Journal of Neurochemistry, 2004, **89**, 654–659 doi:10.1111/j.1471-4159.2004.02382.x

4-Aminopyridine-induced epileptogenesis depends on activation of mitogen-activated protein kinase ERK

Daniela Merlo,*,† Pierangelo Cifelli,*,§ Simona Cicconi,‡ Virginia Tancredi* and Massimo Avoli§,¶

*Dipartimento di Neuroscienze, Università degli Studi di Roma 'Tor Vergata', Roma, Italy
†IRCCS Fondazione Santa Lucia, Roma, Italy
‡Istituto di Neurobiologia e Medicina Molecolare, CNR, Roma, Italy
§Montreal Neurological Institute and Departments of Neurology and Neurosurgery, McGill University, Montréal, Québec, Canada
¶IRCCS Neuromed, Pozzilli (Isernia), Italy

Abstract

Extracellular signal-regulated kinases such as ERK1 [p44 mitogen-activated protein kinase (MAPK)] and ERK2 (p42 MAPK) are activated in the CNS under physiological and pathological conditions such as ischemia and epilepsy. Here, we studied the activation state of ERK1/2 in rat hippocampal slices during application of the K^+ channel blocker 4-aminopyridine (4AP, 50 μM), a procedure that enhances synaptic transmission and leads to the appearance of epileptiform activity. Hippocampal slices superfused with 4AP-containing medium exhibited a marked activation of ERK1/2 phosphorylation that peaked within about 20 min. These effects were not accompanied by changes in the activation state of c-Jun N-terminal kinase (JNK), another member of the MAP kinase superfamily. 4AP-induced ERK1/2 activation was inhibited by the voltage-gated Na^+ channel blocker tetrodotoxin (1 μM). We also found that application of the ERK pathway inhibitors U0126 (50 μM) or PD98059 (100 μM) markedly reduced 4AP-induced epileptiform synchronization, thus abolishing ictal discharges in the CA3 area. The effects induced by U0126 or PD98059 were not associated with changes in the amplitude and latency of the field potentials recorded in the CA3 area following electrical stimuli delivered in the dentate hylus. These data demonstrate that activation of ERK1/2 accompanies the appearance of epileptiform activity induced by 4AP and suggest a cause-effect relationship between the ERK pathway and epileptiform synchronization.

Keywords: 4-aminopyridine, epileptogenesis, extracellular regulated kinase, hippocampal slices, mitogen-activated protein kinase, U0126.

J. Neurochem. (2004) **89,** 654–659.

Extracellular stimuli such as neurotransmitters, neurotrophins, and growth factors regulate neuronal processes including synaptic transmission, neuronal plasticity, morphological differentiation and survival. The extracellular signal-regulated kinases ERK1/2, which are members of the mitogen-activated protein kinase (MAPK) family, are abundantly expressed in mature CNS neurons (Thomas and Hunt 1993; Ortiz *et al*. 1995) and are attractive candidates for coordinating neuronal responses to extracellular signals. Accordingly, the MAPK pathway regulates synaptic transmission through long-term changes in protein synthesis and gene expression under physiological and pathological conditions (Fukunaga and Miyamoto 1998; Sweatt 2001). For instance, MAPK are activated in the rat brain by electroconvulsive shock (Baraban *et al*. 1993) or transient ischemia (Campos-Gonzàles and Kindy 1992). In addition, seizures induced *in vivo* by injection of bicuculline stimulate MAPK tyrosine phosphorylation in hippocampus and somatosensory cortex (Gass *et al*. 1993).

Received December 2, 2003; revised manuscript received January 8, 2004; accepted January 9, 2004.

Address correspondence and reprint requests to Massimo Avoli, MD, PhD, Montreal Neurological Institute, 3801 University Street, Montreal, Quebec H3A 2B4, Canada. E-mail: massimo.avoli@mcgill.ca

Abbreviations used: aCSF, cerebrospinal fluid; 4AP, 4-aminopyridine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ERK, extracellular regulated kinases; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; SAPK, stress-activated protein kinase; TTX, tetrodotoxin.

These observations suggest that stimulation of glutamate receptors and/or neuronal depolarization do activate MAPK during pathological events. In line with this view, ionotropic and metabotropic glutamate receptor agonists can induce MAPK in cultured cortical neurons (Bading and Greenberg 1991; Fiore *et al*. 1993; Kurino *et al*. 1995). Since ERKs can modulate synaptic plasticity and neuronal excitability we have analyzed whether the epileptogenic effects induced by the K^+ channel blocker 4-aminopyridine (4AP) in rat hippocampal slices is associated with MAPK/ERK activation. Previous studies have shown that 4AP causes the appearance of epileptiform activity by enhancing neurotransmitter release at both inhibitory and excitatory synapses by increasing Ca^{2+} entering nerve terminals (Buckle and Haas 1982; Rutecki *et al*. 1987). Moreover, in the hippocampus these epileptiform discharges depend on conductances that are mediated through the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subtype (Perreault and Avoli 1991; Avoli *et al*. 1993). Here, we report that hippocampal slices superfused with 4AP exhibit a specific activation of ERKs that is abolished by the voltage-gated, Na^+ channel blocker tetrodotoxin (TTX). Moreover, we found that application of U0126 or PD98059, which inhibit MEK (the kinase directly upstream of ERK), markedly reduces the epileptiform activity induced by 4AP.

Materials and methods

Hippocampal slice preparation and electrophysiological recordings

Wistar rats (10–24 day old) were decapitated under halothane anesthesia according to the procedures established by the European Union Council of Animal Care. Brains were quickly removed from the skull and placed in cold artificial cerebrospinal fluid (aCSF). Hippocampi were dissected and transverse slices (450 μm) were cut by using a McIllwain tissue chopper (Mickle Laboratory Engineering Co., Gomshall, UK). Slices were then transferred to an interface tissue chamber and maintained between oxygenated aCSF and humidified atmosphere gassed with 95% O_2/5% CO_2 at 33 ± 1°C. The aCSF composition (in mM) was: NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 1.8, $CaCl_2$ 2, $NaHCO_3$ 26 and glucose 10 (pH 7.4).

Field potential recordings were made in the CA3 subfield by using 2 M NaCl filled microelectrodes (resistance = 4–8 MΩ). Signals were fed to a high impedance DC amplifier and displayed on a pen chart recorder. The following drugs were bath applied: 4AP (50 μM; Merck, Darmstadt, Germany), TTX (1μM; Sigma, Frechen, Germany), UO126 (50 μM; Promega, Madison, WI, USA) and PD98059 (50–100 μM; Promega, Milano, Italy). The rate of occurrence and the duration of the 4AP-induced epileptiform activity were measured under control conditions and during drug treatment for periods lasting > 10 min.

Immunoblotting assays

Hippocampal slices used for the electrophysiological experiments were processed for sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblot assays. After treatment, slices were immediately frozen in liquid nitrogen and subsequently washed with ice-cold phosphate-buffered saline and homogenized by sonication in lysis buffer (Tris-acetate 20 mM pH 7.5, NaCl 150 mM, EDTA 1 mM, EGTA 1 mM, Na-pyrophosphate 2.5 mM, Ortovanadate 1 mM, β-glycerolphosphate 1 mM, NaF 100 mM, phenylmethylsulfonyl fluoride 1 mM, leupeptin 1 μg/mL). After sonication, cellular extracts were centrifuged at 4°C for 10 min at 13 000 × *g* and supernatants were recovered. Protein concentration was determined using Bradford reagent (Sigma) and bovine serum albumin as standard. Proteins (10 or 25 μg) were diluted 1 : 1 in 'sample buffer' (Tris-HCl 62.5 mM pH 6.8, sodium dodecyl sulfate 2%, glycerol 10%, dithiothreitol 50 mM, 0.1% bromophenol blue), subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on 10% polyacrylamide gels (Laemmli U.K. 1970) and electrophoretically transferred to nitrocellulose membranes (assayed by staining filters with 'Ponceau'). Membranes were blocked in Tris-buffered saline–Tween-20 (0.1%) with 5% non-fat dry milk for 2 h at room temperature and incubated overnight at 4°C with the primary antibodies at the dilution 1 : 1000 in Tris-buffered saline 0.1% Tween-20 with 5% bovine serum albumin. After wash and incubation for 1 h with the secondary antibodies, immunoreactivity was detected using the chemiluminescence system (ECL, Amersham Biotech, Milano, Italy) and autoradiography. Quantitation was carried out by densitometric film analysis (Fluor-S, Bio-Rad, Milano, Italy). Membranes were first processed to visualize the phosphorylated forms of proteins, dehybridized (Restore Western blot stripping buffer, Pierce, Rockford, IL, USA) and then reprobed with antibodies directed against total proteins for normalization. Protein loading was monitored by reprobing membranes with anti-tubulin antibody (Sigma). Anti-ERK (p42 and p44), anti-JNK (c-Jun N-terminal kinase), phospho-specific anti-ERK and phospho-specific anti-JNK antibodies were purchased from New England Biolabs (NEB, Beverly, MA, USA). Secondary antibodies were acquired from Amersham (Amersham Pharmacia Biotech, Milano, Italy).

Statistical analysis

Throughout the text measurements are expressed as mean ± SEM and *n* indicates the number of slices used for any given experimental protocol. The effects of the treatments are expressed as percentage changes with respect to control conditions following the formula $[(y - y_{control})/y_{control} \times 100]$. Each experiment was repeated at least five times using different batches of proteins. Statistical analysis was carried out by one-way ANOVA and the results were considered statistically significant when p was < 0.05.

Results

As reported in previous studies from our laboratories (Avoli *et al*. 1993; Tancredi *et al*. 1998), field potential recordings obtained in the CA3 subfield of rat hippocampal slices during 4AP application demonstrated the occurrence of interictal (duration = 0.25–0.38 s; interval of occurrence = 1.7–5.5 s) and/or ictal (duration = 3–15 s; interval of occurrence = 80–400 s) discharges (arrows and continuous lines in Fig. 1a, respectively). These epileptiform events appeared within 10–15 min after starting the application of

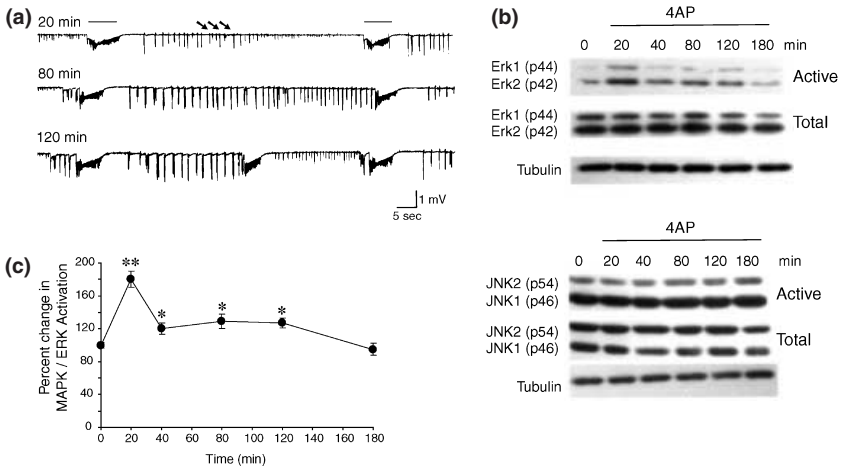

Fig. 1 Effects induced by 4AP on network excitability and endogenous activation of MAPK/ERK1/2 and of SAPK/JNK1/2 in rat hippocampal slices. (a) Interictal (arrows) and ictal (continuous lines) discharges occur in CA3 during 4AP application; numbers on the left of each trace indicate the amount of time of 4AP application. (b) Immunoreactive levels of activated and total forms of ERK1/2 (upper panel) and of JNK1/2 (lower panel) at different time of 4AP application in a slice. (c) Time course of the effects induced by 4AP on ERK1/2 activation in different experiments. Values represent the percentage changes (means ± SEM) in ERK1/2 phosphorylation with respect to control in 11 slices for each time sample; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control values.

4AP-containing medium and remained constant throughout the experiment (up to 3 h) (Fig. 1a).

Hippocampal slices used for electrophysiology ($n = 11$) were then analyzed for the activation of the parallel cascades leading to activation of MAPK/ERK and stress-activated protein kinase (SAPK)/JNK pathways. The band expression of p44 (Erk1) and p42 (Erk2) over a given time period was equivalent in all experiments. Quantification of the immunoreactive levels of the activated kinases normalized by the total amount of the respective kinase for each slice revealed that 4AP treatment increased ERK1/2 phosphorylation by approximately 80% (Figs 1b, upper panels and c). The activation of ERKs by 4AP peaked about 20 min after treatment and remained about 30% above the basal level for at least 2 h. The levels of total ERKs remained constant during 4AP treatment, suggesting that there was no effect on ERK protein expression. Moreover, there was no detectable change in the activation state of JNK1/2 (Fig. 1b, lower panels).

Next, we studied the effects induced by the voltage-gated Na^+ channel blocker TTX (1 μM) on ERK1/2 activation under control conditions and in the presence of 4AP. A near 50% inhibition of ERK phosphorylation was seen in both cases after 40 min of treatment with either TTX ($n = 6$) or TTX + 4AP ($n = 6$) (Figs 2a and b, respectively). However, TTX never abolished ERK phosphorylation completely. As expected, TTX application readily blocked the stimulus-induced population responses seen with field potential recordings in control medium as well as the appearance of epileptiform discharges during 4AP application (data not shown).

Finally, we established the role played by ERK activation in 4AP-induced epileptiform discharges by using the specific MEK inhibitor U0126 (DeSilva *et al.* 1998; Favatta *et al.* 1998; Yuan *et al.* 2002). MEK is the kinase directly upstream of ERK and it is therefore responsible for its activation. As illustrated in Fig. 3(a), application of medium containing 50 μM of U0126 inhibited the baseline phosphorylation of both ERK1 and ERK2 by approx. 50% within 20 min ($n = 8$ slices), while 80% inhibition was seen after 40 min. As illustrated in Fig. 3(b), administration of U0126 together with 4AP abolished the increases in ERK1/2 phosphorylation seen when 4AP only was added to the medium ($n = 8$; cf. Figs 1b, upper panel and c). In addition, ERK1/2 phosphorylation decreased by approximately 40%, and it was almost undetectable after 40 and 120 min of treatment with U0126 + 4AP, respectively (Fig. 3b).

Field potential recordings obtained during these experiments revealed a marked reduction of the interictal discharges (not illustrated) and a complete abolition of the ictal-like activity in all slices treated with U0126 + 4AP ($n = 12$) (Fig. 3c). As illustrated in Fig. 3(c) (inserts), the disappearance of epileptiform discharges was not accompanied by any significant change in the amplitude and latency of the field responses generated by CA3 pyramidal cells following electrical stimuli delivered in the dentate hylus.

We also analyzed the effects induced by PD98059, another MEK inhibitor (Endo and Launey 2003; Karasewski and Ferreira 2003; Salvarezza *et al.* 2003), on the spontaneous epileptiform discharges and on the stimulus-induced field responses recorded in CA3 during 4AP application. Concentrations of PD98059 ranging between 50 and 80 μM

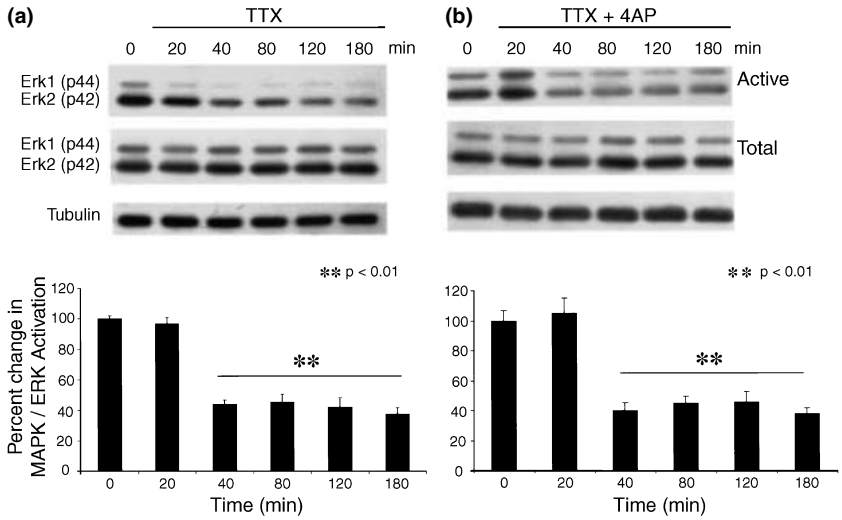

Fig. 2 Time dependence of the effect of TTX on the endogenous activation of MAPK/ERK1/2. Slices were treated with medium containing TTX only (a) or TTX + 4AP (b). The immunoreactive levels of activated and total kinases at the indicate times are shown for two representative experiments in the upper panels; normalized values (means ± SEM; $n = 6$ at each time) of activated kinases are illustrated in the lower panels. $^{**}p < 0.01$ vs. control values.

failed in consistently reducing the development of 4AP-induced epileptiform activity ($n = 3$ slices; not illustrated). In contrast, epileptiform synchronization along with ictal discharges were effectively abolished by 100 μM PD98059 ($n = 3$, Fig. 3d). These effects which were accompanied by a decrease in ERK1/2 phosphorylation, were not associated with any significant change in the field potential responses induced by dentate hylus stimulation (inserts in Fig. 3d).

Discussion

The main findings obtained from this study can be summarized as follows: (i) hippocampal slices respond to 4AP application by generating epileptiform discharges and by displaying a marked activation of ERK1/2, but not of JNK1/2; (ii) TTX application abolishes the epileptiform activity and prevents 4AP-induced ERK activation; (iii) application of the ERK pathway inhibitors U0126 or PD98059 in the presence of 4AP reduces and eventually abolishes epileptiform synchronization without affecting stimulus-induced synaptic responses.

The K^+ channel blocker 4AP induces epileptiform synchronization in hippocampal networks by enhancing neurotransmitter release at both inhibitory and excitatory synapses, presumably by increasing Ca^{2+} entering at nerve terminals (Buckle and Haas 1982; Rutecki *et al.* 1987; Perreault and Avoli 1991; Avoli *et al.* 1993). An involvement of the MAP kinase cascade following stimulation of glutamate receptors and/or depolarization has been previously reported under physiological and pathological conditions (Baraban *et al.* 1993; Bading and Greenberg 1991; Fiore *et al.* 1993; Gass *et al.* 1993; Kurino *et al.* 1995; Perkinton *et al.* 1999). Since the MAPK pathway can modulate synaptic plasticity and neuronal excitability (Sweatt 2001), we hypothesized that ERK activation plays a role in the enhancement of synaptic transmission induced by 4AP and thus in the implementation of epileptiform synchronization. Indeed, our findings support a role for ERK1/2 activation in 4AP-induced epileptiform activity.

First, we have observed that ERK activation is associated with the appearance of epileptiform activity; such a finding is consistent with the mechanism of 4AP action since ERK activity is regulated by neurotransmitters as well as by intracellular Ca^{2+} elevations. Second, ERK activation remains approximately 30% above the basal level for at least 2 h, indicating that continuous neurotransmitter release occurring during 4AP treatment maintains ERK phosphorylation elevated, which, in turn, may play a role in the enhancement of synaptic transmission. This view is supported by the findings obtained by applying the ERK pathway inhibitors U0126 or PD98059. We have found that U0126 markedly reduced ERK phosphorylation after approximately 20 min of treatment in control medium, while in the presence of 4AP this effect was delayed. Moreover, both U0126 and PD98059 reduced interictal discharges and completely abolished ictal activity induced by 4AP, indicating a cause–effect relationship between ERK pathway activation and epileptiform synchronization. Interestingly, the blockade of 4AP-induced ictal events by U0126 or PD98059 was not associated with any change in the in the amplitude and latency of the field potential responses to electrical stimuli delivered in the dentate hylus. Hence, the blockade of 4AP-induced epileptiform by both MEK inhibitors was presumably not caused by a non-specific depressant effect basal neuronal excitability. Finally, we have found that application of the voltage-dependent Na^+ channel blocker TTX, although unable to completely inhibit basal level of ERK1/2 phosphorylation, prevents 4AP-induced ERK activation. This finding indicates that ERK activation occurs through an action potential-dependent mechanism.

We have also found that 4AP treatment leading to the appearance of epileptiform activity was not accompanied by

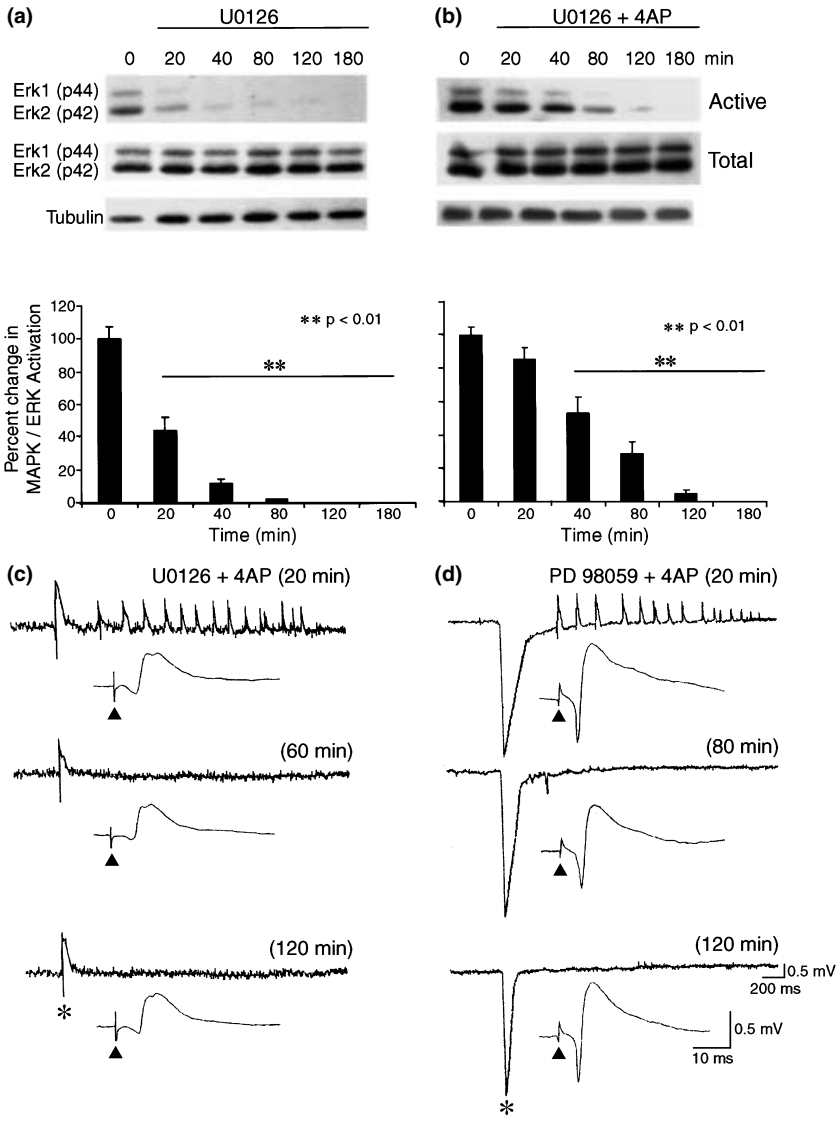

Fig. 3 Time dependence of the effect of U0126 on the endogenous activation of MAPK/ERK1/2 in hippocampal slices that were treated for different times with U0126 (a) or U0126 + 4AP (b). Immunoreactive levels of activated and total kinases at the indicate times in two representative experiments are shown in the upper panels. The normalized amounts of activated kinases obtained from eight slices for each time frame are shown in the lower panels. Bars in the plot represent means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control values. (c and d) Spontaneous, field potential activity recorded from the CA3 area at different times during application of medium containing U0126 + 4AP (c) or PD98059 + 4AP (d); traces shown in the inserts illustrate at faster time base, the field responses to electrical stimuli delivered in the hylus. Note in both experiments the decrease and/or disappearance of the repetitive discharge associated with the ictal-like event that follows the initial negative-going field potential (asterisks); note also that in both cases the disappearance of ictal activity is associated with no change in the amplitude and latency of the stimulus-induced field response.

any detectable change in the activation state of SAPK/JNK1/2. This pathway has been shown to be activated during seizures, though more transiently than ERK (Mielke *et al*. 1999; Jeon *et al*. 2000). These previous findings are not in contrast with our results since JNK activation was observed in kainic acid model of epilepsy which may involve different intracellular molecular mechanism when compared with the 4AP model. MAP-kinase families have, in fact, distinct upstream activators and different substrate specificities for transcription factors. Furthermore, opposite effects of ERK and JNK/p38 MAP-kinase signaling have been observed in cultured fetal neurons (Heidenreich and Kummer 1996).

Clearly, ERK activation leads to phosphorylation of several target proteins having multiple functions on neural activity. Thus, a key question is: what are the downstream effectors that contribute to the induction and/or maintenance of enhanced synaptic transmission during 4AP treatment. A possibility is that ERK regulates the shal-type K^+ channel Kv4.2 that has been shown to be a substrate for MAPK (Adams *et al*. 2000). Yuan *et al*. (2002) have indeed reported that Kv4.2 is the target of MAPK phosphorylation in the dendrites of CA1 pyramidal neurons as well as that its phosphorylation leads to an increase in action potential amplitude and in dendritic excitability.

Early studies of ERK activation in the hippocampus have focused on NMDA receptor coupling to ERK. However, more recently, hippocampal ERK activation has been identified during activation of metabotropic glutamate, muscarinic and dopamine receptors (Roberson *et al*. 1999; Berkeley *et al*. 2001, 2002). In addition, Perkinton *et al*. (1999) have reported that in striatal neurons AMPA receptor leads to Ca^{2+}-dependent activation of ERKs. A similar situation may occur in our experiments in which 4AP-induced epileptiform discharges in the CA3 subfield are

caused by a glutamatergic mechanism that depends on the activation of AMPA receptors (Perreault and Avoli 1991; Avoli *et al*. 1993).

Acknowledgements

This study was supported by the Italian Ministry of University and Research (MIUR), the Canadian Institutes of Health Research (Grant MT-8109) and the Savoy Foundation. We thank Dr P. McPherson for constructive criticism on an early draft of this report.

References

Adams J. P., Anderson A. E., Varga A. W., Dineley K. T., Cook R. G., Pfaffinger P. J. and Sweatt J. D. (2000) The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. *J. Neurochem.* **75**, 2277–2287.

Avoli M., Psarropoulou C., Tancredi V. and Fueta Y. (1993) On the synchronous activity induced by 4-aminopyridine in the CA3 subfield of juvenile rat hippocampus. *J. Neurophysiol.* **70**, 1018–1029,.

Bading H. and Greenberg M. E. (1991) Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. *Science* **260**, 912–914.

Baraban J. M., Fiore R. S., Sanghera J. S., Paddon H. B. and Pelech S. L. (1993) Identification of p42 mitogen-activated protein kinase as a tyrosine kinase substrate activated by maximal electroconvulsive shock in hippocampus. *J. Neurochem.* **60**, 330–336.

Berkeley J. L., Gomeza J., Wess J., Hamilton S. E., Nathanson N. M. and Levey A. I. (2001) M1 muscarinic acetylcholine receptors activate extracellular signal-regulated kinase in CA1 pyramidal neurons in mouse hippocampal slices. *Mol. Cell. Neurosci.* **18**, 512–524.

Berkeley J. L., Decker M. J. and Levey A. I. (2002) The role of muscarinic acetylcholine receptor-mediated activation of Extracellular signal-regulated kinase 1\2 in pilocarpine-induced seizures. *J. Neurochem.* **82**, 192–201.

Buckle P. J. and Haas H. L. (1982) Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. *J. Physiol.* **326**, 109–122.

Campos-Gonzàles R. and Kindy M. S. (1992) Tyrosine phosphorylation of microtubule-associated protein kinase after transient ischemia in the gerbil brain. *J. Neurochem.* **59**, 1955–1958.

DeSilva D. R., Jones E. A., Favatta M. F., Jaffe B. D., Magolda R. L., Trzaskos J. M. and Scherle P. A. (1998) Inhibition of mitogen-activated protein kinase kinase blocks T cell proliferation but does not induce or prevent anergy. *J. Immunol.* **160**, 4175–4181.

Endo S. and. Launey T. (2003) Nitric oxide activates extracellular signal-regulated kinase 1/2 and enhances declustering of ionotropic glutamate receptor subunit 2/3 in rat cerebellar Purkinje cells. *Neurosci. Lett.* **350**, 122–126.

Favatta M., Horiuchi K. Y., Manos E. J. *et al.* (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J. Biol. Chem.* **273**, 18623–18632.

Fiore R. S., Murphy T. H., Sanghera J. S., Pelech S. L. and Baraban J. M. (1993) Activation of p42 mitogen-activated protein kinase by glutamate receptor stimulation in rat primary cortical cultures. *J. Neurochem.* **61**, 1626–1633.

Fukunaga K. and Miyamoto E. (1998) Role of MAP kinase in neurons. *Mol. Neurobiol.* **16**, 79–95.

Gass P., Kiessling M. and Bading H. (1993) Regionally selective stimulation of mitogen activated protein (MAP) kinase tyrosine phosphorilation after generalized seizures in the rat brain. *Neurosci. Lett.* **162**, 39–42.

Heidenreich K. A. and Kummer J. L. (1996) Inhibition of p38 mitogen-activated protein kinase by insulin in cultured fetal neurons. *J. Biol. Chem.* **271**, 9891–9894.

Jeon S. H. and Bae C. D. and. Park J. B. (2000) Activation of JNK and p38 in rat hippocampus after kainic acid induced seizure. *Exp. Mol. Med.* **32**, 227–230.

Karasewski L. and Ferreira A. (2003) MAPK signal transduction pathway mediates agrin effects on neurite elongation in cultured hippocampal neurons. *J. Neurobiol.* **55**, 14–24.

Kurino M., Fukunaga K., Ushio Y. and Miyamoto E. (1995) Activation of mitogen-activated protein kinase in cultured rat hippocampal neurons by stimulation of glutamate receptors. *J. Neurochem.* **65**, 1282–1289.

Laemmli U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Mielke K., Brecht S., Dorst A. and Herdegen T. (1999) Activity and expression of JNK1, p38 and ERK kinases, c-Jun N-terminal phosphorylation, and c-jun promoter binding in the adult rat brain following kainate-induced seizures. *Neuroscience* **91**, 471–483.

Ortiz J., Harris H. W., Guitart X., Terwillinger R. Z., Haycock J. W. and Nestler E. J. (1995) Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. *J. Neurosci.* **15**, 1285–1297.

Perkinton M. S., Sihra T. S. and Williams R. J. (1999) Ca (2+)-permeable AMPA receptors induce phosphorylation of cAMP response element-binding protein through a phosphatidylinositol 3-kinase-dependent stimulation of the mitogen-activated protein kinase signalling cascade in neurons. *J. Neurosci.* **19**, 5861–5874.

Perreault P. and Avoli M. (1991) Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J. Neurophysiol.* **65**, 771–785.

Roberson E. D., English J. D., Adams J. P., Selcher J. C., Kondratick C. and Sweatt. J. D. (1999) The mitogen-activated protein kinase cascade couples PKA, PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. *J. Neurosci.* **19**, 4337–4348.

Rutecki P. A., Lebeda F. J. and Johnston D. (1987) 4-Aminopyridine produces epileptiform activity in hippocampus and enhances synaptic excitation and inhibition. *J. Neurophysiol.* **57**, 1911–1924.

Salvarezza S. B., Lopez H. S. and Masco D. H. (2003) The same cellular signaling pathways mediate survival in sensory neurons that switch their trophic requirements during development. *J. Neurochem.* **85**, 1347–1358.

Sweatt J. D. (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* **76**, 1–10.

Tancredi V., D'Antuono M., Nehlig A. and Avoli M. (1998) Modulation of epileptiform activity by Adenosine A1 receptor-mediated mechanisms in the juvenile rat hippocampus. *J. Pharmacol. Exp. Ther.* **286**, 1412–1419.

Thomas K. L. and Hunt S. P. (1993) The regional distribution of extracellularly regulated kinase-1 and 2 messenger RNA in the adult rat central nervous system. *Neuroscience* **56**, 741–757.

Yuan L. L., Adams J. P., Swank M., Sweatt J. D. and Johnston D. (2002) Protein kinase modulation of dendritic K+ channels in hippocampus involves a mitogen-activated protein kinase pathway. *J. Neurosci.* **22**, 4860–4868.