express kainate receptor subunits[8], and certain subunits have been localized on dendritic spines[29]. It may therefore seem surprising that the synaptic activation of kainate receptors has not been reported previously. However, in most experiments the synaptic activation of AMPA receptors was eliminated using CNQX (or a related quinoxalinedione), and so kainate receptor-mediated responses would also have been antagonized.

The identity of the kainate receptor subtype(s) involved in mossy fibre synaptic transmission is not known, although it clearly involves a receptor subunit combination that does not exhibit rapid and complete desensitization and has a linear I–V relationship[6,12,30]. The function of kainate receptors at this synapse can only be a matter of speculation at present. However, the fact that their activation is facilitated during high-frequency transmission raises the possibility that kainate receptors are involved in synaptic plasticity in this pathway, which classically exhibits NMDA receptor-independent long-term potentiation[17,18]. □

Methods

Experiments were performed on transverse hippocampal slices (400 μm) obtained by using a vibroslice[16] from Wistar rats 13–17 days old. Slices were collected and maintained in medium comprising (mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 10 D-glucose (bubbled with O_2 /CO_2: 95%/5%). After at least 1 h equilibration and recovery time, slices were transferred to a submerged style recording chamber and perfused with the same medium (at a rate of approximately 2 ml min^{-1}) at room temperature. Whole-cell patch-clamp recordings were obtained from the CA3 region, using the 'blind approach' with glass microelectrodes (5–7 mΩ; seal resistance ~10 GΩ) filled with a solution comprising (mM): 120 $CsMeSO_4$, 1 NaCl, 1 $MgCl_2$, 10 BAPTA, 5 N-(2,6-dimethyl-phenylcarbamoylmethyl)-trimethylammonium bromide (QX-314), 5 HEPES (adjusted to pH 7.3). In most experiments, 4 mM Mg-ATP was included, as it was found to prevent rundown of the synaptic currents. EPSCs were evoked by low-frequency stimulation of the mossy fibre and associational-commissural pathways alternately, using 20-μs pulses delivered through bipolar electrodes (Fig. 1a). Each pathway was stimulated once every 30 s, with 15 s separating the stimulation of each pathway. Two successive records per input were averaged to provide 1 min time points and for presentation of corresponding traces (the time points illustrated being indicated on the graphs by numbers). Apparent differences in stimulus artefacts are due to sampling. Access resistance was monitored continually and neurons discarded if this parameter changed by more than 20%. Unless otherwise stated, neurons were voltage-clamped at −60 mV. Drugs were applied by addition to the perfusing medium.

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Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons

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Glutamate, the neurotransmitter at most excitatory synapses in the brain, activates a variety of receptor subtypes that can broadly be divided into ionotropic (ligand-gated ion channels) and metabotropic (G-protein-coupled) receptors. Ionotropic receptors mediate fast excitatory synaptic transmission, and based on pharmacological and molecular biological studies are divided into NMDA and non-NMDA subtypes. The non-NMDA receptor group is further divided into AMPA and kainate subtypes[1]. Virtually all fast excitatory postsynaptic currents studied so far in the central nervous system are mediated by the AMPA and NMDA subtypes of receptors. Surprisingly, despite extensive analysis of their structure, biophysical properties and anatomical distribution, a synaptic role for kainate receptors in the brain has not been found[2]. Here we report that repetitive activation of the hippocampal mossy fibre pathway, which is associated with high-affinity kainate binding[3] and many of the kainate receptor subtypes[4–8], generates a slow excitatory synaptic current with all of the properties expected of a kainate receptor. This activity-dependent synaptic current greatly augments the excitatory drive of CA3 pyramidal cells.

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We used standard electrophysiological techniques to determine whether the synaptic release of glutamate can activate kainate receptors. GYKI 53655 (1-(4-aminophenyl)-3-methylcarbamyl-4-methyl-7,8-methylenedioxy-3,4-dihydro-5H-2,3-benzodiazepine; 30 μM), which is known to block AMPA (α-amino-3-hydroxy-5-methylisooxazole-4-propionic acid)-receptor-mediated responses[9,10] but not kainate receptor-mediated responses[11,12], blocks the mossy fibre field excitatory postsynaptic potential (EPSP) (>90%) (Fig. 1A,a). However, if repetitive stimuli are given in the presence of GYKI 53655, a slow component appears (Fig. 1A,b, trace 3), which is antagonized by CNQX (6-cyano-7-nitroquinoxaline-2,3-dione; 30 μM) (Fig. 1A,b, trace 4). The results of this experiment are shown graphically in Fig. 1A,c. GYKI 53655 blocks the response to single stimuli, but in the continued presence of GYKI 53655, a response is restored by six stimuli at 30 Hz. This response is mediated by mossy fibres, as it is blocked by LCCG-1 (2*S*,3*S*,4*S*-CCG1(25,1′*S*,2′*S*)-2-(carboxycyclopropryl)glycine; 10 μM), which blocks mossy fibre responses by activating presynaptic inhibitory group II metabotropic glutamate receptors that are selectively expressed on mossy fibre terminals[13]. Finally, application of CNQX antagonizes the GYKI 53655-resistant slow component, confirming that it was mediated by non-NMDA receptors.

In this same experiment, responses to stimulation of the associational/commissural fibres were alternated with the mossy fibre responses (Fig. 1B). GYKI 53655 completely blocked the synaptic response to a single associational/commissural stimulus, leaving only a presynaptic fibre volley (Fig. 1B,a), and there was no recovery of a response with a repetitive stimulation (Fig. 1B,b). Thus CNQX had no effect in the presence of GYKI 53655. The finding that repetitive activation of mossy fibres, but not associational/commissural fibres elicits a GYKI 53655-resistant, CNQX-sensitive slow synaptic response was seen in eight separate experiments.

Although the mossy fibre slow component was blocked by CNQX (>80% at 30 μM), it was about 10-fold less sensitive than the fast component (>80% at 3 μM), consistent with previous data on kainate receptors[5,14,15]. The slow component was also blocked by the non-NMDA receptor antagonists NBQX (10 μM) ($n = 3$) and kynurenate (1 mM) ($n = 3$), but was unaltered by the NMDA-receptor antagonist AP5 (100 μM) ($n = 5$), the metabotropic glutamate-receptor antagonist MCPG (1.5 mM) ($n = 4$), the glutamate transport blocker trans-PDC (500 μM) ($n = 5$), or by NS-102 (3 μM) ($n = 5$), an antagonist of low- but not high-affinity kainate receptors[14,15].

Whole-cell recording demonstrated that the presence and size of the GYKI 53655-resistant component were extremely sensitive to the frequency of stimulation. The GYKI 53655-resistant component was very small at low-frequency stimulation (Fig. 2A,a), but was always discernible with higher stimulus rates (Fig. 2A,b and 2A,c). When the stimulus frequency was changed from 0.05 Hz to 0.2 Hz, the response increased by 182 ± 7% ($n = 4$). This is very similar to the facilitation observed for the same change in frequency for the AMPA excitatory postsynaptic currents (EPSCs) (194 ± 13%, $n = 4$) (ref. 16). The decay of the GYKI 53655-resistant component ($\tau = 103 \pm 7$ ms, $n = 8$) was much slower than that for the GYKI 53655-sensitive component ($\tau = 9.1 \pm 1.2$ ms, $n = 6$) (Fig. 2A,d). In addition, the rise time (10–90%) of the GYKI 53655-resistant EPSC (6.8 ± 0.3 ms) was slower than the AMPA EPSC (2.9 ± 0.4 ms) recorded in the same cell ($n = 4$). The amplitude was strongly dependent both on the frequency of stimulation (Fig. 2B,a; $n = 6$) and on the number of stimuli in a train (Fig. 2B,b; $n = 6$). However, this enhancement was not associated with a change in the decay of the response. For example, τ at 1 Hz was 82 ms and at 5 Hz was 78 ms (Fig. 2B,a), and τ for two stimuli was 64 ms, and for three stimuli was 66 ms (Fig. 2B,b). GYKI 53655 completely blocked the EPSCs evoked by associational/commissural stimulation in the CA3 region (Fig. 2C; $n = 4$), as well as Schaffer collateral stimulation in the CA1 region (data not shown), and repetitive stimulation did not restore transmission. The GYKI 53655-resistant component of the mossy fibre response was invariably blocked (>90%) by CNQX (50 μM) (Fig. 2C; $n = 6$).

Further evidence consistent with the GYKI 53655-resistant slow EPSC being mediated by kainate receptors was provided by the action of cyclothiazide, which markedly potentiates AMPA receptor responses[17] and AMPA receptor EPSCs in other regions[18] yet has no effect on kainate receptor responses. Cyclothiazide enhanced the fast, GYKI 53655-sensitive EPSC (Fig. 2D,a; $n = 4$) but had no effect on the slow GYKI 53655-resistant EPSC (Fig. 2D,b; $n = 4$). Thus the response remaining in GYKI 53655 cannot be due to incomplete blockade of AMPA receptors. The GYKI 53655-resistant EPSC reverses at about 0 mV, and the I–V relationship exhibits slight outward rectification (Fig. 2E; $n = 4$). This finding suggests that the kainate receptors involved in this response are of the edited, Ca^{2+}-impermeable form, as unedited receptors show strong inward rectification[19]. The slow time course of the GYKI 53655-resistant EPSC raises the possibility that it might depend on the continued presence of glutamate. However, the time to half-decay of the EPSC was not increased by the glutamate uptake blocker trans-PDC (500 μM) ($n = 4$), (average change = +2.2 ± 3.2%), which has been shown to enhance the spread of glutamate away from mossy fibre synapses[20].

We next tested for the presence of postsynaptic kainate receptors on CA3 pyramidal cells (Fig. 3A). CA3 pyramidal cells were approximately 30 times more sensitive to bath-applied kainate than were CA1 pyramidal cells, in agreement with previous results[21]. These high-affinity kainate receptors are localized at the mossy fibre

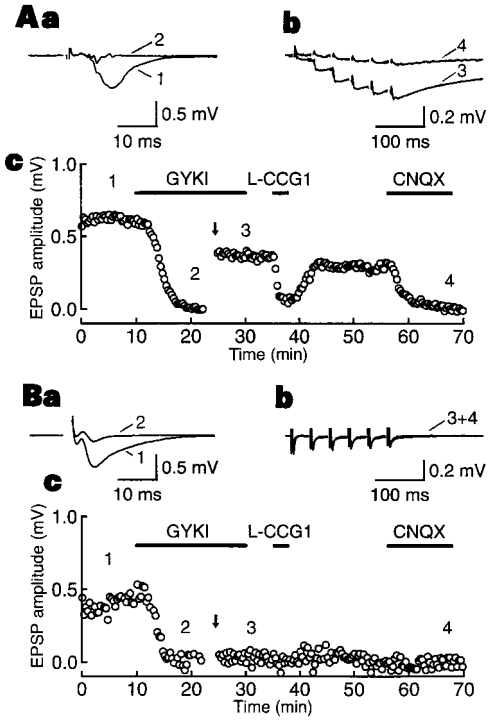

Figure 1 Repetitive activation of mossy fibres but not associational/commissional fibres activates a GYKI 53655 resistant, CNQX-sensitive synaptic potential, as revealed by field-potential recording. **A**, Mossy fibre field-potential records to single (**a**) and repetitive (**b**) stimulation were obtained at the times indicated by the numbers in the graph (**c**). Note the higher gain and slower time scale in **b** then in **a**. **B**, Associational/commissional responses were also recorded in this same experiment. Responses to single (**a**) and repetitive (**b**) stimulation were obtained at the times indicated by the numbers in the graph (**c**). The arrows in **A**, **c** and **B**, **c** indicate the time at which stimulation was changed from single stimuli (0.2 Hz) to repetitive stimuli (6 stimuli at 30 Hz given every 5 s). All traces are an average of 10–30 sweeps.

synapses (Fig. 3B), as brief puff application of kainate to stratum lucidum evoked a large inward current, whereas the identical application in stratum radiatum gave no response ($n = 4$). Repositioning the pipette in stratum lucidum again evoked an inward current. Puff application of kainate (10 μM) to stratum radiatum in the CA1 region close to the cell body layer failed to evoke any inward current ($n = 5$). Application of GYKI 53655, which completely blocked the AMPA receptor miniature EPSCs (downward deflections), had no effect on the stratum lucidum kainate response (Fig. 3C; $n = 4$). CNQX, however, completely blocked this response (Fig. 3C; $n = 4$). Like GYKI 53655, cyclothiazide also had no effect on the kainate responses (Fig. 3D,a), while having the expected enhancing effect on the size of the miniature EPSCs in the same experiment (Fig. 3D,b; $n = 3$). These results, using new pharmacological tools, directly demonstrate the presence of kainate receptors localized to the proximal dendrites of CA3 pyramidal cells, extending previous data on kainate responses associated with the mossy fibre projection[22].

If the GYKI 53655-resistant EPSC is mediated by glutamate released onto kainate receptors, application of glutamate should also activate these receptors. Puff application of glutamate to stratum lucidum evoked a response that was typically only partially sensitive to GYKI 53655, even though the AMPA-receptor miniature EPSCs in this condition were entirely blocked (Fig. 4A). Addition of CNQX blocked the glutamate response remaining in GYKI (Fig. 4A; $n = 4$). In contrast, puff application of glutamate to stratum radiatum evoked an inward current that was completely blocked by GYKI 53655 (Fig. 4B; $n = 4$).

Although our results demonstrate the presence of postsynaptic kainate receptors on CA3 pyramidal cells, several studies have proposed that kainate receptors are present presynaptically not only on the mossy fibre terminals[23–25], but also on the terminals of Schaffer collaterals in the CA1 region[26]. Given the depolarizing action of these receptors, it can be anticipated that their activation on synaptic terminals would depolarize the terminals and as a consequence release glutamate, as has been demonstrated for presynaptic nicotinic receptors on mossy fibres[27]. We therefore investigated whether application of kainate changes the frequency of AMPA-receptor miniature EPSCs, both when applied in stratum lucidum of the CA3 region and in stratum radiatum of the CA1 region. Puff application of kainate had little effect on the frequency of miniature EPSCs, either in the CA3 region (Fig. 4C,a) or in the CA1 region (Fig. 4C,b). Bath application of kainate also failed to alter the frequency of miniature EPSCs (Fig. 3A), whereas depolarization of the terminals with potassium (15 mM) increased the frequency considerably (data not shown). These results, together with the finding that GYKI 53655 and cyclothiazide had no effect on the size of the kainate-evoked response in CA3 cells, suggest that the predominant effect of kainate receptor activation is postsynaptic.

The finding that only the mossy fibres evoke a GYKI 53655-resistant EPSC, together with the selective action of kainate in stratum lucidum, provides a physiological basis for the remarkably selective distribution of high-affinity kainate binding to stratum lucidum[3]. If the kainate receptors in stratum lucidum have high affinity, why is it that at low-frequency stimulation most of the EPSCs are mediated by low-affinity AMPA receptors? If the AMPA and kainate receptors are colocalized at synaptic sites, the small size of the kainate receptor EPSC might be explained by a small single-channel conductance[28] and/or a low density of receptors, and the dramatic increase in the size of the response observed with high stimulus frequencies can be explained by the marked increase in transmitter release[20]. Alternatively the kainate receptors could be excluded from the synaptic cleft, and with high stimulus frequencies

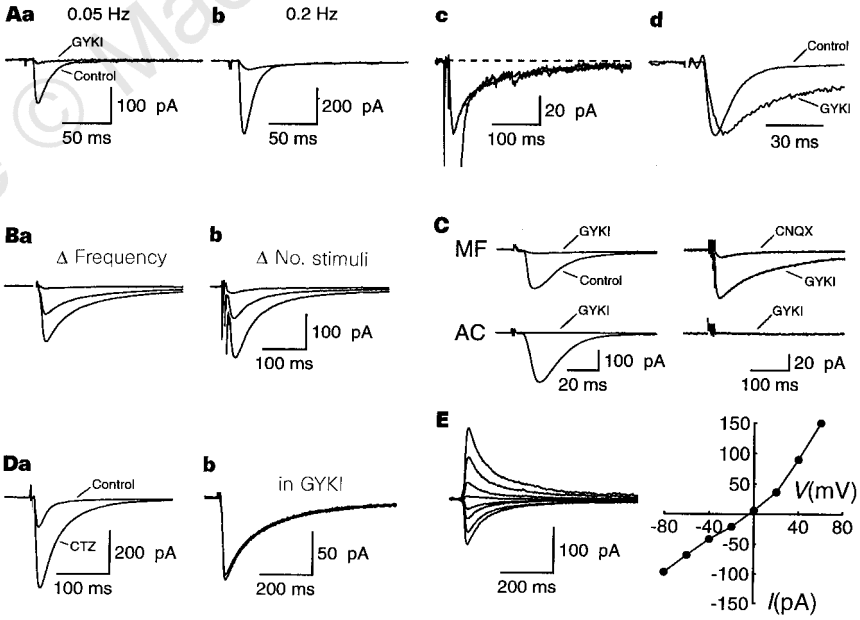

Figure 2 Characterization of the GYKI 53655 (GYKI)-resistant EPSC with whole-cell recording. **A**, Responses to two frequencies of stimulation in the absence and presence of GYKI (30 μM) are shown. All records are from the same cell. Increasing the frequency of stimulation from 0.05 Hz (**a**) to 0.2 Hz (**b**) greatly increases the size of the control and slow GYKI-resistant component. Note the decrease in gain for the records in **b**. **c**, Records in **b** at a much higher gain. **d**, The GYKI-resistant component has been scaled to the peak of the AMPA-receptor component. **B**, All responses were obtained in the presence of GYKI (30 μM). Increasing the frequency of stimulation (0.05 Hz, 1 Hz and 5 Hz) (**a**) or the number of stimuli in a train applied at 200 Hz (1, 2 and 3 stimuli) (**b**) greatly increases the response. **C**, GYKI blocks the response to single mossy fibre (MF) and associated/commissional (AC) stimulation (stimulus rate, 0.05 Hz) (left traces). Repetitive stimulation (4 stimuli at 200 Hz) (right traces) evokes a slow inward current at mossy fibres that is blocked by CNQX, but fails to restore a response at associational/commissional fibres. Note the increased gain and slower time scale for the records on the right. **D**, Cyclothiazide (100 μM) (CTZ) markedly increases the size of the mossy fibre AMPA-receptor EPSC (**a**) but has no effect on the mossy fibre EPSC evoked in the presence of GYKI (**b**). **E**, A family of superimposed current traces recorded in the presence of GYKI at different membrane potentials is shown. The peak current is plotted against membrane potential on the right. All traces are an average of 10–30 sweeps.

glutamate spills over onto these receptors. The similar degree of facilitation of the AMPA and kainate-mediated EPSCs, and the lack of change in the decay time constant of the kainate EPSC at high levels of glutamate release or when glutamate uptake is blocked[29], favours the first possibility. The slow kinetics of the GYKI 53655-resistant component contrasts with the rapid desensitization of kainate receptors, although functional receptors containing the KA1 subunit, which is selectively expressed in CA3 pyramidal cells[4], have not been studied. Further investigation will be necessary to reconcile the properties of kainate receptors to the kinetics of the EPSC mediated by these receptors.

Our results indicate that the synaptic release of glutamate from mossy fibres can activate postsynaptic kainate receptors as well as AMPA and NMDA receptors[30]. The kainate-receptor EPSC shows a remarkable use-dependence, such that it is minimal at low-frequency stimulation. This use-dependence, as well as the slow kinetics of the kainate EPSC, provide a mechanism for augmenting pyramidal-cell excitability during high levels of granule-cell activity. It will be of

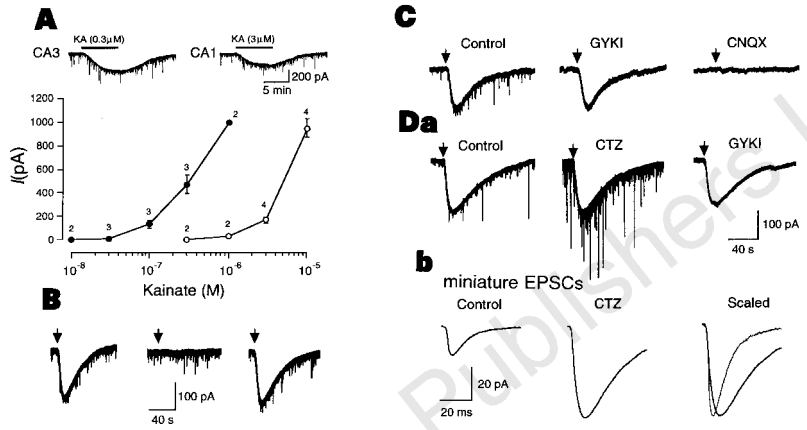

Figure 3 Characterization of responses to applied kainate. **A**, Graph illustrating the different sensitivity of CA1 (open circles) and CA3 (filled circles) pyramidal cells to bath-applied kainate. Responses to different concentrations of kainate were measured after the responses had reached equilibrium. The responses to the highest concentrations are underestimated because it was difficult to reach full equilibrium. All experiments were done in the presence of tetrodotoxin (0.3 μM). Chart records of typical responses are shown above the graph. **B**, Puff application of kainate (3 μM) on the surface of stratum lucidum (left) evokes an inward current. An identical puff applied 300 μm away in stratum radiatum (middle) evokes no response. Repositioning of the pipette over stratum lucidum (right) again evokes an inward current. **C**, Application of GYKI 53655 (GYKI) (30 μM) has little effect on the response evoked by puff application of 3 μM kainate but blocks the miniature EPSCs. The response is blocked by CNQX (25 μM). **D**, Application of cyclothiazide (100 μM) (CTZ) has no effect on the kainate response (**a**) but markedly increases the size of the miniature EPSCs seen as downward deflections (**a**) and as averaged traces (**b**). Miniature EPSCs were aligned and averaged (120 events each). Right, the control response has been scaled to the response obtained in cyclothiazide.

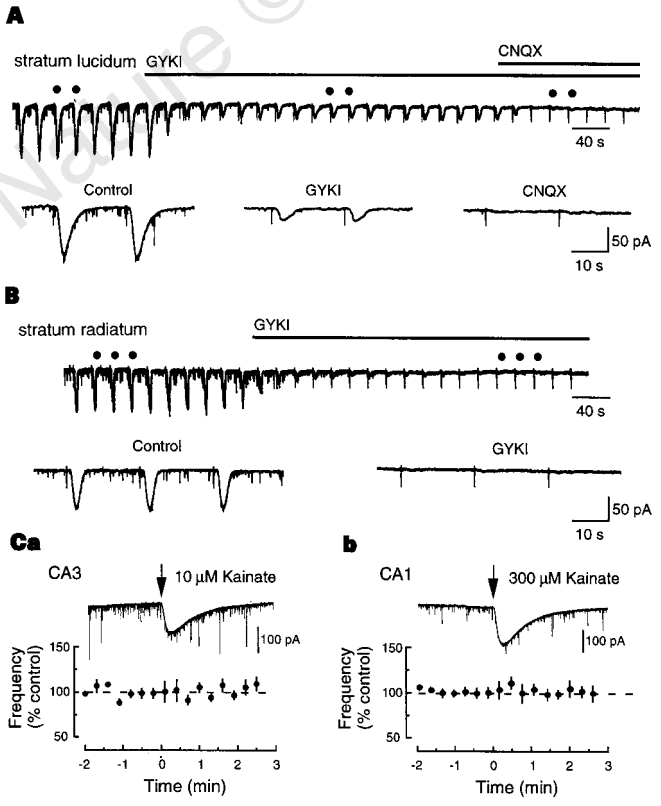

Figure 4 Characterization of the responses to glutamate and the lack of effect of kainate on the frequency of miniature EPSCs. **A**, Puff application of glutamate (3 mM) to the surface of stratum lucidum evokes an inward current that is only partly blocked by GYKI 53655 (GYKI) (30 μM). The remaining current is blocked by CNQX (50 μM). The responses identified by the filled circles above the continuous record are shown at a faster time scale beneath the continuous record. **B**, Puff application of glutamate (3 mM) to the surface of stratum radiatum evokes an inward current that is completely blocked by GYKI (30 μM). Sample records at a faster time scale are shown below the continuous record. **C**, Puff application of 3 μM kainate (200 kPa for 20 ms) to stratum lucidum in the CA3 region (**a**) or 300 μM kainate to stratum radiatum in the CA1 region (**b**) fail to alter the frequency of miniature EPSCs. The sample traces are from a chart recorder, and are shown at the same speed as the graphs below. Both graphs plot the frequency of miniature EPSCs against time (4 cells for each graph).

interest to determine whether kainate receptors in other regions of the central nervous system, such as cerebellum and neocortex[4–8,31], have a similar function. □

Methods

Hippocampal slices (400–500 μm thick) were prepared from guinea-pigs (5–15 days old) and were maintained at room temperature for at least 1 h in a submerged chamber containing artificial cerebrospinal fluid (ACSF) equilibrated with 95% O_2 and 5% CO_2. They were then transferred one at a time to a superfusing chamber[30]. The ACSF contained (in mM): 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, 11 glucose. For all whole-cell recording experiments the $MgSO_4$ and $CaCl_2$ were increased to 4 mM and pictotoxin (100 μM) and AP5 (50 μM) were present. Experiments were done at room temperature. Whole-cell recording electrodes were filled with a solution containing (in mM): 122.5 caesium gluconate, 10 CsCl, 10 HEPES, 10 EGTA, 8 NaCl, 2 MgATP, 0.3 Na_3GTP, 1 $CaCl_2$, 10 glucose. The resistance of the patch pipette ranged between 2 and 4 MΩ. Access resistances ranged between 8 and 15 MΩ, and were not allowed to vary by more than 15% during the course of the experiment. No series resistance compensation was used. Field recordings were performed with a glass electrode placed in the stratum lucidum. Bipolar tungsten electrodes were placed in the granule-cell layer to stimulate mossy fibres and in the stratum radiatum to stimulate associational/commissural fibres. A picospritzer was used to puff-apply glutamate and kainate. The pressure varied from 100 to 200 kPa, and the duration of the puff varied from 20 to 100 ms. The drugs were prepared in Fast Green (0.01%) (Sigma) to help localize the application visually. Average values are expressed as mean ± s.e.m. Drugs used were: L-glutamic acid, kainate, kynurenate, cyclothiazide, picrotoxin, L-AP4 (Sigma), CNQX, NBQX, D-AP5, (+)-MCPG and LCCG-1 (Tocris Cookson), L-trans-pyrrolidine-2,4-dicarboxylic acid, NS-102 (Research Biochemicals International), and tetrodotoxin (CalBiochem).

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Muscle force is generated by myosin heads stereospecifically attached to actin

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Muscle force is generated by myosin crossbridges interacting with actin. As estimated from stiffness[1,2] and equatorial X-ray diffraction[3] of muscle and muscle fibres, most myosin crossbridges are attached to actin during isometric contraction, but a much smaller fraction is bound stereospecifically[4–7]. To determine the fraction of crossbridges contributing to tension and the structural changes that attached crossbridges undergo when generating force, we monitored the X-ray diffraction pattern during temperature-induced tension rise in fully activated permeabilized frog muscle fibres. Temperature jumps[8] from 5–6 °C to 16–19 °C initiated a 1.7-fold increase in tension without significantly changing fibre stiffness or the intensities of the (1,1) equatorial and (14.5 nm)$^{-1}$ meridional X-ray reflections. However, tension rise was accompanied by a 20% decrease in the intensity of the (1,0) equatorial reflection and an increase in the intensity of the first actin layer line by ~13% of that in rigor. Our results show that muscle force is associated with a transition of the crossbridges from a state in which they are nonspecifically attached to actin to one in which stereospecifically bound myosin crossbridges label the actin helix.

Muscle force results from changes in myosin crossbridges, or 'heads', that are attached to thin filaments[9,10]. Changes in the intensities, $I_{1,0}$, $I_{1,1}$, of the main equatorial X-ray reflections[3] (1,0) and (1,1), and stiffness measurements[1,2] show that 50–75% of myosin heads are attached to the thin filaments in fully activated muscle contracting isometrically. Surprisingly, the intensity, I_{1a}, of