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The primary afferent depolarizing action of kainate in the rat

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1 Dorsal roots (L3–L7) isolated from immature (1–9 day old) rats were depolarized selectively by kainate (1–100 μM). L-Glutamate (25–1000 μM), but not L-aspartate, mimicked the action of kainate. N-methylaspartate had no activity on these preparations and quisqualate was thirty times less active than kainate.

2 Depolarizations evoked by L-glutamate (100–1000 μM) faded rapidly in the presence of L-glutamate. Depolarizations evoked by kainate were depressed during the fade induced by L-glutamate.

3 Certain electrically evoked C-fibre volleys in dorsal roots or leg nerves of rats at any age were selectively depressed or abolished in the presence of kainate. The effect of kainate was more selective than that of γ-aminobutyric acid or capsaicin.

4 Prolonged treatment of dorsal roots with kainate did not appear to be deleterious to C-fibres.

5 It is suggested that certain primary afferent C-fibres possess kainate receptors which may be activated physiologically by L-glutamate released at their central terminations.

Introduction

Although central and peripheral unmyelinated nerves are depolarized by depressant amino acids including γ-aminobutyric acid (GABA) (Brown & Marsh, 1978) axons in general are considered to be unresponsive to excitant amino acids (Evans, 1980; Curtis *et al.*, 1984). However, it has been reported that immature dorsal root fibres are directly depolarized by kainic acid (Davies *et al.*, 1979). The latter phenomenon has been recently invested with more importance by the preliminary report that the kainate sensitivity is confined to primary afferent C-fibres in both immature and mature rats (Evans, 1985). Thus in this paper a fuller account is presented of the selective action of kainate on primary afferent C-fibres.

Methods

All experiments in this study were *in vitro* and the majority were carried out on isolated dorsal roots of immature (1–9 day old) rats. Dorsal root preparations consisted of the dorsal root (usually L5) severed or stripped from its point of emergence from the spinal cord together with the dorsal root ganglion and 1–2 mm of peripheral nerve. Such dorsal root preparations were used either for measurement of action potentials, as described below, or were placed across a grease seal to record the d.c. level between peripheral and central ends. The peripheral end was placed inside the grease barrier which was positioned 2–3 mm from the central end. The central end was bathed with Ringer and drug solutions.

In some experiments d.c. recordings were made from the central ends of dorsal roots attached to hemisected spinal cord preparations from immature rats.

The methods of superfusing Ringer solution and drugs and recording of d.c. potentials from dorsal roots placed across a grease seal were similar to those described previously (Evans & Watkins, 1978). Preparations were maintained at 25°C, except where indicated otherwise, by means of a Grant Instruments SE15 thermostat water bath and circulation pump.

Stimulation and recording of compound action potentials

Triphasic compound action potentials were recorded (a.c. coupling time constant, 30 ms) via glass suction electrodes placed at an appropriate site on the peripheral nerve or dorsal root. Suction electrodes were

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made by flaming the ends of borosilicate tubing (1.5 mm o.d., 0.2 mm wall thickness) such that an orifice, of diameter not more than 1/3 of the thickness of the nerve under investigation, was formed. Suction electrodes were filled with perfusing medium in contact with a silver wire. Sufficient negative pressure was applied to cause the area of nerve in contact with the orifice to expand into the orifice for a distance of up to one third of its diameter.

Nerves were stimulated at 0.1 or 0.2 Hz with supramaximal cathodal pulses of 1 ms duration through suction electrodes similar to, but having a wider orifice than, those used for recording. Stimulating electrodes were placed not more than 9 mm from the recording electrode. Conduction velocities were calculated from the distance between centres of the two electrodes and the time, between the stimulus artefact and the first negative deflection produced by the volley of interest. Indifferent electrodes of similar construction were placed in the bathing medium close to recording and stimulating electrodes. The stimulus intensity for each preparation was adjusted between the lowest intensity required for a maximal volley and the intensity at which anodal block occurred. This varied from 2–40 times threshold for the lowest threshold fibres and was dependent to some extent on the distance between recording and stimulating electrodes.

Preparation of nerves

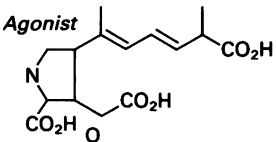
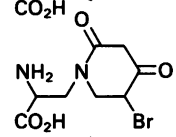
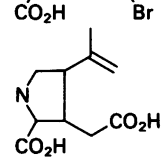
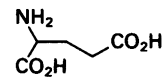
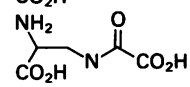
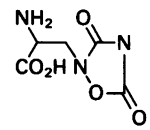
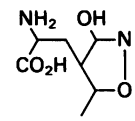
Careful desheathing of peripheral nerves was necessary to allow successful recording of drug effects. Superfusing medium was applied to the whole of the nerve between and in contact with recording and stimulating electrodes.

Molar potency ratios, relative to kainate, for depolarization of dorsal root fibres were determined on at least three preparations for each test compound by bracketing responses to test compound between responses produced by 2 and 5 μM kainate. The parallel displacement of such responses from the line plotted for kainate on a log concentration scale gave the potency ratios.

Amino acids

N-methyl-D-aspartate, L-α-aminoadipate, L-α-aminopimelate, L-α-aminosuberate, D and L forms of homocysteate, willardiine and bromowillardiine were provided by Drs A.W. Jones and J.C. Watkins. β-N-oxalyl-α, β-diaminoproprionate (ODAP) was provided by Dr P.B. Nunn, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) was provided by Dr P. Krogsgaard-Larsen and domoate by Professor T. Takemoto. Other chemicals were obtained from commercial sources.

Table 1 Mean equimolar potency ratios ± s.e.mean for depolarization of dorsal root fibres in the immature rat (number of preparations tested in parentheses).

Agonist		*Potency*
Domoic		36.3 ± 4.7 (3)
Bromowillardiine		3.1 ± 0.73 (5)
Kainic		1.0
Glutamic		0.077 ± 0 (9)
ODAP		0.038 ± 0.016 (3)
Quisqualic		0.032 ± 0.007 (4)
AMPA		0.015 ± (4)

ODAP = β-N-oxalyl-α, β diaminoproprionate;
AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate

Results

Depolarization of dorsal root fibres

The depolarizing effects of excitant amino acids were compared with the depolarizing effect of GABA on isolated dorsal roots. Three features were evident as illustrated in Figures 1 and 2. GABA always gave maximum depolarizing responses which were at least 2 fold greater than the maximum effect of kainate (Figure 1). Depolarizations produced by L-glutamate, at concentrations greater than approximately 4 times threshold, faded markedly during the L-glutamate application (Figures 1, 2 and 3). Thus the maximal depolarizing effect of L-glutamate was always lower than that produced by kainate (Figure 1). Finally kainate produced after-hyperpolarizing responses

(Figures 1 and 2). In four preparations subjected to a full range of kainate doses, the mean effective concentration for half maximal response was 8.7 μM ± 1.3 s.e.mean.

Dorsal roots from the youngest animals (1 day post partum) were the most suitable for these measurements giving larger depolarizations than could be measured in L3–L5 of older animals. Excitants, in contrast to GABA, had no effect when applied to the dorsal root ganglion with the central end of the dorsal root placed on the recording electrode. Furthermore, excitants had no effect on the d.c. polarity of dorsal roots or vagus nerves from mature animals. The sensitivity to GABA of these preparations has been reported previously (Brown & Marsh, 1978).

Comparison of a series of excitants showed a more selective structure-activity relationship than that reported for spinal neurones (Biscoe *et al.*, 1976). Relative potencies for those compounds with potencies greater than 0.01 (relative to kainate = 1) are presented in Table 1. Due to the small amplitude of responses it was difficult to rank compounds with molar potencies less than 0.01. However the potency of L-homocysteate lay between 0.01 and 0.002 and the following compounds were considered to be inactive having molar potencies less than 0.002: L-aspartate, L-α-aminoadipate, L-α-aminopimelate, L-α-aminosuberate, N-methyl-D-aspartate, D-aspartate, D-glutamate, D-homocysteate and willardiine. The selectivity for kainate relative to quisqualate and NMDA is illustrated in Figure 2. It can be seen that the relative potencies are quite different when neurones contributed to the response (lower trace b).

The sensitivity to kainate was reduced considerably during fade induced by L-glutamate. This fade is illustrated in Figure 3 which shows a fifty fold rightward displacement of the kainate dose-response curve produced by 250 μM L-glutamate. It can be seen that there was no significant reduction in sensitivity to GABA.

As illustrated above, the depolarizations evoked by kainate did not fade (Figures 1, 2 and 3) and correspondingly depolarizations evoked by L-glutamate were not reduced, in the presence of kainate, any more than would have been expected from occlusion of the effect of one depolarizing agent by another.

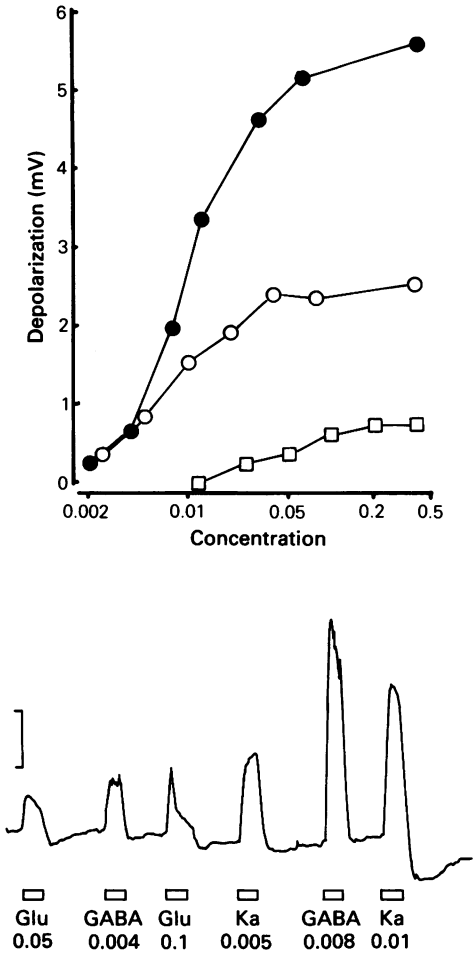


Figure 1 Comparison of the depolarizing action of γ-aminobutyric acid (GABA, ●), L-glutamate (Glu, □) and kainate (Ka, ○) on an isolated dorsal root preparation. Amino acids were applied for 2 min intervals at the concentrations (mM) indicated. Depolarizing responses (calibration 0.25 mV) are shown below the dose-response plot. See legend of Figure 2 under (a) for recording method.

Selectivity of kainate for C-fibres

Measurement of depolarizing responses from whole nerves, as above, does not indicate which fibre groups contribute to the depolarization produced by an agonist. Thus compound action potentials were recorded in order to compare the action of kainate on fibres of different conduction velocity. It was found that conduction in the slowest fibres was selectively depressed by kainate. The action of GABA was less selective (Figure 4). This selectivity of action of kainate is illustrated further by Figure 5 in which it is seen that kainate, at levels over one hundred times greater than that necessary to depress volleys in the slowest conducting fibres, had no significant effect on faster conducted volleys. In fourteen immature (less than 9 days post partum) preparations the mean conduction velocity of the kainate-sensitive compound action potential was

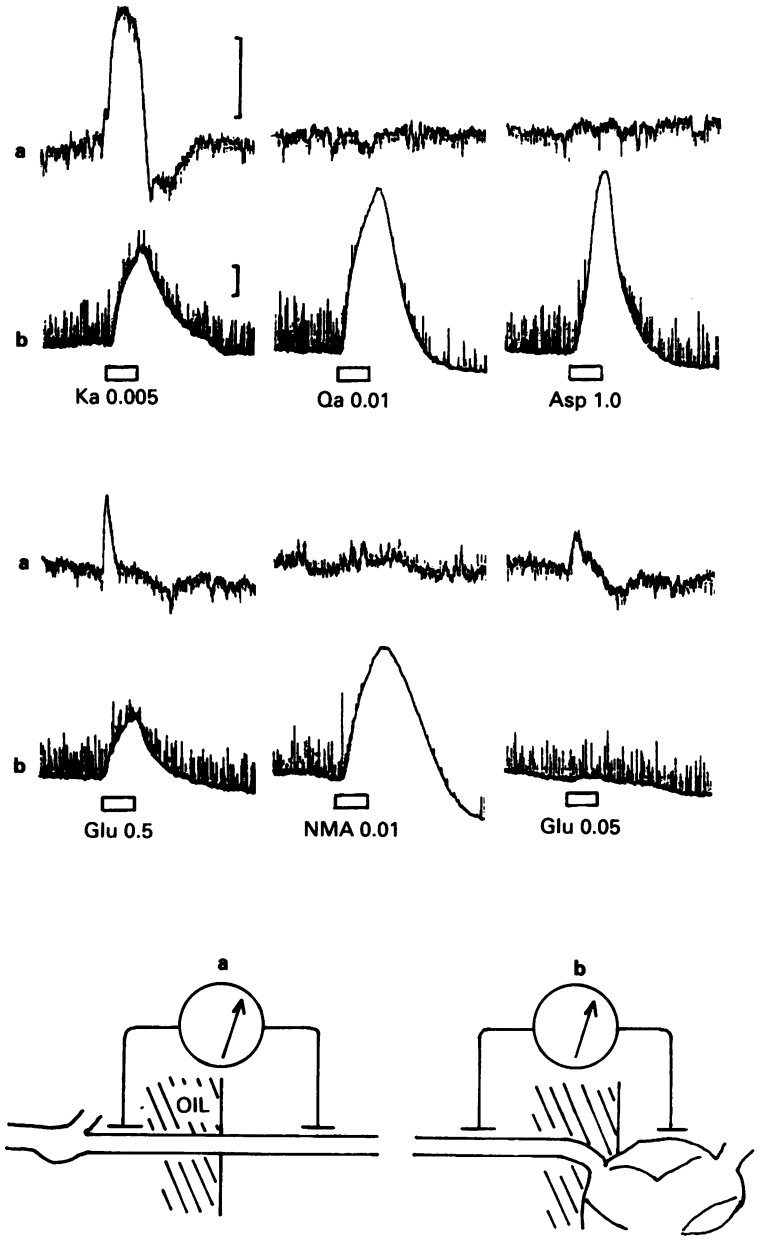


Figure 2 Comparison of the depolarizing effects of L-aspartate (Asp), L-glutamate (Glu), N-methyl-D-aspartate (NMA) and quisqualate (Qa) on an isolated dorsal root (a) and a dorsal root attached to a hemisected spinal cord (b). Methods of recording (a) and (b) were as follows: traces (a) and (b) are synchronous. The two preparations were bathed in the same medium. Amino acids were applied for 90 s at the concentrations indicated below the record. Note that only kainate and L-glutamate depolarize the isolated dorsal root whereas all 4 amino acids depolarize the neuronal preparation. Vertical calibration 0.5 mV. The break between each panel represents an interval of 20 to 60 min. Other details as for Figure 1.

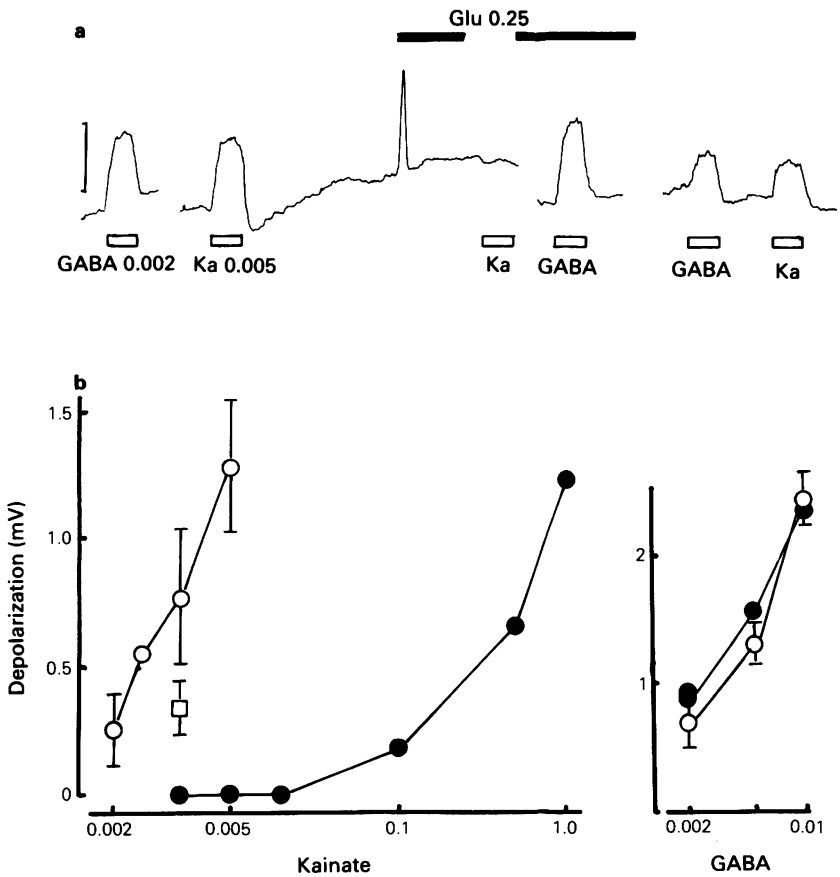


Figure 3 Depolarization of isolated dorsal root preparation by γ-aminobutyric acid (GABA) and kainate (Ka) showing desensitizing action of L-glutamate (Glu). (a) Pen recorder trace. GABA and kainate applied for 2 min intervals at the concentrations (μM) indicated. L-Glutamate was applied as indicated above the record. Note the L-glutamate-induced depolarization which faded rapidly. Vertical calibration 0.25 mV. (b) Dose-response plots of data: (○), before application of L-glutamate; (●), in the presence of 250 μM L-glutamate; (□) responses to kainate obtained 70 min after introducing L-glutamate-free Ringer solution. Standard deviations of 3 or more responses are indicated by the vertical bars.

0.34 m per s ± 0.014 (s.e.mean). It was of interest to compare this value with the value obtained at 37°C. Thus in three preparations the conduction velocity of the kainate-sensitive component at 25°C was 54.5% ± 4.7 (s.d.) of the velocity recorded at 37°C. There were no components with velocities faster than 4 m per s at 25°C in these immature animals.

These kainate-sensitive volleys with large amplitudes in the C-fibre range, sometimes in excess of 1 mV, were easily recorded from immature dorsal roots. Consequently they were used for the majority of experiments in this study. However, mature dorsal roots (40–90 days post partum) were found also to have kainate-sensitive C-fibre volleys. Thus in five mature preparations kainate sensitive volleys had a mean velocity of 0.39 m per s ± 0.027 (s.e. mean). This is not significantly different from the values recorded in immature dorsal roots ($P > 0.05$ from Student's t test).

Kainate sensitivity of C-fibres in a mature dorsal root is illustrated by Figure 6. In Figure 6A it can be seen that as in immature nerves, kainate-sensitivity was confined to the slowest conducting elements. Kainate-sensitive volleys were more difficult to locate in mature peripheral nerves but contrary to a preliminary report (Evans, 1985) they were present. Thus kainate-sensitive volleys, of mean velocity 0.47 m per s ± 0.04 (s.e. mean, not significantly different, $P > 0.05$, from the values recorded with mature dorsal roots), were observed in three out of three

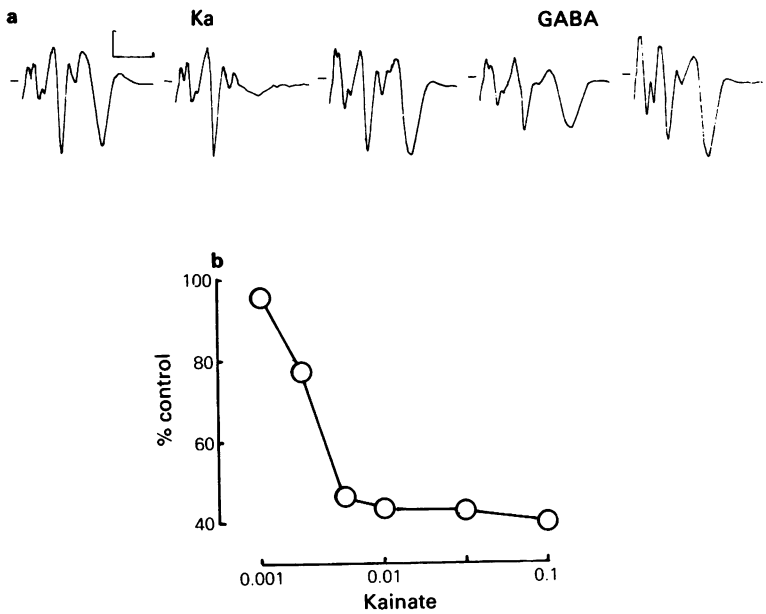


Figure 4 Effect of kainate on evoked volleys in L5 dorsal root of 5 day old rats. (a) Oscillographic records showing effect of kainate 20 μM (Ka) and γ-aminobutyric acid (GABA) 200 μM. Recording electrode on central end of dorsal root, stimulating electrode 3.6 mm more distal. The amino acids were applied for 5 min followed by a 20 min wash period. (b) Dose-response plot from a similar preparation to (a) showing the size of the kainate-sensitive component as a percentage of the amplitude before application of kainate. Calibration in (a) 0.5 mV, 5 ms.

sciatic nerves and five out of eight sural nerves and were absent from the one tibial nerve examined.

Since primary afferent C-fibres are depolarized by capsaicin (Ault & Evans, 1980; Pini, 1983) it was of interest to compare the actions of capsaicin and kainate in the present experiments. It was found that capcaisin, unlike kainate, depressed all volleys within the C-fibre range of conduction velocity (< 2 m per s). This less selective action of capsaicin, relative to kainate, is illustrated in Figure 6B. As was expected for this very lipophilic compound, the times taken to reach maximal depressant effects and times taken for recovery following applications of capsaicin were very prolonged compared to kainate.

The relationship of chemical structure to activity for depression of kainate-sensitive C-fibre volleys was more easily determined via depolarizing responses recorded from immature dorsal roots as described above. Thus only glutamate, which is a likely endogenous ligand for this receptor, was tested on C-fibre volleys. A consistent depression of C-fibre volleys by L-glutamate (0.05–1 mM) was not observed. However, an interaction with the kainate receptor was evident since the effect of kainate was reduced by L-glutamate, as illustrated in Figure 7, presumably due to desensitization.

Effect of prolonged and high doses of kainate

Prolonged treatment of spinal cord preparations with kainate produces irreversible depolarization of motoneurones associated with loss of spontaneous and reflex synaptic activity (Evans, 1980). The excitotoxic action of kainate is well documented (Olney, 1980). In the present experiments prolonged treatment of dorsal roots with high concentrations of kainate had no apparent deleterious effect on kainate-sensitive C-fibre volleys. This is illustrated in Figure 8 which shows in the lower trace the abolition of synaptic activity following 35 min treatment with 0.5 mM kainate whereas the C-fibre volley recorded from the dorsal root reappeared on washout of kainate.

Discussion

The results show that a certain population of primary afferent C-fibres are endowed with kainate receptors. It is presumed that the depression of the C-fibre volleys by kainate was a consequence of depolarization of C-fibres as shown directly by the d.c. recordings from dorsal roots of immature animals. Certainly the two effects showed a similar dose-response re-

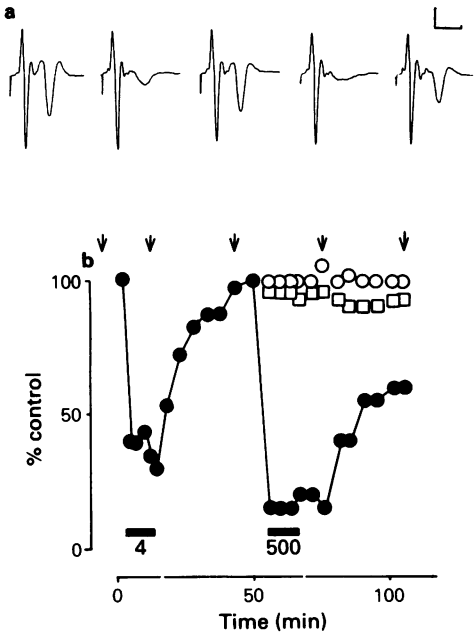


Figure 5 Volleys evoked in L5 dorsal root of three day old rat. Oscillographic records shown in (a) were obtained at the points indicated by arrows above (b). In (b) the amplitudes of each of three components have been plotted as a percentage of the initial amplitude. Kainate was applied at the concentrations (μM) indicated beneath the plot: (●) kainate-sensitive volley (0.27 m per s); (○) the small intermediate volley (0.4 m per s); (□) the large initial component (0.65 m per s). Calibration in (a) 0.25 mV, 10 ms. The recording electrode was placed at the central end of the dorsal root and the stimulating electrode was 3.6 mm towards the dorsal root ganglion.

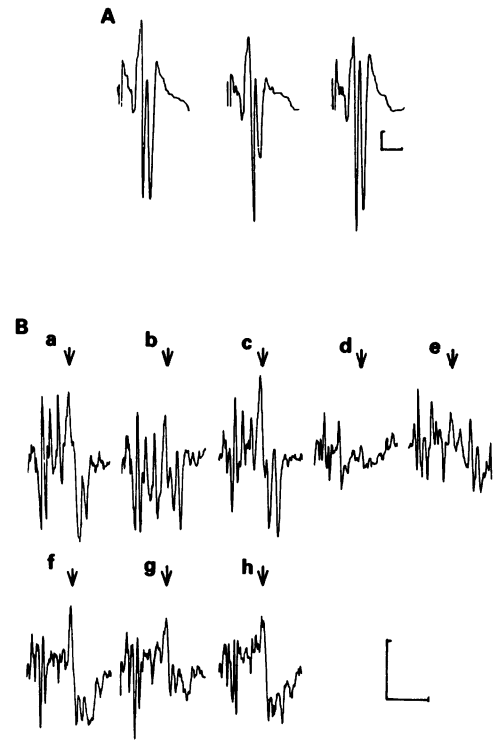


Figure 6 (A) Volleys recorded in Lissauer's tract following dorsal root stimulation (80 day old rat). Electrodes 5.1 mm apart. Left hand trace control, centre trace 5 min after introduction of 10 μM kainate and right hand trace 20 min after introduction of kainate-free Ringer solution. The faster volley corresponds to a conduction velocity of 0.41 m per s and the slower, kainate-sensitive, volley to a velocity of 0.34 m per s. Calibration 50 μV, 10 ms. (B) C-fibre volleys in L5 dorsal root (40 day old rat). Consecutive records. (a) Control, (b) 5 min after introduction of 200 μM kainate, (c) 20 min following washout of kainate, (d) 5 min after introduction of 2 μM capsaicin, (e) 20 min following washout of capsaicin, (f) 90 min following washout of capsaicin, (g) 5 min after introduction of 10 μM kainate, (h) 20 min following washout of kainate. Calibration as for (A). The stimulus was applied 6 ms before start of trace. The conduction velocities illustrated range from 0.29 to 0.64 m per s. The kainate-sensitive component (0.32 m per s) is indicated by arrows. The recording electrode was placed 2 mm from the central end of the dorsal root with the stimulating electrode 5.1 mm distal to it.

lationship. The depolarization of C-fibres induced by kainate could not be observed directly in mature roots or in peripheral nerves. Furthermore the maximum amplitude of C-fibre volleys recorded from immature dorsal roots was much higher than could be recorded from nerves of mature rats. It has been shown that the developing sympathetic chain has a very low proportion of satellite cells to nerve fibres (Aquayo *et al.*, 1973). If developing dorsal roots similarly possessed a low proportion of satellite cells it could explain the relatively greater amplitude of C-fibre potentials recorded from such immature nerves. Supporting cells presumably lower the shunt resistance to extracellular potential differences.

Pharmacological receptors for excitant amino acids have been classified into at least three types. While the existence of one of these types, the N-methylaspartate receptor, is supported by rigorous pharmacological criteria (Evans *et al.*, 1982) the distinction between receptors activated by kainate or quisqualate is not so clear cut. The thirty fold lower potency of quisqualate, relative to kainate, in depolarizing primary afferent C-fibres in the present study tends to support the proposed distinction between receptors activated by these two amino acids (McLennan & Lodge, 1979).

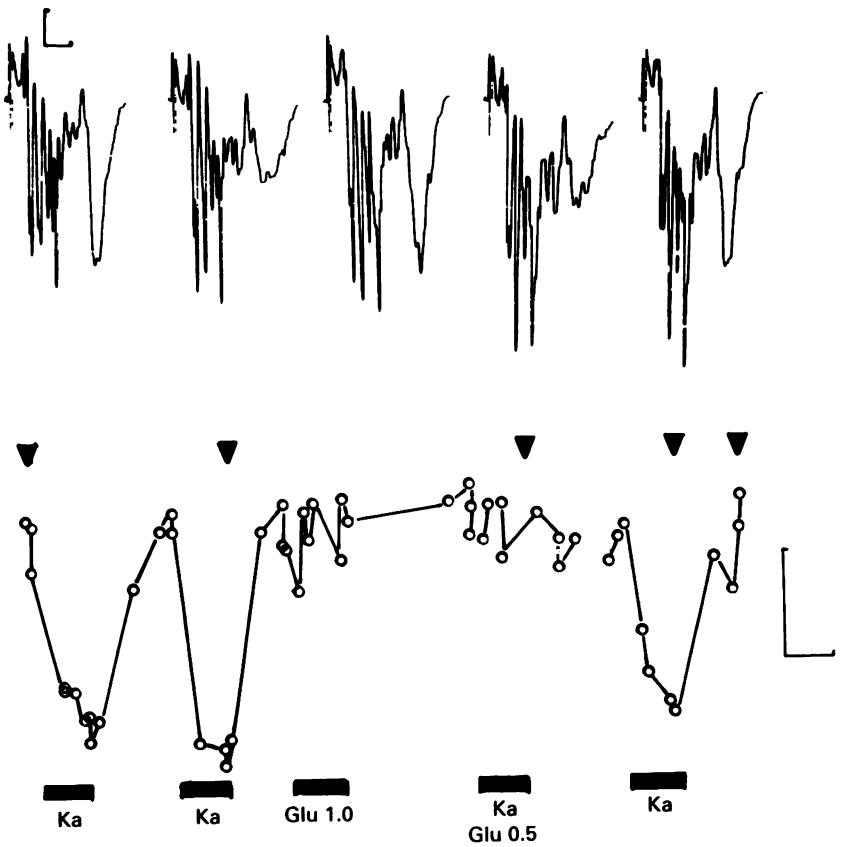


Figure 7 Effect of 5 μM kainate (Ka) on C-fibre potentials in L5 dorsal root of six day old rat. Mean amplitudes of three consecutive responses are plotted against time. The upper oscillographic records were obtained at the times indicated by arrows above the trace. The break before the final kainate application represents an interval of 70 min. L-glutamate was applied at 0.5 and 1.0 mM as indicated. Period of application of amino acids indicated by bars below the plot. Vertical calibration 100 μV. Horizontal calibration 10 ms for upper and 10 min for lower records.

The rank order of potencies of domoate, kainate, L-glutamate and N-methylaspartate for depolarization of C-fibres (see Table 1) is comparable to their rank order for displacement of labelled kainate from brain membrane preparations (Slevin *et al.*, 1983). A very significant feature of the structure activity relationship at the C-fibre kainate receptor is the clear distinction between the two endogenous excitants L-aspartate, with no significant activity, and L-glutamate with one tenth the potency of kainate.

It is interesting that addition of a bromide atom to position 2 of the uracil ring results in a conversion from a potent quisqualate agonist willardiine (Evans *et al.*, 1980) to a potent kainate agonist bromowillardiine (Davies *et al.*, 1982) (Table 1). ODAP, which has been identified previously as a non-N-methylaspartate agonist (Pearson & Nunn, 1981), is identified, through low potency in depolarizing dorsal roots, as a quisqualate-like agonist in the present study. Thus the neuropathological effects associated with ODAP (Chase *et al.*, 1985) may be a consequence of the activation of quisqualate receptors.

It was not surprising that consistent depression of the C-fibre volley by L-glutamate could not be demonstrated. The d.c. recordings from immature dorsal roots showed a rapid time course of desensitization to L-glutamate with a half time of less than 30 s. Thus at a stimulation rate of 0.1 Hz only one or possibly two recordings could have occurred during the depolarizing phase of the action of L-glutamate. The desensitizing action of L-glutamate was easily demonstrated on C-fibre volleys. It is unusual that cross-desensitization between kainate and L-glutamate did not appear to be mutual (i.e. L-glutamate and not kainate produced

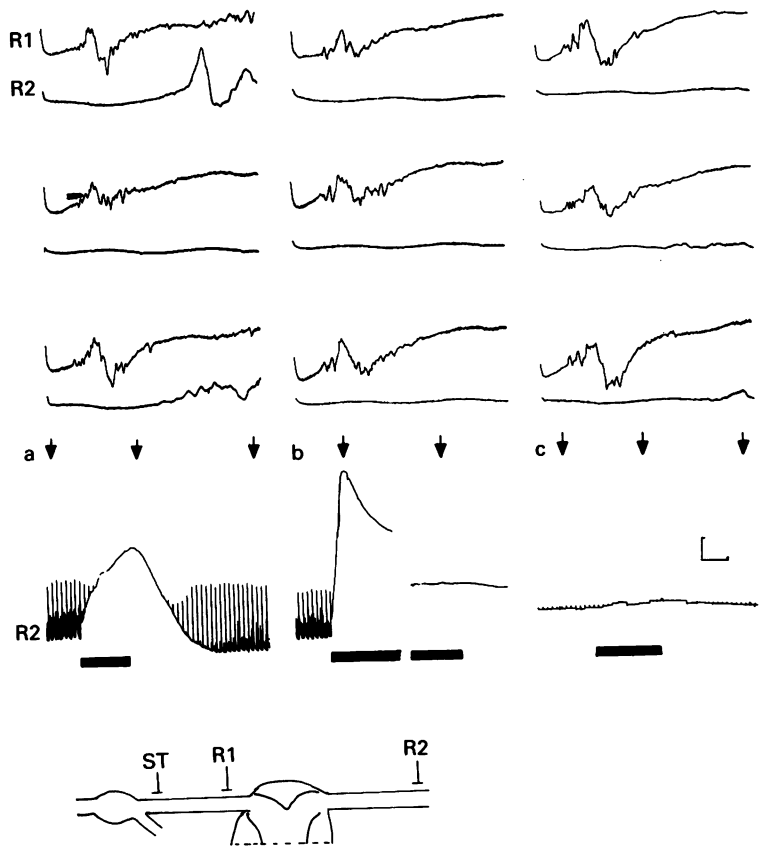


Figure 8 Effect of kainate on dorsal root C-fibre volley and ventral root reflex. L5 segment of 2 day old rat hemisected spinal cord preparation: (a), (b) and (c) are consecutive chart recordings of central root polarity from R2 in the diagram below. Oscillograph traces recorded at the respective times indicated by the arrow heads above the chart recording are shown in descending order above (a), (b) and (c). The third trace above (b) was recorded 14 min after washout of kainate (not indicated on the chart record). Introduction of kainate 10 μM in (a) and (c) and 500 μM in (b) is indicated by the bars underneath the record.

The upper trace of each oscillographic panel was recorded at R1 on the L5 dorsal root and the lower trace was recorded at R2 on the L5 ventral root. L5 dorsal root was stimulated via ST at 0.05 Hz.

The break in record (b) represents an interval of 20 min. Intervals of 30 and 50 min respectively occurred between (a) and (b) and (b) and (c). Note that each treatment with kainate produced a reversible reduction in the C-fibre volley (R1), whereas the ventral root potential (R2) was irreversibly depressed indicating loss of neuronal activity.

The upward deflections in the chart records (a and b) represent an integration of the slower (polysynaptic) components in the ventral root potential which showed some recovery, apparent also in the lower oscillographic trace above (a), following the initial brief application of kainate.

Calibration, (a), (b) and (c) 0.5 mV and 2 min. Oscillographic traces, upper 0.23 mV, lower 0.9 mV; horizontal 5 ms. Distance between ST and R1 = 2.5 mm.

desensitization to both agonists). Perhaps the desensitization process involves a different site, with which kainate is unable to interact, from the one that mediates depolarization.

The use of kainate as a brain lesioning agent which spares fibres of passage is believed to depend upon the absence of kainate receptors from axons (Coyle *et al.*, 1978). The present study supports other findings (McBean & Roberts, 1981; Ferkany *et al.*, 1982; Collins *et al.*, 1983, see also Coyle, 1983) which suggest that the absence of excitatory amino acid receptors from axons or terminals (Evans, 1980; Curtis *et al.*, 1984) is not a general phenomenon. Nevertheless it appears that depolarization of primary afferent C-

fibres by kainate is not deleterious (see Figure 8). Thus the excitotoxic hypothesis (Olney, 1983) is untenable for kainate-sensitive C-fibres.

The action of kainate was more discretely localized than that of GABA or capsaicin which depolarized fibres over a wider range of conduction velocity. Kainate always depolarized the slowest conducting fibres in dorsal roots.

The synaptic activation of kainate receptors by L-glutamate has been described for the junction between photoreceptors and bipolar cells in the retina (Slaughter & Miller, 1983) and it is interesting to speculate on a possible functional role for the C-fibre kainate receptors which can be activated by L-glutamate. A-fibre volleys have been shown to produce increased excitability of C-fibre afferent terminals in the spinal cord (Calvillo, 1978; Hentall & Fields, 1979; Fitzgerald & Woolf, 1981) and A-fibres have been shown to inhibit C-fibre inputs to projection cells in the spinal cord (Fitzgerald, 1981). Perhaps such effects are mediated by L-glutamate, released either from A-fibres or interneurones, which acts at kainate receptors on C-fibres. GABA is the usual candidate for such a presynaptic conditioning role. However, it may be significant that primary afferent C-neurones compared to A-neurones have been reported to possess a low density of GABA receptors (Desarmenien *et al.*, 1984).

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