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Minireview

Kainate-evoked modulation of gene expression in rat brain*

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Kainate is a glutamate analog that produces neuronal excitation resulting in seizures within hours following its intraperitoneal injection into adult rats. Then, at 2–3 days after the treatment, neurodegeneration of apoptotic character can be observed in limbic system. As a consequence, plastic reorganization and glial reactivation phenomena occur. These physiological and pathological responses are reflected by specific changes in gene expression, that can be dissected according to their spatio-temporal patterns. The early phase of gene expression observed in all hippocampal subfields appears to reflect a sudden burst of spiking activity. Changes in mRNA levels restricted to dentate gyrus are suggestive of a link to neuronal plasticity. The late gene expression response implies its correlation either to neuronal cell death or glial reactivation, depending on cellular localization of gene products. Thus analysis of the temporal and spatial gene expression pattern in the hippocampus after kainate treatment may provide clues revealing specific phenomena to which gene expression could be attributed.

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CA, Cornu Ammonis (hippocampal subfields); EMSA, electrophoretic mobility shift assay; GFAP, glial fibrillary acidic protein; KA, kainate [2-carboxy-4(1-methylethenyl)-3-pirrolidinacetate]; NMDA, *N*-methyl-D-aspartate; PTZ, pentylenetetrazole.

KAINATE TREATMENT AS A MODEL SYSTEM

Understanding of the mechanisms of long term modulations of brain functions is one of major goals of molecular neurobiology. Recently, it has been repeatedly stressed that genomic responses play a significant role in these phenomena. Many studies have established that excitation of neurons results in activation of a number of genes. Thus model systems employing compounds that depolarize nerve cells, such as the natural major excitatory neurotransmitter, L-glutamate and its analogs (excitatory amino acids) are becoming valuable experimental tools. One of the most widely studied excitatory amino acids is kainic acid [2-carboxy-4(1-methyl-ethenyl)-3-pirrolidinacetic acid], or to be more precise kainate (KA) (for review see: Sperk, 1994). This compound shares structural similarities with L-glutamate and can activate ionotropic non-NMDA (*N*-methyl-D-aspartate) glutamate receptors in the brain. In fact there is a class of these receptors that has been named "kainate receptors". However, KA may act on AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors as well (see: Kaczmarek *et al.*, 1997). Thus, two main subtypes of non-NMDA ionotropic receptors for excitatory amino acids in the brain can be stimulated upon kainate binding.

Given either systematically or in the form of intracerebral injections in adult rats, KA provides a useful model system to study brain function in a complex physio-pathological situation (Schwob *et al.*, 1980; Ben-Ari *et al.*, 1981; Ben-Ari, 1985; Sperk, 1994). Initially, within a few dozens of minutes following intraperitoneal injection (i.p.), kainate evokes seizures that may last up to several hours. The seizures are dependent on activation of glutamate receptors. Within a few days — sometimes as early as one day after the injection — a massive neurodegeneration occurs in specific brain areas. The most strongly affected are pyramidal neurons of CA3 and CA1 (Cornu Ammonis) subfields of the hippocampus as well as entorhinal cortex. Other brain regions, e.g., neocortex are affected by neuronal cell loss to a variable frequency and degree. Notably, dentate gyrus of the hippocampus is consistently spared from the neurodegeneration (Sperk, 1994). On the contrary, the granule neurons of the region are involved in plastic changes including axonal sprouting, believed to be a form of functional brain recovery from the severe damage evoked by kainate in the neighboring areas (Sperk, 1994). At least four intermingled processes could be distinguished as a consequence of kainate administration: i. excessive neuronal firing resulting in seizures; ii. neuronal cell loss; iii. neuronal plasticity; and iv. glial reactivation. Despite this apparent complexity of the model, analysis of the temporal and spatial gene expression pattern in the hippocampus after kainate treatment may provide clues revealing specific phenomena to which gene expression could be attributed.

KAINATE AND TRANSCRIPTION FACTORS

Our interest in kainate has been motivated by a search for model systems that involve a massive neuronal activation dependent on glutamate receptors. In our first experiments, we investigated whether such a situation may involve elevation of DNA binding of AP-1 transcription factor, as we have shown for *in vitro* cultured neurons and glia (Condorelli *et al.*, 1993, 1994). With the aid of electrophoretic mobility shift assay (EMSA) we have found that, indeed, treating the rats with 10 mg/kg i.p. of sodium kainate results in robust accumulation of AP-1 DNA binding activity in hippocampus and entorhinal cortex at 2–6 h after the KA administration (Kaminska *et al.*, 1994a; Przewlocki *et al.*, 1995; Kaminska *et al.*, 1994b). These structures are known to be especially prone to kainate-evoked effects, as indicated by electrophysiological and histological approaches (Sperk, 1994).

At 24 h after the KA administration AP-1 content was decreased, similarly as it was shown for AP-1 activation produced by another proconvulsive agent — pentylenetrazole (PTZ) (Kaminska & Kaczmarek, 1993; Lukasiuk & Kaczmarek, 1994; Przewlocki *et*

al., 1995). However, contrary to PTZ effects, KA administration provoked a second wave of AP-1 DNA binding activity, that was observed 48–72 h following the insult (Kaminska *et al.*, 1994a). This second AP-1 peak corresponded well in time with neurodegeneration. This temporal coincidence led us to ask whether kainate may produce a programmed cell death process, or apoptosis. Indeed, we demonstrated apoptotic features of KA-evoked cell loss, such as intranucleosomal DNA fragmentation (Filipkowski *et al.*, 1994; Kaminska *et al.*, 1994a). These observations were further strengthened by the results reported by Ben-Ari and his colleagues, who documented both a necrotic and an apoptotic character of KA-evoked neuronal death (Pollard *et al.*, 1994a, b).

To investigate the composition of AP-1, that can be formed by various dimeric combinations of Fos and Jun proteins (Morgan & Curran, 1991) we initially employed an EMSA-superhift procedure. In the untreated animals, AP-1 appears to be composed predominantly of FosB and JunD. Two–six hours following the KA treatment c-Fos and JunB are becoming major DNA binding AP-1 components. At three days after the KA administration, again FosB and JunD comprise majority of AP-1 (Kaminska *et al.*, 1994a, see also Kaminska *et al.*, 1996). These observations were supported by northern blot hybridization to measure mRNA levels and immunocytochemical technique to visualize the proteins belonging to the Fos and Jun families (Kaminska *et al.*, 1994a) (Fig. 1).

DNA binding activities of two other transcription factors were also investigated. Neither CREB nor Octamer DNA binding activities were found to be modified by kainate administration (Kaminska *et al.*, 1994a). However, AP-1 is clearly not the only transcription factor whose expression is modulated in response to KA treatment. We have observed an increase in *zif268* mRNA at 2–6 h following KA injection, as well as CREM/ICER mRNA accumulation at 6–24 h thereafter (Konopka *et al.*, in press).

NEURONAL ACTIVATION-LINKED GENE EXPRESSION

The fact that KA administration results in an elevation of transcription factors, and that there are at least two phases of the process,

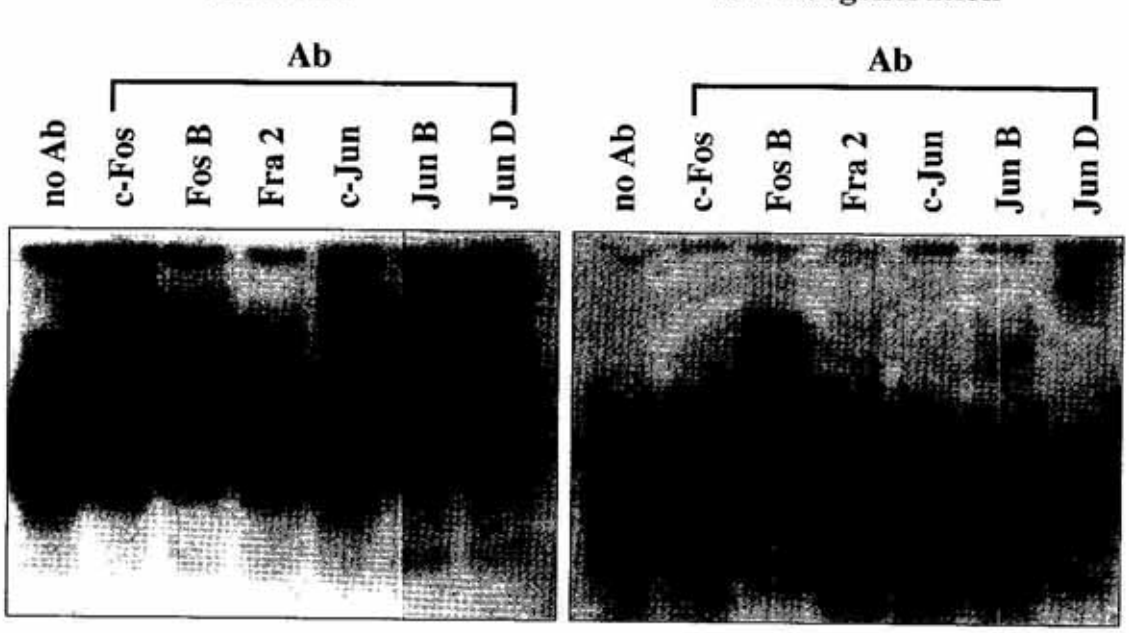

Figure 1. Different protein composition of AP-1 transcription factor at various phases following intraperitoneal kainate administration to rats.

Nuclear protein extracts were isolated from hippocampi at either 2 h (activation) or 72 h following injection of 10 mg/kg of KA, and then subjected to EMSA supershift procedure with antibodies specific to various AP-1 components (Ab), as indicated at the top.

raises the question of possible targets for these regulators of gene expression. Studying the levels of mRNA for a number of genes, it has been found that the gene expression response can be spatio-temporally divided to reflect various neuronal and glial responses to KA.

The first group of possible targets for the initial phase of the KA-evoked activation of AP-1 and/or its cognates includes three genes coding for proteins related to synaptic release, namely, secretogranin II (sg II), clathrin heavy chain (chc) and heat shock protein 70 cognate protein (hsc 70) (Nedivi *et al.*, 1993; Konopka *et al.*, 1995). With the aid of *in situ* hybridization we have found that sg II mRNA was present in the hippocampal dentate gyrus, as well as in Cornu Ammonis of the hippocampus proper. KA treatment led to an increase in sg II mRNA level in all subfields of the hippocampus in pyramidal and granule cell layers. In the case of hsc 70, its mRNA could be observed in non-treated animals only in the dentate gyrus. However, KA injection evoked a profound elevation of this mRNA in pyramidal and granule cells throughout the whole hippocampus (Fig. 2). Clathrin heavy chain mRNA was expressed in the hippocampi of non-treated rats at the lowest level of all these three genes examined. KA treatment provoked a dramatic increase of these mRNA levels in the dentate gyrus granule cells and, to a lesser extent, in other hippocampal subfields as well.

Northern type of analysis of gene expression revealed that all three genes were characterized by similar time-course of expression, with a slight elevation of mRNA abundance at 2 h post-treatment (not shown), maximal increase at 6–24 h, and a decrease thereafter (see, e.g., Fig. 2). To find out whether the observed elevation of gene expression was dependent on previous protein synthesis we applied cycloheximide pretreatment (3 mg/kg, subcutaneously, 30 min before KA injection). Cycloheximide admini-

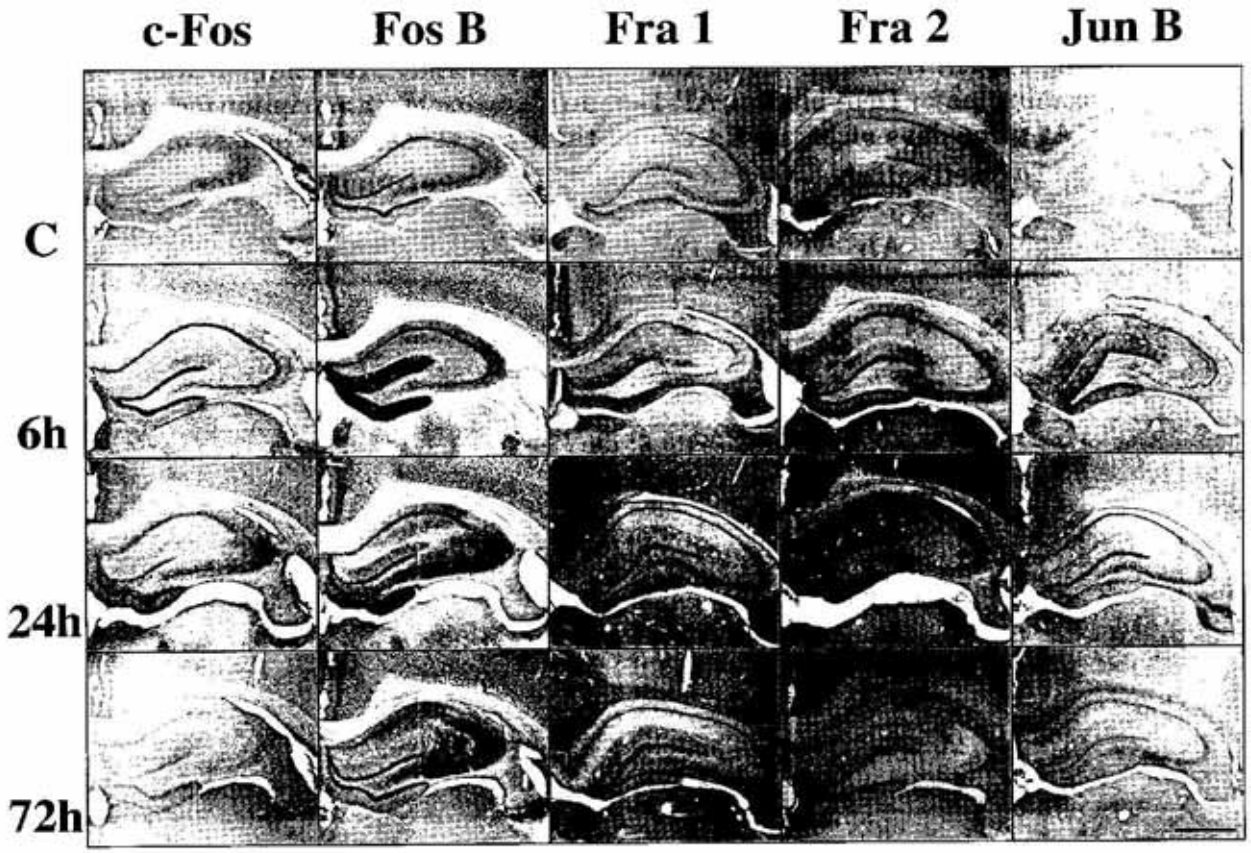

Figure 2. Expression of AP-1 proteins after kainate administration to rats.

The animals were treated i.p. with KA and killed at various times, as indicated, after the treatment (C, control). The brains were isolated and processed for immunocytochemical analysis with specific antibodies to the proteins indicated. Scale bar, 1 mm.

stration abolished KA-induced activation of clathrin and secretogranin II. In the case of hsc 70 this effect was also clear, although not so pronounced. Cycloheximide alone had no effect on control levels of mRNAs of chc and sg II, and a slight inductive effect on hsc 70 (Konopka *et al.*, 1995). Such a pattern of gene expression strongly suggests that their activation is of a secondary character, i.e., depends on previous gene expression (e.g., genes coding for either AP-1 or Zif/268 transcription factors).

Another gene — TIMP-1 (tissue inhibitor of metalloproteinases-1) behaves similarly to those just described. A marked increase in its expression was observed by northern hybridization at 6–24 h after kainate administration (Biedermann *et al.*, unpublished). *In situ* hybridization revealed that KA treatment leads to TIMP-1 mRNA accumulation in neuronal cell layers of all hippocampal subfields. We have noted a virtual overlap of spatial distribution of c-Fos immunoreactivity and TIMP-1 mRNA in hippocampi of KA-treated rats. Moreover, Bugno *et al.* (1995) demonstrated the dependence of TIMP-1 expression on AP-1 in cells cultured *in vitro*. Hence, we decided to pursue further studies on the possible role of AP-1 in control of TIMP-1 expression in brain. Using EMSA, we have analyzed protein binding to TIMP-1 regulatory elements (Biedermann *et al.*, unpublished). The TIMP-1 promoter carries a non-perfect AP-1 binding region in the vicinity of STAT/ETS elements (Bugno *et al.*, 1995). The DNA binding assay revealed that the oligonucleotide containing these sequences is bound by proteins whose levels increase at 6 h after KA administration. Moreover, this binding is abolished if AP-1 sequence is mutated. These results strongly implicate AP-1 in regulation of KA-driven TIMP-1 expression in hippocampus.

Recently, we have applied the RNA differential display technology to clone genes that are activated at 6 h after KA administration (Dabrowski *et al.*, unpublished). So far, we have obtained a number of genes that are potentially KA-driven. For two of them we have been able to confirm on northern blots

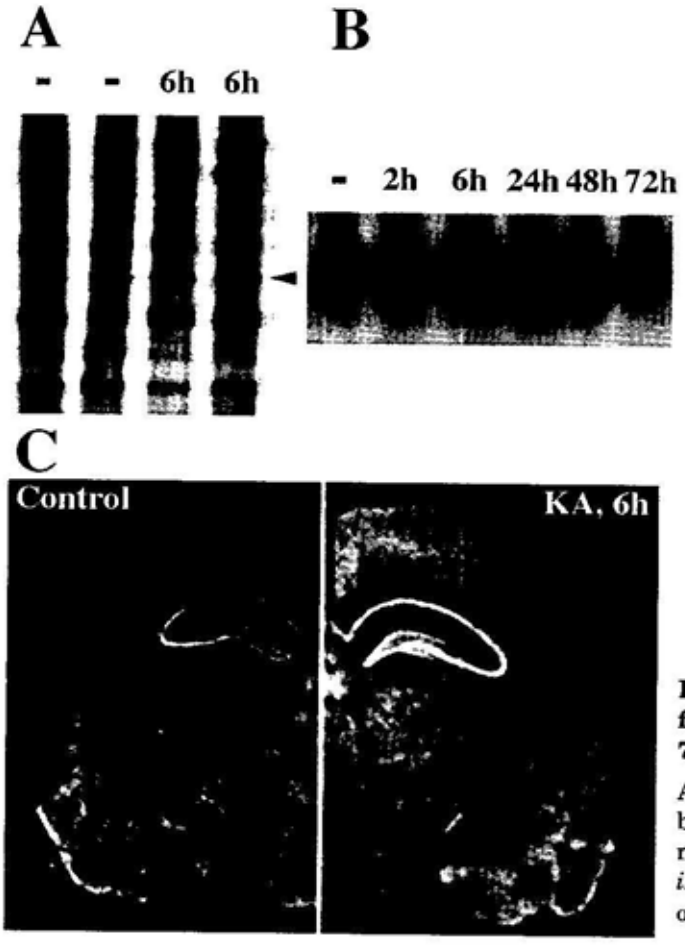

Figure 3. Evidence of upregulation of mRNA for the heat shock cognate 70 kDa protein (hsc 70) after kainate treatment.

A, RNA differential display, arrow points to hsc 70 band; B, northern blot of the time course of hsc 70 mRNA expression after kainate administration; C, *in situ* hybridization; dark field autoradiograms obtained after reaction to hsc 70 gene probe.

their upregulation by KA-induced seizures. A fragment of an autoradiogram of a differential display gel showing the up-regulated band of hsc 70 is shown in the Fig. 3. Hsc 70 was already known to be KA-regulated. The other identified gene was found to be a mammalian suppressor of Sec4 (Mss4), a soluble protein that functions as a GTP/GDP exchange factor for small (monomeric) G proteins of the Rab family (Burton *et al.*, 1994). The Rab proteins are involved in neuronal endocytosis (de Hoop *et al.*, 1994) and injection of Mss4 into squid giant nerve termini enhances neurotransmitter release (Burton *et al.*, 1994).

All the genes described in this chapter share similar features of expression pattern. Their mRNA accumulation is secondary (i.e., protein synthesis-dependent), delayed and prolonged in comparison with the initial phase of AP-1 components' expression. Moreover, expression of these secondary genes can be demonstrated in all hippocampal subfields. These observations suggest that expression of these genes results from neuronal excitation *per se*, and appears to exemplify a physiological response to enhanced spiking activity.

KAINATE-EVOKED DECREASE IN DYSTROPHIN mRNA EXPRESSION

Whereas the finding that a change in gene expression occurs in all hippocampal subfields indicates that this response might be a consequence of neuronal activation, limitation of the modulation of expression to the dentate gyrus only may suggest a possible role of gene expression in plasticity. We came across such a situation while analyzing expression of dystrophin gene in rat hippocampus (Gorecki *et al.*, in press). We have hypothesized that modulation of dystrophin mRNA levels by KA treatment may provide clues for elucidating a possible role of this protein in functioning of neurons in adult brain. This study has revealed that a low, albeit clear, expression of dystrophin mRNAs could be observed in the pyramidal cell layer of all CA subfields of the hippocampus proper as well as in the granule cell layer of the dentate gyrus. Similar spatial distribution of the hybridization signal was also seen 1 h after kainate insult. However, at 6 h after the treatment, there was no discernible hybridization signal of dystrophin in the dentate gyrus, whereas its expression in the CA1 subfield remained unchanged and in CA3 was even increased. At 24 h after KA administration, the pattern of the gene expression came back to that of control animals. At 72 h, the expression persisted at apparently control levels in the dentate gyrus and decreased in the CA subfields, concomitantly with neurodegeneration observed in these areas on the histologically stained parallel sections. Such a pattern of dystrophin mRNA expression in response to kainate challenge may imply that the gene product is involved in establishment/maintenance of synaptic contacts. Thus, studies on KA-regulated gene expression appear to reveal potential involvement of dystrophin in neural plasticity.

KAINATE PROVOKED NEURODEGENERATION AND GENE EXPRESSION

Kainate induces conditions similar to human temporal lobe epilepsy that is accompanied by neuropathological alterations including neuronal death and activation of glia. Hence studies on the neurodegenerative phase of brain response to kainate are of great importance. Our initial observation that KA treatment results in neuronal apoptosis coincident with the second wave of AP-1 elevation motivated us to search for genes whose expression is upregulated later than 24 h after the insult.

Two such genes were identified. The first codes for GFAP (glial fibrillary acidic protein) that is a specific component of astroglial intermediate filaments. GFAP mRNA accumulation in various brain regions is increased dramatically at 24 h following KA administration and remains elevated for at least 3 days thereafter (Hetman *et al.*, 1995). This increase is reminiscent of GFAP mRNA response to brain injury (Condorelli *et al.*,

1990). The results of immunocytochemical analysis confirmed that the GFAP increase was confined to reactive astroglia.

In parallel with GFAP mRNA accumulation, we have also observed increase in cathepsin D mRNA (Hetman *et al.,* 1995). Cathepsin D is a major lysosomal aspartic protease whose physiological function remains poorly known. Cathepsin D message was detectable in hippocampus, limbic cortex and neocortex in control animals and its level increased at all time-points examined, i.e., 6, 24, and 72 h as well as 7 days after KA administration. The moderate increase — 1.5–2-fold above the control level at 6 and 24 h — was followed by a more pronounced, 4–6 fold increase above control level, at 3 and 7 days after kainate treatment. The peak values were reached at 72 h in the limbic cortex and neocortex. The greatest increase in mRNA was observed in the hippocampus and limbic cortex 7 days after the treatment. Cathepsin D mRNA level in neocortex increased earlier but did not reach values as high as in the two other brain regions (Hetman *et al.,* 1995).

Using immunohistochemical technique we identified the expression pattern of cathepsin D protein in rat brain at 72 h after kainate treatment (Hetman *et al.,* 1995). In brains of control animals, we observed a wide distribution of cathepsin D immunoreactivity that was confined to granules localized mainly to perikaria of neurons in the hippocampus, limbic cortex, and also in neocortex. In kainate-treated animals, the increased cathepsin D immunoreactivity was localized mainly to hippocampus, piriform cortex and amygdala, i.e., limbic structures. An intense increase was also found in temporo-parieto-occipital neocortex in the cases with extensive damage of this brain area. The highest cathepsin D immunoreactivity was found in the regions that showed features of neurodegeneration. On the cellular level, cathepsin D immunoreactivity was found to be increased both in neuronal and glial cells. However, degenerating neurons were the main site of increased cathepsin D immunoreactivity. Thus, cathepsin D expression induced by kainate appeared to be largely related to toxic effects of this compound on neuronal cells.

In conclusion, in the studies on late gene response to KA administration, we demonstrated an increased cathepsin D and GFAP expression on both mRNA and protein levels in kainate damaged rat brain. We have shown that the increased cathepsin D expression is mainly related to neuronal cell death, whereas activation of GFAP reflects reactive gliosis. These findings suggest that cathepsin D might be engaged in the neuronal death and/or regeneration.

CONCLUDING REMARKS: KAINATE TREATMENT AS A WINDOW TO VARIOUS LONG TERM RESPONSES OF BRAIN CELLS

In our studies reviewed herein we have investigated kainate-evoked gene expression. The main rationale behind this work was to understand responses of nerve cells to excitatory activity of glutamate and its analogs. Recent progress in neurobiology has provided multiple examples of the complex nature of effects exerted by excitatory amino acids on brain cells, both glia and neurons (for review see: Kaczmarek *et al.,* 1997). Under physiological conditions, neuronal activity is regulated by glutamate acting as a major depolarizing neurotransmitter. This phenomenon involves mainly non-NMDA ionotropic AMPA receptors. The NMDA receptors appear to be especially important for neuronal plasticity, i.e., reorganization of synaptic circuits. The role of kainate receptors still remains largely unknown. However, kainate treatment results in activation of all three kinds of glutamate ionotropic receptors, either directly or as a neuronal circuit property. Hence, this model system provides an opportunity to follow various physiological neuronal responses to activation of glutamate receptors, that may drive plastic changes or at least display adaptation to a sudden increase of the spiking activity. The latter response has been named "replenishment" to suggest that it is involved in the recovery of the biochemical machinery fa-

tigued as a result of this spiking burst (see for discussion: Kaczmarek, 1995; Kaczmarek & Chaudhuri, 1997).

The early phase of gene expression that is observed in all brain regions responding to KA, especially all hippocampal subfields, may provide valuable information. The fact that we have identified three genes encoding proteins related to synaptic release supports the notion that their response to KA treatment represents a "replenishment" class of reactions. On the other hand, neuronal responses limited to dentate gyrus only, such as a decrease of dystrophin mRNA seem to imply that these reactions may have something to do with the fact that this brain region not only survives kainate challenge, but is even actively engaged in neural plasticity.

Too much of a good thing might be, however, dangerous. Excessive amounts of available glutamate result in neuronal cell death (Danysz *et al.*, 1995). Both necrosis and apoptosis take place in this kind of neuronal response to kainate. Importantly, the programmed cell death is also a form of long term cellular response involving gene expression phenomena (Dragunow & Preston, 1995). Studies on late kainate-driven neuronal gene expression appear to provide a useful tool to get closer to elucidation of molecular mechanisms of apoptosis.

In conclusion, we may say that careful dissection of the spatio-temporal pattern of changes in gene expression that follow kainate administration may help us to understand a variety of phenomena of brain physiology and pathology.

REFERENCES

Ben-Ari, Y. (1985) Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* **14**, 375–403.

Ben-Ari, Y., Tremblay, E., Riche, D., Ghilini, G. & Naquet, R. (1981) Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline and pentetrazole: Metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience* **6**, 1361–1391.

Bugno, M., Graeve, L., Gatsios, P., Koj, A., Heinrich, P.C., Travis, J. & Kordula, T. (1995) Identification of the interleukin-6/oncostatin M response element in the rat tissue inhibitor of metalloproteinases (TIMP-1) promoter. *Nucleic Acids Res.* **23**, 5041–5047.

Burton, J.L., Burns, M.E., Gatti, E., Augustine, G.J. & De Camilli, P. (1994) Specific interactions of Mss4 with members of the Rab GTPase subfamily. *EMBO J.* **13**, 5547–5558.

Condorelli, D.F., Dell Albani, P., Kaczmarek, L., Messina, L., Spampinato, G., Avola, R., Messina, A. & Giuffrida-Stella, A.M. (1990) Glial fibrillary acidic protein messenger RNA and glutamine synthetase activity after nervous system injury. *J. Neurosci. Res.* **26**, 251–257.

Condorelli, D.F., Dell Albani, P., Amico, C., Kaczmarek, L., Nicoletti, F., Lukasiuk, K. & Giuffrida-Stella, A.M. (1993) Induction of primary response genes by excitatory amino acids receptor agonists in primary astroglial cultures. *J. Neurochem.* **60**, 877–885.

Condorelli, D.F., Dell'Albani, P., Amico, C., Lukasiuk, K., Kaczmarek, L. & Giuffrida-Stella, A.M. (1994) Glutamate-receptor driven activation of transcription factors in primary neuronal cultures. *Neurochem. Res.* **19**, 489–499.

Danysz, W., Parsons, C.G., Bresink, I. & Quack, G. (1995) Glutamate in CNS disorders. *Drug News Persp.* **8**, 261–277.

Dragunow, M. & Preston, K. (1995) The role of inducible transcription factors in apoptotic nerve cell death. *Brain Res. Rev.* **21**, 1–28.

Filipkowski, R.K., Hetman, M., Kaminska, B. & Kaczmarek, L. (1994) DNA fragmentation in rat brain after intraperitoneal administration of kainate. *Neuroreport* **5**, 1538–1540.

Gorecki, D.C., Lukasiuk, K., Szklarczyk, A.W. & Kaczmarek, L. (1998) Kainate-evoked changes in dystrophin mRNA levels in the rat hippocampus. *Neuroscience* (in press).

Hetman, M., Filipkowski, R.K., Domagała, W. & Kaczmarek, L. (1995) Elevated cathepsin D expression in kainate-evoked rat brain neurodegeneration. *Exp. Neurol.* **136**, 53–63.

de Hoop, M.J., Huber, L.A., Stenmark, H., Williamson, E., Zerial, M., Parton, R.G. & Dotti, C.G. (1994) The involvement of the small GTP-binding protein Rab5a in neuronal endocytosis. *Neuron* **13**, 11–22.

Kaczmarek, L. (1995) Towards understanding of the role of transcription factors in learning processes. *Acta Biochim. Polon.* **42**, 221–226.

Kaczmarek, L. & Chaudhuri, A. (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: Implications for functional mapping and neural plasticity. *Brain Res. Rev.* **23**, 237–256.

Kaczmarek, L., Kossut, M. & Skangiel-Kramska, J. (1997) Glutamate receptors in cortical plasticity: Molecular and cellular biology. *Physiol. Rev.* **77**, 217–255.

Kaminska, B., Filipkowski, R.K., Zurkowska, G., Lason, W., Przewlocki, R. & Kaczmarek, L. (1994a) Dynamic changes in composition of the AP-1 transcription factor DNA binding activity in rat brain following kainate induced seizures and cell death. *Eur. J. Neurosci.* **6**, 1558–1566.

Kaminska, .B., Lukasiuk, K. & Kaczmarek, L. (1994b) Seizures-evoked activation of transcription factors. *Acta Neurobiol. Exp.* **54**, 65–72.

Kaminska, B. & Kaczmarek, L. (1993) Robust induction of AP-1 transcription factor DNA binding activity in the hippocampus of aged rats. *Neurosci. Lett.* **153**, 189–191.

Kaminska, B., Kaczmarek, L. & Chaudhuri, A. (1996) Visual stimulation regulates the expression of transcription factors and modulates the composition of AP-1 in rat visual cortex. *J. Neurosci.* **16**, 3968–3978.

Konopka, D., Nowicka, D., Filipkowski, R.K. & Kaczmarek, L. (1995) Kainate evoked secondary gene expression in the rat hippocampus. *Neurosci. Lett.* **185**, 167–170.

Konopka, D., Szklarczyk, A.W., Filipkowski, R.K., Trauzold, A., Nowicka, D., Hetman, M. & Kaczmarek, L. (1998) Plasticity- and neurodegeneration-linked CREM/ICER mRNA expression in the rat brain. *Neuroscience* (in press).

Lukasiuk, K. & Kaczmarek, L. (1994) AP-1 and CRE DNA binding activities in rat brain following pentylenetetrazole induced seizures. *Brain Res.* **643**, 227–233.

Morgan, J.I. & Curran, T. (1991) Stimulus-transcription coupling in the nervous system, involvement of the inducible proto-oncogenes fos and jun. *Annu. Rev. Neurosci.* **14**, 421–451.

Nedivi, E., Hevroni, D., Naot, D., Israeli, D. & Citri, Y. (1993) Numerous candidate plasticity-related genes revealed by differential cDNA cloning. *Nature* **363**, 718–722.

Pollard, H., Cantagrel, S., Charriaut-Marlangue, C., Moreau, J. & Ben-Ari, Y. (1994a) Apoptosis associated DNA fragmentation in epileptic brain damage. *Neuroreport* **5**, 1053–1055.

Pollard, H., Charriaut-Marlangue, C., Cantagrel, S., Represa, A., Rabin, O., Moreau, J. & Ben-Ari, Y. (1994b). Kainate induced apoptotic cell death in hippocampal neurons. *Neuroscience* **63**, 7–18.

Przewlocki, R., Kaminska, B., Lukasiuk, K., Nowicka, D.Z., Przewlocka, B., Kaczmarek, L. & Lason, W. (1995) Seizure related changes in the regulation of opioid genes and transcription factors in the dentate gyrus of rat hippocampus. *Neuroscience* **68**, 73–81.

Sperk, G. (1994) Kainic acid seizures in the rat. *Prog. Neurobiol.* **42**, 1–32.

Schwob, J.E., Fuller, T., Price, J.L. & Olney, J.W. (1980) Widespread patterns of neuronal damage following systemic or intracerebral injections of kainic acid, a histological study. *Neuroscience* **5**, 991–1014.