Br. J. Pharmac. (1976), **57**, 359–368

A COMPARATIVE STUDY OF THE EFFECTS OF GLUTAMATE AND KAINATE ON THE LOBSTER MUSCLE FIBRE AND THE FROG SPINAL CORD

A. CONSTANTI[1] & A. NISTRI[2]

Department of Pharmacology, St Bartholomew's Hospital Medical School, University of London, London EC1

1 The depolarizing actions of glutamate and its conformationally restricted analogue kainate were investigated on the lobster muscle fibre and the frog spinal cord using intracellular and extracellular recordings, respectively.

2 Bath-applied kainate was less potent than glutamate on the lobster fibre but more potent on the frog cord. From the log–log transformation of dose-response curves it was proposed that more than one glutamate molecule was necessary to activate both the lobster and the frog receptor sites. In the frog, at least three kainate molecules were thought to be required for receptor activation.

3 The ionic dependence of glutamate and kainate responses appeared different for the two tissues.

4 Some possible explanations of the differential tissue sensitivity to kainate are discussed.

Introduction

Glutamic acid is an acidic amino acid present in high concentrations in nervous tissue and able to produce neuronal excitation when applied exogenously; a neurotransmitter role for this substance is considered likely (Johnson, 1972; Curtis & Johnston, 1974). However, a better understanding of the function of this amino acid is hampered by the lack of specific antagonists and the scarcity of quantitative studies. For this reason little is known about glutamate receptors although binding of this substance to rat brain synaptic membranes (Roberts, 1974) and purified proteins of crustacean muscle (Fiszer de Plazas & de Robertis, 1974) has been described. Kainate, an amino acid structurally related to glutamate, has recently been found to be more potent than glutamate in depolarizing vertebrate central neurones (Shinozaki & Konishi, 1970; Johnston, Curtis, Davies & McCulloch, 1974; McCulloch, Johnston, Game & Curtis, 1974; Biscoe, Evans, Headley, Martin & Watkins, 1975). In contrast, kainate has a weaker depolarizing action than glutamate at the crayfish (Shinozaki & Shibuya, 1974; Takeuchi & Onodera, 1975) and locust (Clements & May, 1974) neuromuscular junctions.

Prompted by these reports on the differing sensitivities of vertebrate and invertebrate tissues to kainate, we attempted a quantitative comparison of the dose-response relationships to bath applied glutamate and kainate on two *in vitro* preparations, the lobster muscle fibre and the frog spinal cord. In both these preparations glutamate is considered to be a putative excitatory transmitter (Kravitz, Slater, Takahashi, Bownds & Grossfeld, 1970; Barker, Nicoll & Padjen, 1975). The ionic dependence of the amino acid-evoked depolarizations in both tissues was also investigated. Some of our results have been published in preliminary form (Constanti & Nistri, 1975).

Methods

Lobster muscle fibre

Claw opener muscles of the first or second walking leg of the lobster (*Homarus vulgaris*) were dissected and prepared for intracellular recording as described in the preceding paper (Constanti & Nistri, 1976). Single superficial muscle fibres were impaled with two glass microelectrodes placed within 50 μm of each other at the centre of the fibre. One electrode filled with 2 M tripotassium citrate recorded the membrane potential while the other filled with 0.6 M potassium sulphate passed constant hyperpolarizing current pulses through the membrane. The resultant electrotonic potentials were recorded as previously described (Constanti & Nistri, 1976).

[1] Present address: Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX.

[2] Present address: Institute of Pharmacology, University of Florence, Viale Morgagni 65, 50134 Florence, Italy.

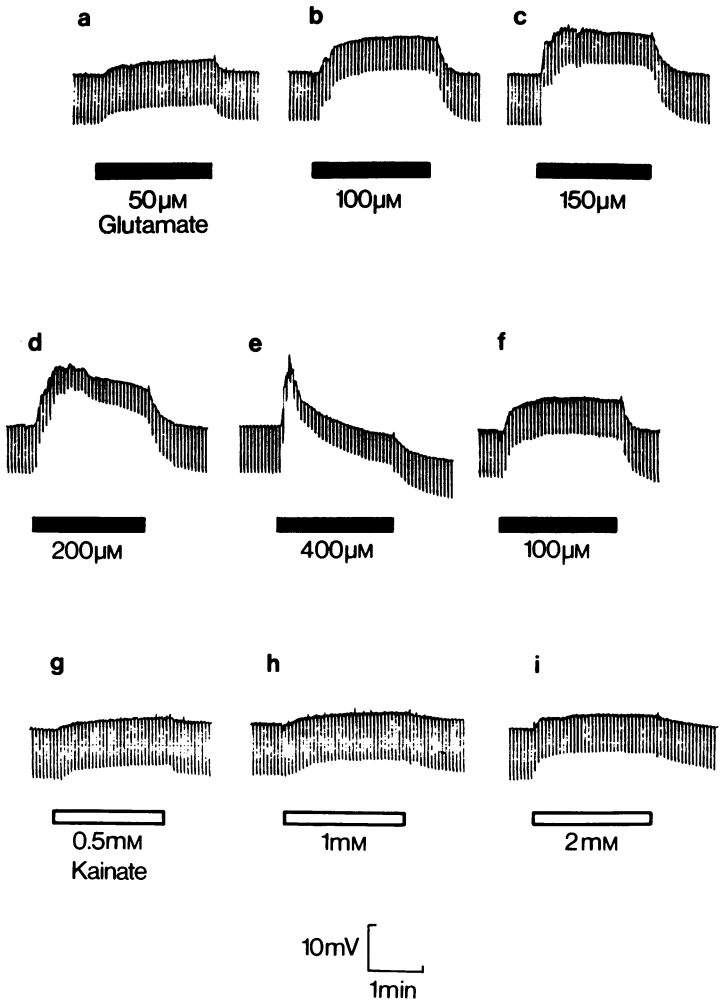

Figure 1 Depolarizations evoked by L-glutamate (filled bars) and kainate (open bars) recorded at the centre of a single lobster muscle fibre. Downward deflections are electrotonic potentials resulting from the application of control intracellular current pulses (800 ms; 2.5×10^{-7} A). (a–e) Show the effects of successively increasing concentrations of glutamate (50 μM to 400 μM); (f) is a recovery response. Each response was separated by at least 5 min washing in drug-free solution; (g–i) show the effects of kainate (0.5 mM to 2 mM). Note the relatively weaker effect of kainate and the rapidly waning effect of glutamate at high concentrations (d and e). All measurements were made on the same fibre (Resting potential = −76 mV).

Frog spinal cord

The method for setting-up and recording from the frog (*Rana temporaria*) isolated spinal cord is given in the preceding paper (Constanti & Nistri, 1976).

Drugs

Glutamate was purchased from Sigma and kainate from Calbiochem.

Results

Lobster muscle fibre

When glutamate (50 μM to 400 μM) was added to the superfusion fluid, a depolarization of the muscle membrane was produced (2–18 mV). This effect was reproducible and had a relatively fast onset and decline rate (Figure 1, a–f). At concentrations higher than 150 μM glutamate often caused a contraction of

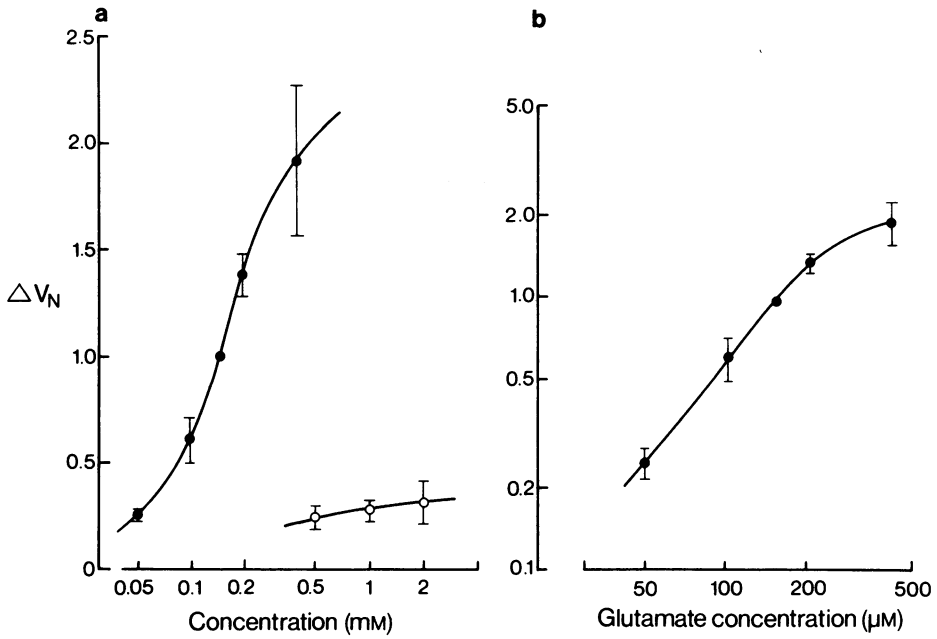

Figure 2 (a) Normalized log dose-response curves for glutamate (●) and kainate (○) in single lobster muscle fibres. Points represent mean of 3 experiments. Vertical lines show s.e. mean. Ordinate scale represents normalized membrane depolarization (ΔV_N); abscissa scale gives concentration of amino acid added to the superfusing solution. ΔV_N was calculated by dividing all glutamate and kainate depolarizations of any single fibre by the response to 150 μM glutamate (approximately half maximal) in that fibre. This allowed data from different muscle fibres to be represented on the same graph. Note that the kainate dose-response curve was almost flat. (b) Log ΔV_N vs. log [glutamate] transformation of the glutamate dose-response curve of Figure 2a. The limiting slope (in the concentration range studied) was 1.24. Error intervals indicated are the transformed standard errors.

the fibre with consequent displacement of the microelectrodes. Attempts to prevent this effect by pretreatment of the muscle with 400 mM glycerol were unsuccessful. However, in a number of preparations in which contractions following high doses of glutamate did not occur, the glutamate-induced depolarization was quite large and tended to fade away despite the continued presence of the drug (Figure 1e). Such an effect is likely to be the result of receptor desensitization (Takeuchi & Takeuchi, 1964). Where possible, log dose-response curves to the effect of glutamate were obtained (Figure 2a), but definite maxima were not attained because of desensitization and the risk of muscle contraction. Where necessary, the glutamate depolarizations were corrected to account for the slight non-linearity in the current/voltage relationship in the depolarizing direction.

The effect of kainate (0.5 mM to 2 mM) on the lobster muscle fibres was different from that of glutamate. Even though both compounds were able to depolarize the preparation, kainate was much less potent than glutamate on a molar basis (Figure 1, g–i) and the kainate dose-response curve was almost flat (Figure 2a). The decline in the effect of kainate was also somewhat slower than that of glutamate and no desensitization to kainate was seen in the concentration range investigated. Since the maximal effect of glutamate was not available, the conventional Hill plot could not be used to study the kinetics of drug-receptor interaction. Instead, the log–log plot (Werman, 1969) was employed in order to estimate the number of glutamate molecules interacting with a single receptor site, bearing in mind that depolarizations rather than conductance changes were being measured in these experiments. Figure 2b shows that in the case of glutamate the plot was linear in the initial portion and had a limiting slope (estimated from the lower two points) of 1.24. This indicates that more than one molecule of glutamate was interacting with the receptor site (see also Dudel, 1975). A similar plot for kainate could not be obtained owing to the flat nature of the dose-response curve.

Owing to the weak depolarizing action of bath-applied kainate on invertebrate muscle (Shinozaki &

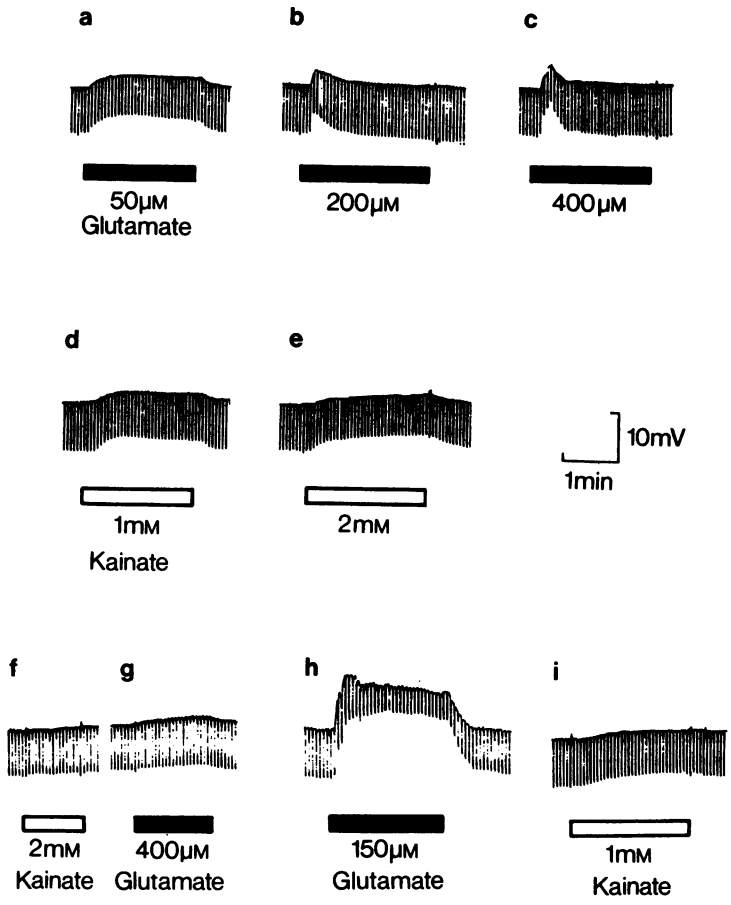

Figure 3 Effect of Na^+ and Ca^{2+} removal on glutamate and kainate depolarizations of lobster muscle fibres (recording continued from that in Figure 1); (a), (b) and (c) show responses to glutamate (50, 200 and 400 μM respectively) applied after 15 min exposure to Na^+-free (Li^+ containing) solution; (d) and (e) show responses to kainate (1 and 2 mM) in the same medium. Note that kainate responses were hardly affected (cf Figure 1h, i) whereas glutamate responses were drastically reduced although not abolished. Residual effects of kainate (2 mM) and glutamate (400 μM) were subsequently reduced after 15 min exposure to Na^+-free, Ca^{2+}-free (Mg^{2+} containing) solution (f and g); (h) and (i) show recovery responses to kainate and glutamate recorded 15 min after return to normal solution.

Shibuya, 1974; Clements & May, 1974) the ionic dependence of this amino acid depolarization has not been investigated. According to Takeuchi & Onodera (1973) the glutamate-evoked depolarization of crayfish muscle is largely dependent upon an increase in Na^+ permeability with a possible contribution from Ca^{2+}. Some experiments were therefore carried out in which the Na^+ content of the lobster saline was replaced with an equimolar amount of Li^+ (the solution was adjusted to pH 7.6 with LiOH). On changing to a Na^+-free medium there was usually a small depolarization (about 2 mV) of the muscle fibre but little change in membrane resistance. After 15 min exposure to this medium the effect of glutamate (prepared in the Na^+-free solution) was drastically reduced (Figure 3, a–c); however, a rapidly fading component of the response to glutamate was still present. The application of glutamate after 5, 20 or 60 min exposure to Na^+-free solution produced similar responses, ruling out the possibility of an insufficient removal of external Na^+ being responsible for the residual glutamate component. It was also interesting to note that small doses of glutamate produced slightly larger responses in Na^+-free medium than in a normal saline solution. In contrast, the action of kainate in Na^+-free solution was practically unchanged (Figure 3, d–e). If Ca^{2+} was also removed and substituted with an equimolar amount of Mg^{2+}, a slight depolarization

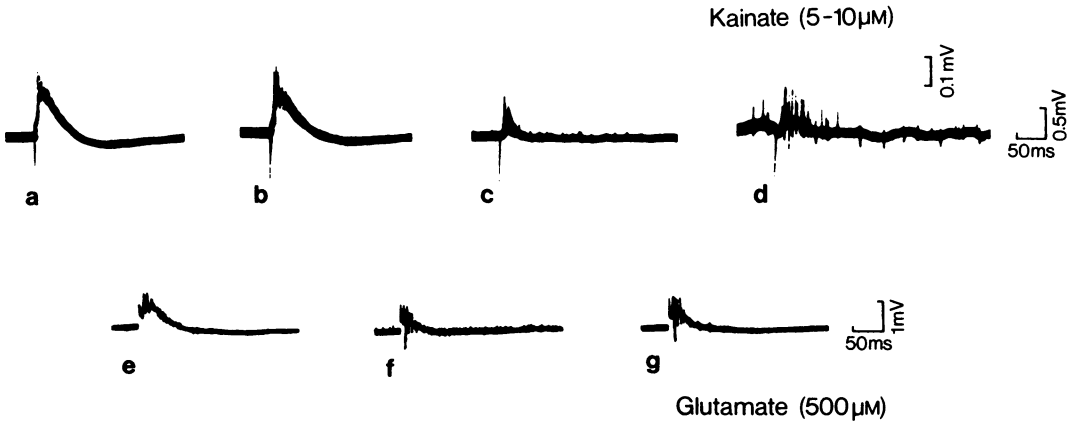

Figure 4 Effects of glutamate or kainate on the ventral root potential (VRP) of the frog isolated spinal cord; (a) and (e) controls; (b) kainate (5 μM) 2 min; (c) kainate (10 μM) 2 min; (d) kainate (10 μM) 4 min, note the higher gain; (f) and (g) glutamate (500 μM) 2 and 4 min respectively. Calibration bars for (a), (b), (c) and (d) 0.5 mV (except for (d) where a 0.1 mV bar is shown); 50 ms. Calibration bars for (e), (f) and (g) 1 mV; 50 ms.

(about 3 mV) of the membrane was produced and the effects of kainate or glutamate (residual) were virtually abolished (Figure 3, f–g). Changing from a normal solution to one containing an additional 21 mM $MgCl_2$ produced a small increase (about 5%) in the membrane resistance (see Takeuchi & Takeuchi, 1971) and also a slight increase in the amplitude of glutamate or kainate-evoked responses. All these actions were easily reversible on returning to normal lobster Ringer. Unfortunately, more detailed studies of the role of Ca^{2+} in these processes were not possible using the present method since the absence of this cation from the bathing solution depolarized the membrane and markedly reduced the membrane resistance (Takeuchi &Takeuchi, 1971) thus preventing a clear interpretation of the data.

Frog spinal cord

In the *in vitro* frog spinal cord both glutamate and kainate produced a depolarization of the ventral and dorsal roots accompanied by intense spike activity. The ventral root potential (VRP) was reduced by the bath application of kainate or glutamate and eventually abolished (Figure 4). A depression of the dorsal root potential (DRP) was also seen. Both these effects were fully reversible on washing. A feature of all these experiments was the higher potency of kainate compared to glutamate on a molar basis. Since glutamate is considered to be an excitatory transmitter of the afferents to spinal motoneurones (Barker *et al.*, 1975), it was necessary to determine whether this substance was acting directly on the motoneurones or indirectly via interneurones. In order to prevent any indirect action as a result of propagated interneuronal activity, the spinal cord was treated with tetrodotoxin (TTX; 1 μg/ml); therefore the d.c. recordings from the ventral root only represented the electrotonic spreading of the depolarization of the motoneurones along their axons (Biscoe *et al.*, 1975).

In the TTX-treated cords the ventral root depolarization produced by glutamate was dose-related, had a fast onset and decline and was often followed by a hyperpolarization (see inset of Figure 5). However, the maximum of the dose-response curve was difficult to obtain in those cases where high doses of glutamate produced a desensitization. This is reflected in Figure 5 by the scattering of the points at doses higher than 8 mM. The glutamate dose-response curve obtained in the absence of TTX was found to be very similar to that obtained in the presence of this toxin. The cord responses to glutamate could be closely reproduced even after many hours provided that the contact time of the drug was not longer than 1 min and 8–10 min elapsed between each administration. Strychnine (10 μM) did not reduce the effects of glutamate, while picrotoxin (10 μM) slightly potentiated the response to glutamate.

Figure 6 (inset) shows the ventral root responses to the application of kainate in a TTX-treated cord. This compound was active at concentrations much lower than those of glutamate and had a relatively slower onset-decline in its rate of action. The recovery of the preparation after a single administration of kainate took 12–20 min of repetitive washing; however, the addition of high doses of kainate (>100 μM) often produced an irreversible depolarization of the cord followed by an insensitivity to glutamate and other neurally active compounds. Provided that kainate was administered at intervals of 15–20 min and doses

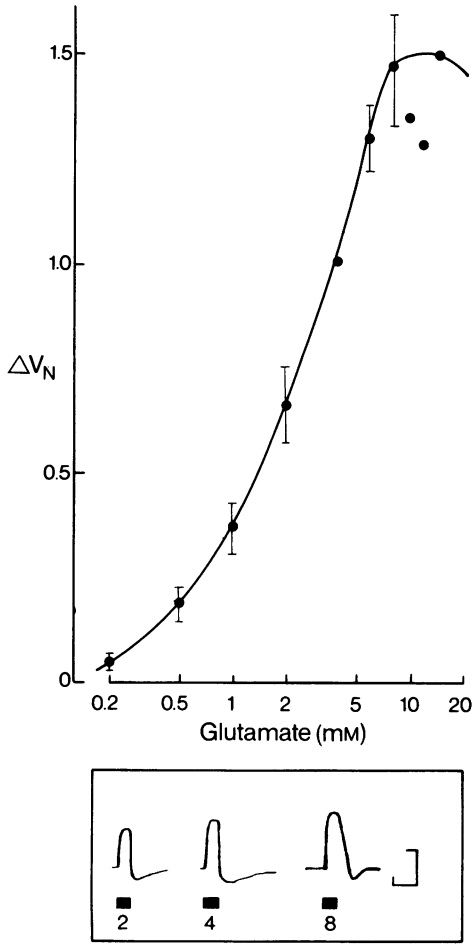

Figure 5 Normalized log dose-response curve for glutamate obtained from a ventral root of tetrodotoxin-treated spinal cords. Points represent mean of 6 experiments. Vertical lines show s.e. mean. Ordinate scale represents normalized cord depolarizations (ΔV_N) which were calculated by dividing the glutamate responses of each cord by the response to 4 mM glutamate of that cord. Abscissa scale: log concentration of glutamate. Inset: examples of cord responses to glutamate (2, 4 or 8 mM). In this and the following figures depolarizations are indicated by upward deflections of the pen. Calibration bars: 1 mV; 1 min.

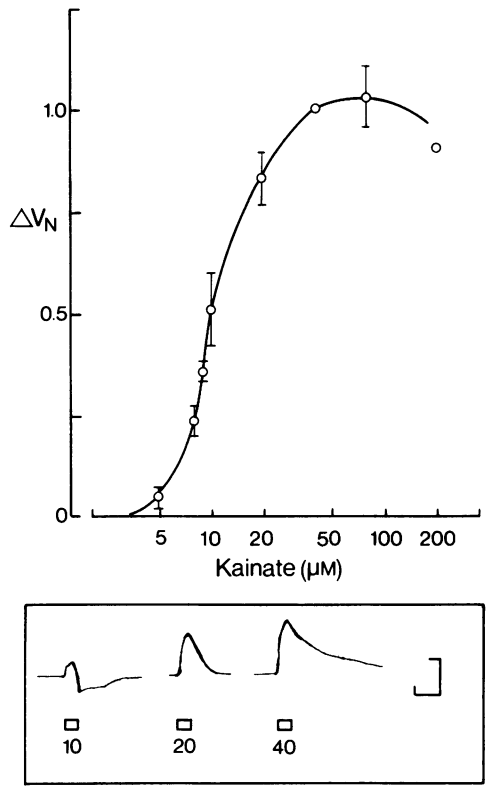

Figure 6 Normalized log dose-response curve for kainate obtained from a ventral root of tetrodotoxin-treated spinal cords. Points represent mean of 4 experiments. Vertical lines show s.e. mean. Ordinate scale represents normalized cord responses (ΔV_N) which were calculated by dividing the kainate responses of each cord by the response to 40 μM kainate of that cord. Abscissa scale: log concentration of kainate. Inset: examples of cord responses to kainate (10, 20 or 40 μM). Calibration bars: 1 mV; 1 min.

higher than 100 μM were avoided, reliable dose-response curves could be obtained as in Figure 6. Of course, the maximum shown in this figure was largely influenced by the desensitization phenomenon and the irreversible effects of large doses. Similar kainate dose-response curves were obtained from cords not treated with TTX.

In order to investigate the kinetics of the drug receptor interaction, log–log plots for glutamate and kainate were prepared (Figure 7a and b). For both glutamate and kainate the plots were linear initially with limiting slopes of 1.45 and 3.33, respectively. This suggests that more than one molecule of these drugs was interacting with a single receptor site. The ionic dependence of the amino acid depolarizations was then studied by substituting over 90% of external Na^+ in the bathing solution with equimolar Li^+. After 90 min in low-Na^+ medium, the responses to glutamate and kainate were almost completely blocked; recovery was achieved 60 min after return to the normal salt solution (Figure 8). The removal of Ca^{2+} and its replacement with equimolar Mg^{2+} did not affect the depolarization produced by glutamate but increased the subsequent hyperpolarization (Figure 8).

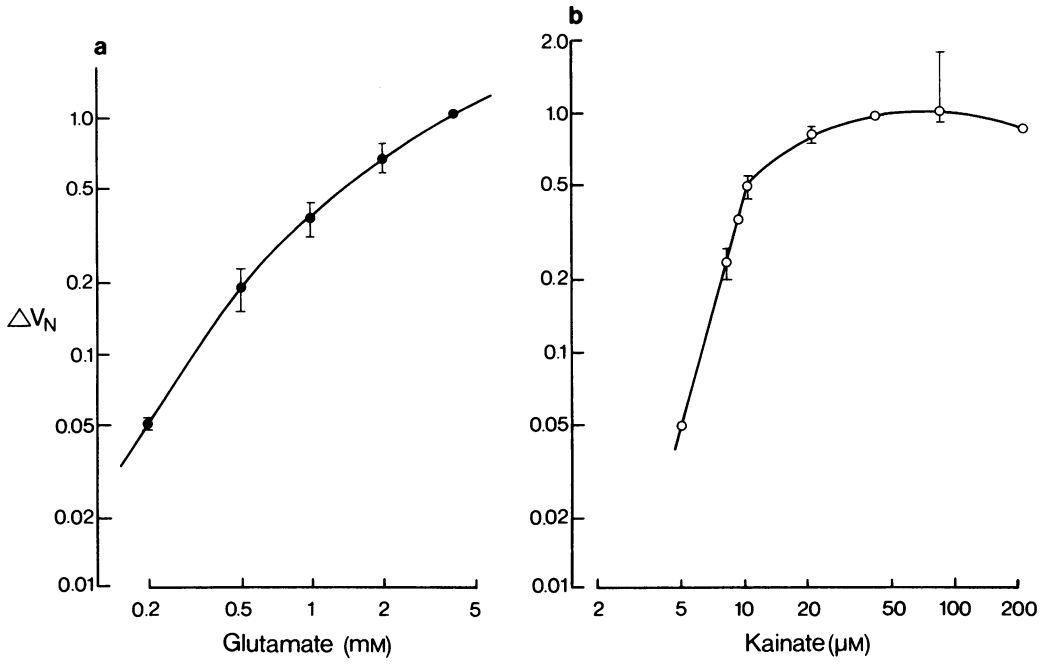

Figure 7 Log–log transformations of the dose-response curves for glutamate (●) and kainate (○) from a ventral root of tetrodotoxin-treated spinal cords; (a) Log ΔV_N vs log [glutamate], slope = 1.45; (b) Log ΔV_N vs log [kainate], slope = 3.33. Error intervals indicated are the transformed standard errors. For further details see Figures 5 and 6.

Similar results were obtained with kainate in a Ca^{2+}-free solution.

Discussion

A quantitative study of the effects of glutamate and related compounds on nervous tissue preparations suffers from several technical limitations. If this substance is applied microiontophoretically almost all neurones are excited. This widespread action complicates the assessment of glutamate as a putative neurotransmitter. Moreover, dose-response curves are difficult to obtain by iontophoretic administration (Curtis, Duggan & Johnston, 1971) despite the different methods proposed (Hill & Simmonds, 1973; Clarke, Forrester & Straughan, 1974). In order to avoid these difficulties and to allow the construction of dose-response curves, we carried out some quantitative experiments on two *in vitro* preparations, the lobster muscle fibre and the frog spinal cord. In both these tissues glutamate and kainate, a structurally related amino acid, were applied via the bathing solutions.

On the lobster muscle fibre where glutamate is considered to be the excitatory transmitter (Kravitz *et al.*, 1970) this substance had a depolarizing effect which showed rapid desensitization at high concentrations (if muscle contraction did not occur). The glutamate-evoked depolarization was largely dependent on external Na^+ (see Gerschenfeld, 1973, for review) and required more than one molecule for the receptor activation. However, it should be emphasized that the log–log plot analysis used in this study can give an underestimate of the true degree of cooperativity when the true limiting slope (attained as glutamate concentration approaches zero) lies outside the physiological range of the measurements.

In contrast, kainate showed a weaker depolarizing effect than glutamate (on a molar basis) which was relatively unaltered in the presence of a Na^+-free solution, but was abolished in a Na^+ and Ca^{2+}-free solution. In spite of the limitations of the Ca^{2+}-free experiments, a role for this divalent cation in the muscle responses to glutamate and kainate can thus be envisaged and is supported by the findings on other crustacean muscle preparations (Takeuchi & Onodera, 1973).

In the frog spinal cord both glutamate and kainate produced dorsal and ventral root depolarization, intense spike activity and depression of the VRP and DRP presumably as a consequence of excessive

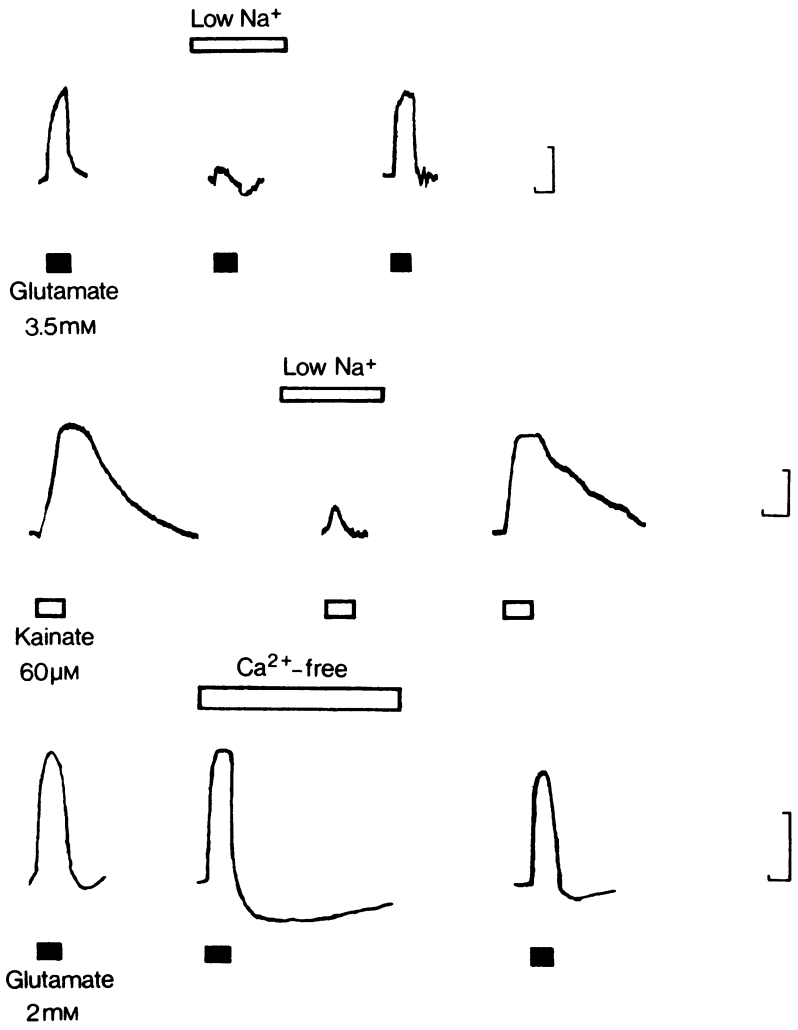

Figure 8 Effects of ionic changes on glutamate and kainate-evoked depolarizations recorded from ventral roots in tetrodotoxin-treated spinal cords. The first response in each row is a control and the last is a recovery (60 min after low Na^+ Ringer and 30 min after Ca^{2+}-free Ringer respectively). The centre responses were obtained after either 90 min in low Na^+ (replaced by equimolar Li^+) medium or 30 min in Ca^{2+}-free (replaced by equimolar Mg^{2+}) medium. Drugs were applied as indicated by the filled bars (glutamate) or open bars (kainate) below the traces. All calibrations: 1 mV; 1 min.

depolarization (Curtis, Phillis & Watkins, 1961). The glutamate dose-response curves obtained in TTX-treated cords, using the depolarization recorded from the ventral root as the response parameter, were similar in shape to those obtained in the absence of this toxin. The same was also found for kainate. This suggests that both drugs were acting directly on the motoneurones. A drug-induced depolarization of the afferent synaptic knobs with consequent discharge of transmitters onto the motoneurones seems unlikely since the glutamate or kainate-induced depolarization was essentially unchanged in Ca^{2+}-free Ringer (containing Mg^{2+}) in which synaptic transmission was blocked. However, a contribution of increased extracellular K^+ to the observed motoneuronal depolarization following the administration of these amino acids cannot be excluded.

In all our experiments on the frog cord, kainate was more potent than glutamate (on a molar basis), thus confirming previous observations on the vertebrate

central nervous system (Shinozaki & Konishi, 1970; Johnston *et al.*, 1974; McCulloch *et al.*, 1974; Biscoe *et al.*, 1975). However, the action of kainate had a slower onset and decline relative to glutamate and was irreversible at high concentrations. The latter finding might explain the considerable *in vivo* neurotoxicity of this drug (Olney, Rhee & Ho, 1974). From the analysis of our results it would appear that glutamate and kainate interact with the frog motoneuronal membrane via a Na^+-dependent mechanism. Moreover, more than one molecule of glutamate seems to be involved in the receptor interaction, while in the case of kainate a number even higher than this can be envisaged. Kainate may thus be considered to act as an agonist on glutamate receptors in this system, as has been suggested in the mammalian central nervous system (Johnston *et al.*, 1974; McCulloch *et al.*, 1974).

If kainate was also acting on glutamate receptors in lobster muscle, then the observed difference in tissue sensitivity to this agent might be explained if the frog spinal glutamate receptors had a relatively higher affinity for kainate. An essentially similar glutamate binding site on both preparations could thus show a different tolerance to variations in agonist structure, the invertebrate site being the more restricted in this respect. A difference in the diffusion rate of kainate through the two tissues could be excluded since, on the lobster muscle where kainate was less potent, only single superficial fibres were used for recording, and these were in direct contact with the superfusing fluid and therefore more accessible to this drug than spinal motoneurones.

At the crayfish neuromuscular junction, both junctional and extrajunctional glutamate receptors have been postulated (Shinozaki & Shibuya, 1974) although only the extrajunctional receptors are thought to be sensitive to kainate (Takeuchi & Onodera, 1975). It is possible, therefore, that kainate was acting on extrajunctional glutamate receptors in both lobster muscle and the frog spinal cord. The different tissue sensitivity to kainate would then be explained if the two preparations contained a different number of such receptors. However, evidence of spinal extrajunctional glutamate receptors is not yet available due to the lack of suitable techniques and the presence of extrajunctional glutamate receptors on lobster muscle needs to be tested experimentally.

In conclusion, our present findings suggest that the receptor sites activated by glutamate in the lobster and the frog do not constitute a homogeneous population. In the absence of specific glutamate antagonists (Nistri & Constanti, 1975), the use of kainate together with other glutamate agonists may be a useful tool with which to characterize these receptor sites.

We thank Prof. J.P. Quilliam for his encouragement and interest in our study, Miss Sheila Harper for excellent technical assistance and Miss Alison Robson for her help in some preliminary experiments. A.C. was supported during this investigation by a grant from the Governors of St Bartholomew's Hospital. Please send reprint requests to A.N. in Florence.

References

BARKER, J.L., NICOLL, R.A. & PADJEN, A. (1975). Studies on convulsants in the isolated frog spinal cord. II. Effects on root potentials. *J. Physiol., Lond.*, **245**, 537–548.

BISCOE, T.J., EVANS, R.H., HEADLEY, P.M., MARTIN, M. & WATKINS, J.C. (1975). Domoic and quisqualic acids as potent amino acid excitants of frog and rat spinal neurones. *Nature, Lond.*, **255**, 166–167.

CLARKE' G., FORRESTER, P.A. & STRAUGHAN, D.W. (1974). A quantitative analysis of the excitation of single cortical neurones by acetylcholine and L-glutamic acid applied micro-iontophoretically. *Neuropharmacology*, **13**, 1047–1055.

CLEMENTS, A.N. & MAY, T.E. (1o74). Pharmacological studies on a locust neuromuscular preparation. *J. exp. Biol.*, **61**, 421–442.

CONSTANTI, A. & NISTRI, A. (1975). Actions of glutamate and kainic acid on the lobster muscle fibre and the frog spinal cord. *Br. J. Pharmac.*, **53**, 437–438P.

CONSTANTI, A. & NISTRI, A. (1976). A comparative study of the action of γ-aminobutyric acid and piperazine on the lobster muscle fibre and the frog spinal cord. *Br. J. Pharmac.*, **57**, 347–358.

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

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergeb. Physiol.*, **66**, 97–188.

CURTIS, D.R., PHILLIS, J.W. & WATKINS, J.C. (1961). Actions of amino-acids on the isolated hemisected spinal cord of the toad. *Br. J. Pharmac. Chemother.*, **16**, 262–283.

DUDEL, J. (1975). Potentiation and desensitization after glutamate induced postsynaptic currents at the crayfish neuromuscular junction. *Pflügers. Arch. ges. Physiol.*, **356**, 317–327.

FISZER DE PLAZAS, S. & DE ROBERTIS, E. (1974). Isolation of hydrophobic proteins binding neurotransmitter amino acids. Glutamate receptor of the shrimp muscle. *J. Neurochem.*, **23**, 1115–1120.

GERSCHENFELD, H.M. (1973). Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.*, **53**, 1–119.

HILL, R.G. & SIMMONDS, M.A. (1973). A method for comparing the potencies of γ-aminobutyric acid antagonists on single cortical neurones using microiontophoretic techniques. *Br. J. Pharmac.*, **48**, 1–11.

JOHNSON, J.L. (1972). Glutamic acid as a synaptic transmitter in the nervous system. A review. *Brain Res.*, **37**, 1–19.

JOHNSTON, G.A.R., CURTIS, D.R., DAVIES, J. &

McCULLOCH, R.M. (1974). Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature, Lond.*, **248**, 804–805.

KRAVITZ, E.A., SLATER, C.R., TAKAHASHI, K., BOWNDS, M.D. & GROSSFELD, R.M. (1970). Excitatory transmission in invertebrates—glutamate as a potential neuromuscular transmitter compound. In *Excitatory Synaptic Mechanisms*, ed. Andersen, P. & Jansen, J.K.S. pp. 85–93. Oslo: Universitets Forlaget.

McCULLOCH, R.M., JOHNSTON, G.A.R., GAME, C.J.A. & CURTIS, D.R. (1974). The differential sensitivity of spinal interneurones and Renshaw cells to kainate and *N*-methyl-D-aspartate. *Expl. Brain Res.*, **21**, 515–518.

NISTRI, A. & CONSTANTI, A. (1975). Effects of glutamate and glutamic acid diethyl ester on the lobster muscle fibre and the frog spinal cord. *Eur. J. Pharmac.*, **31**, 377–379.

OLNEY, J.W., RHEE, V. & HO, O.L. (1974). Kainic acid: a powerful neurotoxic analogue of glutamate. *Brain Res.*, **77**, 507–512.

ROBERTS, P.J. (1974). Glutamate receptors in the rat central nervous system. *Nature, Lond.*, **252**, 399–401.

SHINOZAKI, H. & KONISHI, S. (1970). Actions of several anthelmintics and insecticides on rat cortical neurones. *Brain Res.*, **24**, 368–371.

SHINOZAKI, H. & SHIBUYA, I. (1974). Potentiation of glutamate-induced depolarization by kainic acid in the crayfish opener muscle. *Neuropharmacology*, **13**, 1057–1065.

TAKEUCHI, A. & ONODERA, K. (1973). Reversal potentials of the excitatory transmitter and L-glutamate at the crayfish neuromuscular junction. *Nature, New Biol.*, **242**, 124–126.

TAKEUCHI, A. & ONODERA, K. (1975). Effects of kainic acid on the glutamate receptors of the crayfish muscle. *Neuropharmacology*, **14**, 619–625.

TAKEUCHI, A. & TAKEUCHI, N. (1964). The effect on crayfish muscle of iontophoretically applied glutamate. *J. Physiol., Lond.*, **170**, 296–317.

TAKEUCHI, A. & TAKEUCHI, N. (1971). Anion interaction at the inhibitory post-synaptic membrane of the crayfish neuromuscular junction. *J. Physiol., Lond.*, **212**, 337–351.

WERMAN, R. (1969). An electrophysiological approach to drug-receptor mechanisms. *Comp. Biochem. Physiol.*, **30**, 997–1017.

(Received October 20, 1975.
Revised February 11, 1976.)