fibres. Fig. 1*A* illustrates the failure of L-glutamate to modify the threshold (0·5 μA) of a plantaris Ia afferent fibre (blocking factor greater than 6) which was, however, lowered by potassium.

As has been reported for ventral horn Ia terminations with L-glutamate (Curtis

& Lodge, 1982), excitant amino acids (L-aspartate, DLH, D-homocysteate, NMDA, quisqualate, kainate) readily lowered the threshold of afferent terminations stimulated in the dorsal horn (cutaneous Aα and Aδ; thirty-three terminations), intermediate nucleus (muscle Ia and Ib; five) and ventral horn within motonuclei (muscle Ia and II; 140). Thresholds were also reduced by potassium, P4S and GABA, the latter

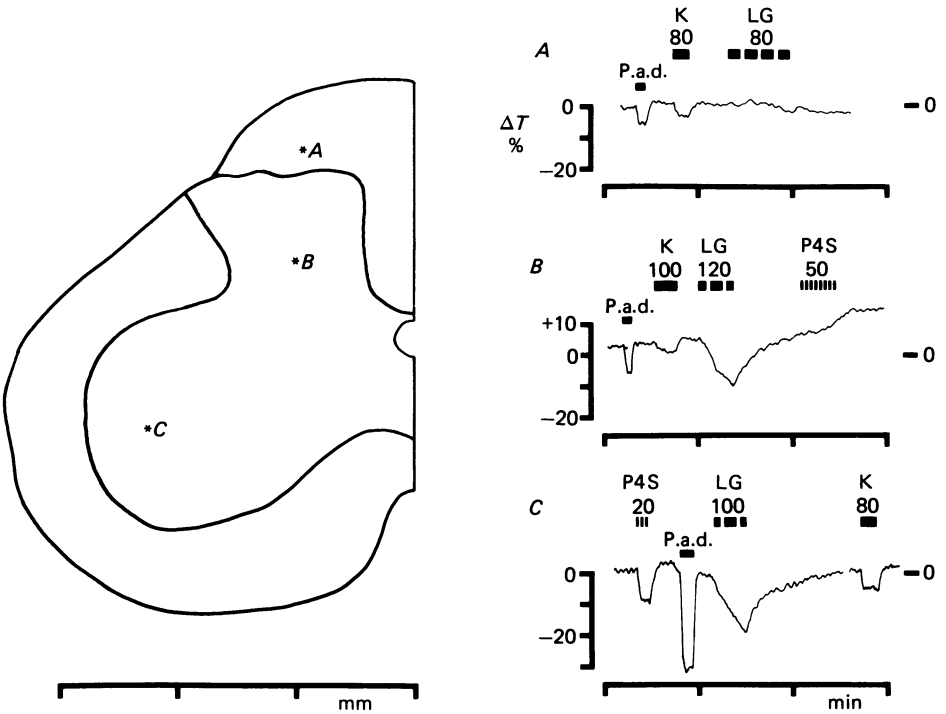

Fig. 1. Changes (ΔT) in the electrical threshold (10 Hz stimulation, 50% response) of Ia fibres (*A* and *B*) and a termination (*C*) stimulated at the sites indicated in the hemi-cross-section of the spinal cord and produced by micro-electrophoretically administered potassium (K), L-glutamate (LG) and piperidine-4-sulphonate (P4S) for the times indicated by the horizontal bars (currents, nA). Primary afferent depolarization (p.a.d.) was produced by tetanic stimulation of the posterior biceps–semitendinosus nerve (four volleys, 2*T*, 320 Hz) 30 ms prior to the testing pulse. *Ordinates*: percentage change in control threshold. *Abscissae*: time in minutes. All results from the same experiment using the same micropipette. *A*: plantaris Ia fibre stimulated in the dorsal column, threshold (*T*) 0·5 μA, anodal blocking factor (BF) > 6. *B*: gastrocnemius Ia fibre in the dorsal horn, *T* 0·9 μA, BF > 6. *C*: gastrocnemius Ia termination near gastrocnemius motoneurones, *T* 0·5 μA, BF 2·4.

frequently having a biphasic action (Curtis *et al.* 1982). Furthermore, both p.a.d. and depolarization by gabamimetics were reduced by BMC which generally had no effect on the reduction of threshold by the excitants. Fig. 1*C* illustrates the effects of P4S, L-glutamate and potassium on a gastrocnemius termination (threshold 0·5 μA, blocking factor 2·4) stimulated in the immediate vicinity of motoneurones.

Excitant amino acids also reduced the threshold of three Ia afferent *fibres* stimulated within the dorsal horn but dorsal to the intermediate nucleus, as

illustrated in Fig. 1*B* for a gastrocnemius fibre (threshold 0·9 μA, blocking factor greater than 6). In this region, remote from Ia terminals in the intermediate nucleus, the thresholds of Ia fibres were reduced by potassium, but not by either P4S or GABA, and the p.a.d. was insensitive to micro-electrophoretic BMC.

At all sites within the grey matter at which excitant amino acids reduced the threshold of primary afferent fibres or terminations, neurones were observed to be excited by these substances, as has been established in numerous previous investigations.

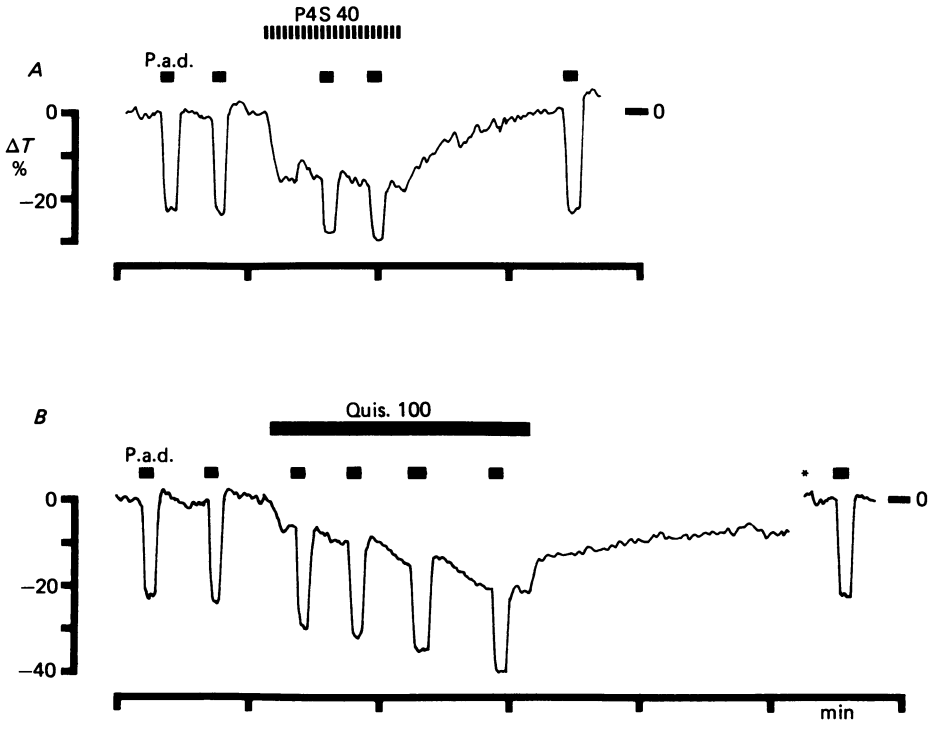

Fig. 2. Changes in the threshold, as in Fig. 1, of a gastrocnemius Ia termination (*T* 0·9 μA, BF 3·2) in the ventral horn produced by p.a.d. and P4S (*A*) and quisqualate (Quis., *B*). At the asterisk the stimulating pulse was adjusted to restore the multiplying factor of the feed-back system to 1·00 (see text).

Mechanism of threshold reduction

In contrast to the reduction of p.a.d. during the depolarization of Ia terminations by GABAmimetics, p.a.d. was not altered during reductions in threshold by either potassium or L-glutamate (Curtis & Lodge, 1982). Thus, whereas a conductance increase apparently accompanies depolarization by GABAmimetics, no such increase was detected during changes in threshold induced by potassium or the excitant amino acid. An example of these differing actions is illustrated in Fig. 2 for a ventral horn gastrocnemius Ia termination (threshold 0·9 μA, blocking factor 3·2). During the 17 % reduction in threshold by P4S (Fig. 2*A*), p.a.d. was reduced from 24 to 16 %, when measured as a percentage of actual threshold at the time, yet when quisqualate

reduced the threshold by 21% (Fig. 2*B*) p.a.d. remained at 25%. Even when thresholds of terminations were reduced by as much as 60–80% by NMDA, D-homocysteate or kainate, p.a.d. was not reduced, although errors in the measurement of the small changes in threshold associated with p.a.d. at these very low levels made comparison difficult.

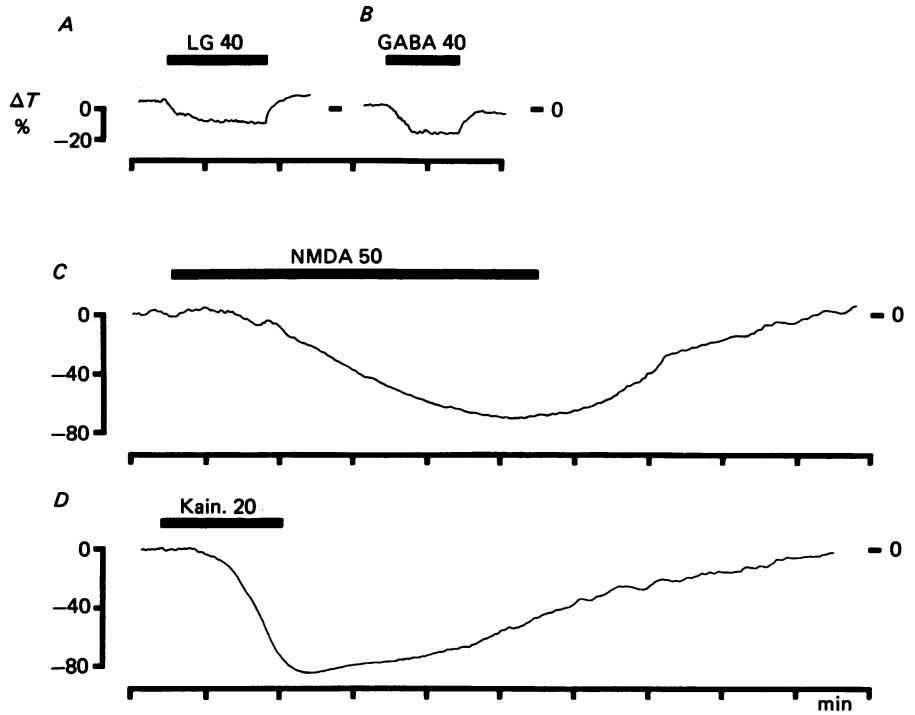

Fig. 3. Changes in the threshold, as in Fig. 1, of a ventral horn gastrocnemius Ia termination (T 0·7 μA, BF not determined) produced in *A*, by L-glutamate (LG), in *B* by GABA, in *C*, by *N*-methyl-D-aspartate (NMDA) and in *D*, by kainate (Kain.).

Relative potencies and time courses

Although only a limited number of comparisons were possible, the relative potencies and time courses of action of excitant amino acids in reducing the thresholds of Ia fibres and terminations were similar to these parameters in relation to the excitation of neurones. L-aspartate and L-glutamate were approximately equipotent with similar time courses of onset and recovery, maximum effects and recovery each occurring within 1 min (Figs. 1*B* and *C* and 3*A*). In contrast, DL-homocysteate, D-homocysteate, NMDA, and especially kainate were much more effective, producing large and reversible reductions in threshold for smaller ejecting currents when allowance is made for dilution in NaCl of the solutions within the micropipettes. Furthermore, the onsets and offsets were generally much slower than with L-glutamate, as illustrated in Fig. 3*C* and *D*. Quisqualate (Fig. 2*B*) was also more effective in reducing the threshold of Ia terminations than was L-glutamate, but the time course of recovery was usually faster than that following NMDA or kainate.

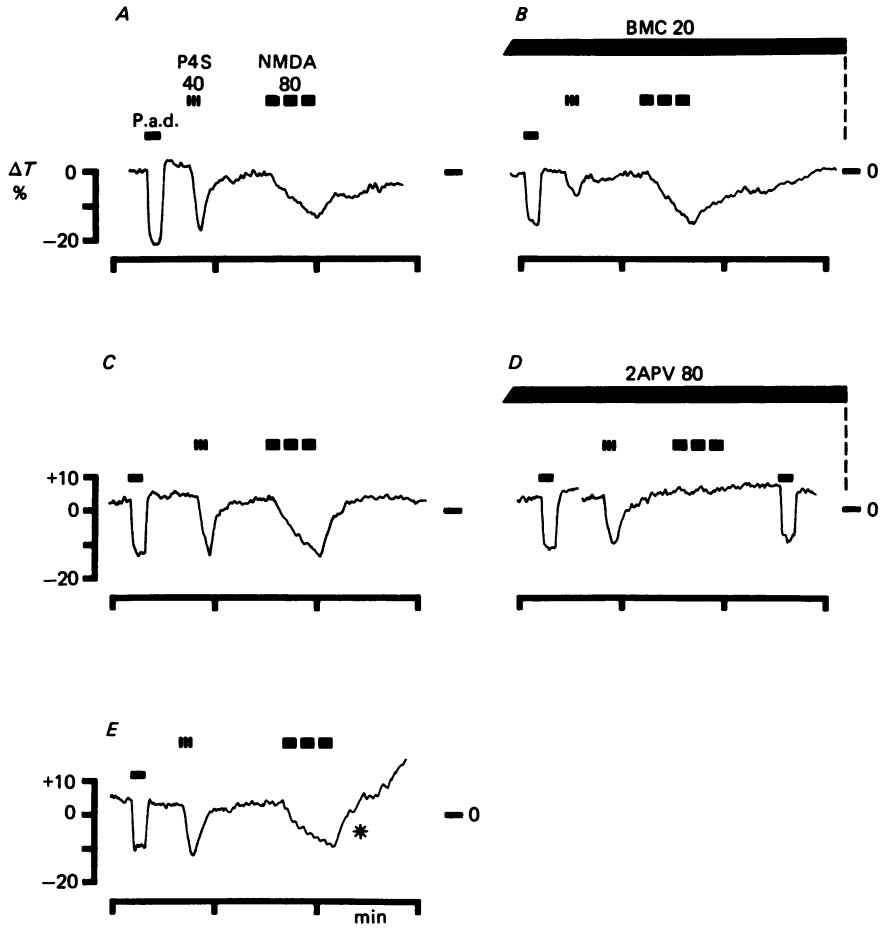

Fig. 4. Changes in the threshold, as in Fig. 1, of a gastrocnemius Ia termination (T 0·8 μA, BF 3·8) simulated in the ventral horn, and produced by p.a.d., P4S and NMDA. *A*: before, *B*: during the ejection of bicuculline methochloride (BMC) which commenced 5 min earlier and ceased at the vertical dashed line. *C*: 4 min after *B*. *D*: during the ejection of 2-aminophosphonovalerate (2APV) which commenced 2 min earlier and ceased at the vertical dashed line. *E*: 2 min after *D*. At the asterisk sudden movement of the termination in relation to the micropipette prevented further investigation.

Amino acid antagonists

BMC. With ejecting currents adequate to block the depolarization of Ia terminations by GABA or P4S, and to attenuate considerably p.a.d., BMC had no effect on the reduction of threshold of twenty-four of these structures by excitant amino acids, as illustrated in Fig. 4*A–C*.

The threshold of this lateral gastrocnemius Ia termination (threshold 0·8 μA, blocking factor 3·8) was reduced during p.a.d. and by P4S and NMDA. When ejected for 8 min, BMC (20 nA) reduced p.a.d. from 23 to 14% and the effect of P4S from 18 to 8% without reducing the effect of NMDA (Fig. 4*A–C*). BMC, ejected with currents as high as 80 nA, was also without effect on the reduction of threshold of

twenty-three of twenty-four other terminations by L-glutamate, DL-homocysteate, D-homocysteate, quisqualate and kainate.

With only one termination did BMC modify the effect of NMDA and quisqualate. In this instance, with an amount of BMC that abolished a 15% reduction in threshold by P4S and reduced p.a.d. from 22 to 11%, the onsets of the effects of NMDA and quisqualate were slowed although the maximum reductions in threshold were unaffected. Since both excitants had unusually rapid actions on this particular termination stimulated within the gastrocnemius motonucleus, both may have excited interneurones which synapse with the Ia terminal, an initial depolarization mediated by GABA being replaced by a bicuculline-insensitive component associated with the excitation of other neurones. Other evidence, however, suggests that GABA releasing interneurones of the p.a.d. pathway are generally not close to motoneurones (Jankowska, McCrea, Rudomin & Sykova, 1981; Curtis & Lodge, 1982; Curtis *et al.* 1982).

Excitant amino acid antagonists. Although an extensive study was not made, D-α-aminoadipate (Biscoe, Evans, Francis, Martin & Watkins, 1977; Lodge, Headley & Curtis, 1978) diminished the reduction of threshold of three terminations by L-aspartate without affecting p.a.d. or depolarization by GABA. Furthermore, 2APV (Davies & Watkins, 1982; Peet, Leah & Curtis, 1983) reduced the effects of NMDA on the threshold of three fibres; with one of these three the action of NMDA was reduced to a greater extent than that of quisqualate. The reversible abolition by 2APV of the action of NMDA on one Ia termination is illustrated in Fig. 4*C-E*, there being no reduction in either p.a.d. or the depolarization by P4S.

Such observations suggest a similarity between membrane receptors associated with the depolarization of neurones by these excitant amino acids and those associated with the reduction of the threshold of primary afferent terminations.

DISCUSSION

In view of the evidence cited in the Introduction, it is reasonable to assume that reductions in the electrical threshold of extracellularly stimulated afferent fibres and terminals within the spinal cord are associated with membrane depolarization, as had been demonstrated, for example, *in vivo* by Sastry (1979). Unlike the depolarization of primary afferents by GABAmimetics which is restricted to terminal regions (Curtis & Lodge, 1982), the reduction by excitant amino acids of the threshold of primary afferent fibres appears to be related to their proximity to neurones rather than being confined to terminals. Hence, although a direct depolarizing action of these amino acids at receptors on afferent terminals cannot be fully excluded, observations such as those of Fig. 1 strongly support the proposition that the depolarization of fibres and terminations is indirect and secondary to neuronal excitation. Evidence in support of the direct activation of excitant amino acid receptors on afferent terminals (perhaps associated with enhanced transmitter release; see Ransom, Bullock & Nelson, 1977; Brookes, 1978) would be difficult to obtain in the present type of investigation, unless the use of a more extensive series of agonists and antagonists revealed differences between the effects of different excitant amino acids on neurones and terminals. Since depolarization by excitant amino acids was not reduced by BMC, except in the one example discussed above, it was unlikely to be the consequence of the excitation of interneurones which release GABA at synapses upon afferent terminals.

Synaptic excitation of spinal neurones *in vivo* has been shown to be associated with increases in extracellular potassium activity (a^o_K; reviewed by Somjen, 1979). Under *in vitro* conditions, superfused L-glutamate also increases a^o_K in the vertebrate spinal cord, an effect, which like the associated neuronal depolarization, is independent of synaptic transmission and neuronal action potentials (Kudo & Fukuda, 1976; Sonnhof, Grafe, Richter, Parekh, Krumnikl & Linder, 1978; Evans, 1980; Sonnhof & Buhrle, 1980; Vycklický, Vyskočil, Kolaj & Jastreboff, 1982; Buhrle & Sonnhof, 1983). Excitant amino acids raise a^o_K in the fluid bathing cultured mammalian central neurones (Hosli, Hosli, Landolt & Zehntner, 1981), and increases in a^o_K are also produced when excitant amino acids are ejected micro-electrophoretically into the cat cerebral cortex (Lux, 1974), frog (Sonnhof & Buhrle, 1980) and cat spinal cord (Engberg, Flatman, Lambert & Lindsay, 1983).

Although elevation of a^o_K might be expected from an amino-acid-induced efflux of potassium produced by, or underlying, neuronal depolarization (see Buhrle & Sonnhof, 1983), as well as from the high frequency discharge of action potentials, alterations in extracellular ion activity during the ejection of excitant (and other) amino acids would be complex. In addition to ion movements associated directly or indirectly with neuronal depolarization and repetitive firing, another factor to be considered is the co-transport of ions, particularly sodium, with amino acids taken up by neurones and glia (see Curtis *et al.* 1982). In the superfused frog cord, L-glutamate reduces the extracellular sodium and especially calcium (a^o_{Ca}) activity (Buhrle & Sonnhof, 1983), and considerable reductions in a^o_{Ca} accompany the micro-electrophoretic ejection of glutamate, aspartate and DLH in the cat cerebral cortex (Heinemann & Pumain, 1980). Profound changes in extracellular ion levels in, and in the morphology of, the extracellular space of the cat spinal cord during the ejection of excitant amino acids are suggested (Engberg *et al.* 1983) by concomitant increases in tissue resistance (see also Curtis *et al.* 1982), decreases in extracellular volume, the recording of large negative 'focal' potentials with extracellular micro-electrodes, as well as increases in a^o_K. Thus the aetiology of the reduced threshold of afferent fibres and terminations may be more complex than mere elevation of a^o_K, and reduction of a^o_{Ca} may be an additional important factor producing membrane depolarization (see Curtis, Perrin & Watkins, 1960).

The extent and time course of changes in afferent fibre threshold produced by a particular excitant amino acid would be determined by its potency as an excitant, its spread within spinal tissue, and the efficiency and kinetics of membrane processes which restore extracellular ion levels to normal values. Recording conditions were never sufficiently stable to determine if reductions in termination threshold were consistently followed by prolonged elevations, as might be expected from electrogenic ion pumping. Electrogenic sodium pumping appears to be important as an explanation of the hyperpolarization of primary afferent fibres which follows depolarization induced by superfusing the isolated frog spinal cord with L-glutamate (Padjen & Smith, 1983). It is significant that in the same tissue the rise of a^o_K in the dorsal horn produced by L-glutamate is followed by a depression below the normal level (Fig. 4*A*, Sonnhof & Buhrle, 1980).

Whatever the cause of the reduction of the threshold of primary afferent fibres by excitant amino acids, the effect is clearly not restricted to terminals. Micro-

electrophoretic potassium depolarizes afferent fibres and terminals, and, based on the absence of a reduction in concomitant p.a.d., a conductance increase of the terminal membrane was not detected (Curtis & Lodge, 1982). That relatively large reductions in threshold of Ia afferent terminations by excitant amino acids were also not associated with changes in p.a.d. suggests that the conductance of the membrane of Ia terminals was not affected by these amino acids. This lack of effect on p.a.d. also suggests that excitant amino-acid-induced alterations in the extracellular environment did not alter the synaptic release of GABA from axo-axonic terminals which were also presumably depolarized. Thus, if the same factors are important for the synaptic release of Ia transmitter as for GABA, the extent and nature of the conductance increase at synapses activated by GABA on Ia terminals is apparently more important than depolarization *per se* to the reduction of excitatory transmitter release which underlies presynaptic inhibition. This being so, although elevation of a_K^o associated with neuronal excitation by afferent volleys may contribute *in vivo* to depolarization of the terminals of other afferent fibres (see Shefner & Levy, 1981), such *depolarization* may be relatively unimportant to the presynaptic inhibition of transmitter release. Certainly, in the case of extensor muscle Ia afferent terminations, the reduction by BMC of p.a.d. generated by impulses in low threshold flexor afferent fibres suggests that any contribution by elevated a_K^o levels is of minor significance as a cause of p.a.d. (Curtis & Lodge, 1982).

In conclusion, the present observations are consistent with an indirect depolarization of primary afferent fibres and terminals in the cat spinal cord by excitant amino acids as a consequence of changes in the extracellular medium resulting from neuronal excitation, of which elevation of a_K^o may be an important, but not the only, factor. Although further investigation is required, there was no specific evidence for the occurrence of excitant amino acid receptors on afferent terminations. In particular, depolarization of afferent terminations, detected as a reduction of threshold, appeared not to be associated with a change of membrane conductance, as might be expected if activation of excitant amino acid receptors resulted in the opening of ion channels and a redistribution of ions across the membrane. As initially proposed by Evans (1980), the absence of excitant amino acid receptors on afferent fibres and terminals could account for the apparent insensitivity of these structures to the neurotoxic effects of certain excitant amino acids which destroy some types of neurone (Olney, 1978), such toxicity presumably being a direct consequence of profound and prolonged disturbances of intracellular ion levels. The greater sensitivity to depolarization by superfused L-glutamate of primary afferent fibres which have synapses upon motoneurones than of fibres establishing polysynaptic connexions in the amphibian spinal cord (Shapovalov *et al.* 1983) may reflect the different intraspinal courses, the nature and the sites of termination, and the proximity to neurones of particular groups of afferents rather than different mechanisms of depolarization at terminals.

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