Kainate-induced uptake of calcium by synaptosomes from rat brain

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Kainic acid induces a rapid increase in $^{45}Ca^{2+}$ uptake by crude synaptosomal fractions isolated from rat brain. This enhanced Ca^{2+} permeability occurs with a half-time of approx. 1 s, similar to the fast phase of depolarization-induced calcium uptake. The depolarization-induced uptake of calcium is inhibited 85% by 3 mM $CoCl_2$, 80% by 100 μM quinacrine and 50% by 15 μM trifluoperazine while these agents had little effect on the kainate-induced uptake. It is proposed that kainate induces receptor-mediated opening of a class of calcium channels with properties different from those of the voltage-dependent channels.

Kainic acid *Calcium uptake* *Synaptosome*

1. INTRODUCTION

Kainic acid, a structural analogue of glutamate with potent neurotoxic and neuroexcitatory activities, has been extensively utilized to produce selective damage of neurons [1,2]. Many neurophysiological studies carried out during the past few years have suggested mechanisms of kainate toxicity in the brain. It has commonly been proposed that the toxicity of kainate is due to tissue hypoxia resulting from an induced hyperactivity of the neurons [3–5].

Consistent with this hypothesis are biochemical studies (particularly on brain slices), which showed that kainate caused increase in intracellular contents of water and sodium [6], influx of $^{45}Ca^{2+}$ and $^{22}Na^{+}$ into the inulin-impermeant space [7], release of aspartate and glutamate [8–11] and increase of extracellular potassium [12]. On the other hand, results of Pinard and co-workers [13] provide evidence that hypoxia cannot be considered responsible for the damage produced by kainate. They suggested that the damage is caused by release of an endogenous toxic factor which is associated with an excessive rise in intracellular calcium concentrations.

In [11] we reported that addition of kainic acid to synaptosomes isolated from rat brain caused increased internal Ca^{2+}, depolarization of the plasma membrane and release of aspartate and glutamate but not of GABA. It was proposed that the toxicity of kainic acid is mediated by Ca^{2+}, the kainic acid acting on presynaptic receptors which cause opening of calcium channels and influx of Ca^{2+} into the presynaptic terminals. Here we report a test of this hypothesis by examining the effect of kainate on the very rapid influx of Ca^{2+} into synaptosomes which has been considered to be channel mediated. These data suggest that kainate causes opening of Ca^{2+} channels which have a half-time for closing of approx. 1 s; these channels have different properties from the voltage-dependent Ca^{2+} channels.

2. MATERIALS AND METHODS

Male Sprague-Dawley rats (200–250 g) were used

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throughout. Crude synaptosomal preparations (P_2 fraction) were prepared from cortex homogenates by centrifugation for 10 min at 20000 × *g* of the initial low-speed supernatant (3 min at 1000 × *g*). The P_2 fractions were suspended in modified Krebs-Henseleit saline (140 mM NaCl, 5 mM KCl (or 3 mM), 5 mM $NaHCO_3$, 1.3 mM $MgSO_4$, 1 mM Tris-phosphate and 10 mM Tris-Hepes, buffered to pH 7.4, and supplemented with 10 mM glucose and 0.1 mM $CaCl_2$ to make a final protein concentration of approx. 0.8–1 mg/ml.

2.1. *Measurement of calcium uptake*

For $^{45}Ca^{2+}$ uptake 0.4-ml portions of resuspended P_2 pellet were preincubated for 10 min at 30°C. Uptake of $^{45}Ca^{2+}$ was initiated by rapid addition of an equal volume of dilution medium. For nondepolarizing conditions the composition of the dilution medium was the same as the incubation medium except for the presence of $^{45}Ca^{2+}$ (1.25 mM final concentration, 1 μCi/ml) while for depolarizing conditions the NaCl was also replaced by KCl. In some experiments the dilution media also contained the following reagents at the indicated final concentrations: kainic acid (0.5–10 mM), cobalt chloride (3 mM), trifluoperazine (15 μM) or quinacrine (100 μM) (see table and figures for details).

At the appropriate times (1,2,5,10,20 or 40 s) uptake of $^{45}Ca^{2+}$ was stopped by the addition of 5 ml ice-cold 'stopping solution' (140 mM NaCl, 3 mM KCl, 1.3 mM $MgCl_2$, 5 mM $NaHCO_3$, 1 mM Tris-phosphate, 3 mM EGTA and 10 mM Tris-Hepes; pH 7.4). Each sample was immediately filtered through glass fiber filters in a Millipore vacuum filtration apparatus. The filters were rapidly washed twice with 5-ml aliquots of cold incubation medium and then dried in air. The radioactivity was measured in the Searle Delta 300 liquid scintillation counter using a solution for counting aqueous samples. Protein concentrations were determined by the method of Lowry et al. [14] with bovine serum albumin as the standard.

2.2. *Statistical analysis*

All values are presented as means ± SE. Comparison of means was performed using either the paired *t*-test or analysis of variance. When post hoc analysis was required, the Tukey's HSD test was employed.

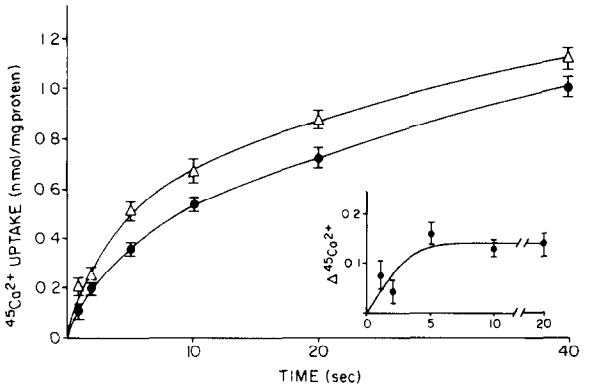

Fig.1. Effect of kainate on uptake of $^{45}Ca^{2+}$ into the synaptosomal fraction. Synaptosomes were preincubated for 10 min at 30°C in standard medium in the presence of 5 mM K^+ and 0.1 mM $CaCl_2$. At time zero, 400-μl aliquots were added to equal volumes of media containing $^{45}Ca^{2+}$ (final concentration 1.25 mM) and 0 (●) or 5 mM kainate (△). Uptake was terminated at the indicated times by injection of 5 ml ice-cold stopping solution and rapidly filtering. Each point represents a mean ± SE of 5 experiments.

3. RESULTS

3.1. *Effect of kainic acid on $^{45}Ca^{2+}$ uptake*

$^{45}Ca^{2+}$ uptake by crude synaptosomal preparation was studied in the presence or absence (control) of 5 mM kainic acid (fig.1). The initial rate of uptake of $^{45}Ca^{2+}$ in both cases was quite rapid and then declined to a minimum by about 20 s incubation. The presence of 5 mM kainic acid resulted in a statistically significant increase in the initial rapid rate of $^{45}Ca^{2+}$ uptake under nondepolarizing conditions. The same data are plotted in the inset of fig.1 as the difference between uptake of $^{45}Ca^{2+}$ in the presence and absence of kainic acid. The kainate-induced increase in Ca^{2+} uptake occurs during the first 3 s, with a half-time of approx. 1 s.

The effect of kainic acid on $^{45}Ca^{2+}$ uptake is dependent on the concentration of toxin (fig.2); addition of increasing concentrations (0.5, 1.0, 5.0 and 10 mM) caused progressive activation of $^{45}Ca^{2+}$ uptake (increases of 10, 17, 30 and 39%, respectively).

Statistically significant differences become apparent at 1 mM ($p < 0.05$). Moreover, addition of 5 mM kainic acid produced a significantly greater

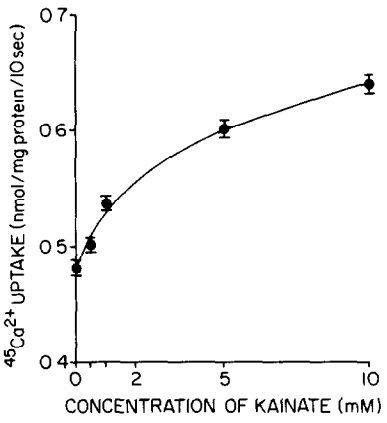

Fig.2. Rate of $^{45}Ca^{2+}$ uptake as a function of kainate concentration. $^{45}Ca^{2+}$ uptake into synaptosomal fraction was measured at 10 s in the presence of various concentrations of kainate (0–10 mM) in the standard medium as described in section 2. Values are means ± SE for 5 experiments.

($p<0.05$) uptake of $^{45}Ca^{2+}$ than did 1 mM but the effect plateaued thereafter.

3.2. *Effect of kainic acid on voltage-dependent calcium uptake*

It is well established that depolarization of the synaptosomal membranes with high external K^+ leads to an enhanced uptake of Ca^{2+} [15–18]. In our preparations of P_2 fraction, there was little change in Ca^{2+} uptake as the external K^+ concentration was raised from 3 to 5 mM but further increase in K^+ up to 70 mM caused large (up to 5-fold) increases in calcium uptake (fig.3). Quinacrine (100 μM), Co^{2+} (3 mM) and trifluoperazine (15 μM) inhibited the effect of 70 mM K^+ by 80, 85 and 50% respectively (not shown).

Addition of 5 mM kainic acid under nondepolarizing conditions caused increase in $^{45}Ca^{2+}$ uptake of 27 ± 4% compared to control and this change was statistically significant ($p<0.01$). The effects of kainate at K^+ concentrations greater than 12 mM were not statistically significant.

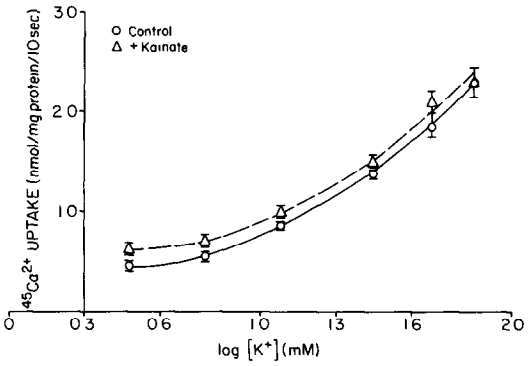

Fig.3. Effect of kainate on depolarization-induced Ca^{2+} uptake by synaptosomes. $^{45}Ca^{2+}$ uptake into crude synaptosomal fraction was measured at 10 s in the presence of difference K^+ concentrations (3–70 mM) and without (○) or with (△) 5 mM kainate. Values are means ± SE for 4 experiments.

3.3. *The effect of putative calcium channel blockers on $^{45}Ca^{2+}$ uptake in the presence of kainate*

The effect of kainic acid on the uptake of $^{45}Ca^{2+}$ was measured in the presence of different calcium channel blockers: trifluoperazine, quinacrine and Co^{2+}. The results are presented in table 1. It should be noted that addition of trifluoperazine (final concentration 15 μM) or quinacrine (final concentration 100 μM) either did not change or on-

Table 1

Effect of kainate on Ca^{2+} uptake by synaptosomes in the presence of putative calcium channel blockers

Additions	$^{45}Ca^{2+}$ uptake (nmol/mg protein per 10 s)		
	− kainate	+ kainate (5 mM)	Difference
None	0.45 ± 0.01	0.55 ± 0.01	0.10
Trifluoperazine (15 μM)	0.46 ± 0.01	0.55 ± 0.02	0.09
Quinacrine (100 μM)	0.38 ± 0.01	0.47 ± 0.02	0.09
$CoCl_2$ (3 mM)	0.27 ± 0.01	0.35 ± 0.01	0.08

The uptake of Ca^{2+} was initiated by diluting the synaptosomal fraction (P_2) with an equal volume of standard medium (3 mM K^+) containing $^{45}Ca^{2+}$ in the presence or absence of 5 mM kainate. The putative calcium channel blockers were present in the dilution medium to give the final concentrations indicated. $^{45}Ca^{2+}$ uptake was measured for the first 10 s after dilution (see section 2) and the results presented as means ± SE for 5 experiments

ly slightly changed uptake of $^{45}Ca^{2+}$ in nondepolarized synaptosomal preparations; 3 mM Co^{2+}, however, decreased this rate by about 40%. On the other hand, stimulation of Ca^{2+} uptake by kainate is not affected by quinacrine or trifluoperazine and only slightly, if at all, by cobalt.

4. DISCUSSION

The present results suggested a possible mechanism for toxicity of kainic acid. The toxic significantly increased calcium uptake into the synaptosomal fraction, apparently by modulating calcium channels. Uptake of calcium by voltage-dependent calcium channels has been reported to be biphasic with a fast phase occurring in the first 1–3 s and a slow phase which continues for 15–30 s [16,19,20]. The data presented in fig.1 show kainic acid-induced uptake was complete in 3–4 s. The approximately exponential decrease in the rate of calcium uptake with time is consistent with uptake occurring through a channel which closes with a half-time of approx. 1 s, too fast for more accurate determination. The dependence of the calcium uptake on kainate concentration suggests a saturable binding site (receptor) with a half-maximal effect occurring at 1–2 mM.

A major class of calcium channels in the synaptosomal membrane is the voltage-dependent channels (review [21,22]), and we therefore experimentally tested the possibility that the effect of kainate was on the voltage-dependent channels. Depolarization of the synaptosomal membrane by increasing the extrasynaptosomal $[K^+]$ caused a marked increase in rapid calcium influx (fig.3). The rate was 5-fold greater with 70 mM K^+ than with 5 mM K^+ (see also [17,19,20]). The increase in calcium uptake induced by high $[K^+]$ was through the voltage-dependent Ca^{2+} channels. This is consistent with the fact that it is inhibited by cobalt, trifluoperazine and quinacrine. Cobalt and many other polyvalent ions (La^{3+}, Ni^{2+}, Mo^{2+}, Mg^{2+}) have been reported to block calcium currents in many preparations [21,22]. Trifluoperazine has been reported to block calcium currents and to inhibit depolarization-dependent uptake into synaptosomes [23] at 15–20 μM. Quinacrine has been reported by Baba and co-workers [24] to block selectively voltage-dependent calcium channels although it is better known as a phospholipase A_2 inhibitor [25,26]. The voltage-dependent Ca^{2+} channels are not, however, responsible for the increased influx of Ca^{2+} by kainate. If the effect of kainate were on the voltage-dependent channels it should be inhibited by these agents and there is no detectable effect of trifluoperazine or quinacrine on the kainate-induced calcium uptake. A similar pattern was observed for cobalt which inhibited depolarization-dependent calcium uptake about 80% while having little or no effect on the kainate-induced calcium uptake.

Measurement of the depolarization-dependent calcium uptake in the presence and absence of kainate showed that the effect of kainate was statistically significant only at low external $[K^+]$. The overall pattern is, however, consistent with kainate-induced calcium uptake being additive to the depolarization-dependent uptake at all external K^+ values. It is clear that kainate has not simply shifted the calcium uptake to lower external $[K^+]$ by either partially depolarizing the synaptosomal membranes or by causing the channels to open at lower voltages. The same increase in calcium uptake is observed at 3 and 5 mM external K^+ and our previous results [11] had shown that only at high (5–10 mM) kainate could a small (5–7 mV) depolarization of the synaptosomal membranes be observed.

The above date suggest that kainate binds to a receptor which activates a class of calcium channels which is different from the voltage-dependent channels. Channels are probably involved because the increase in calcium uptake is turned on and off very rapidly. Maximal stimulation is observed in measurements taken 1 s after the synaptosomes are diluted into a medium containing kainate, indicating that transport across the membrane is not required, i.e. the receptor is on the external surface of the plasma membrane. The kainate-induced increase in calcium uptake 'turns off' on a time scale (about 1 s) similar to that for the voltage-dependent channels. The toxicity of kainate may be due in part to the metabolic disturbances resulting from increased calcium in neurons having this kainate receptor.

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