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Glutamate-evoked gene expression in brain cells – Focus on transcription factors

Review Article

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Summary. L-glutamate (L-glu) is major excitatory neurotransmitter in mammalian central nervous system. Besides short term effects on neuronal activity, L-glu affects also long term neuronal and glial responses. Neuromodulatory function of L-glu may in part be dependent on its ability to activate transcription factors – proteins regulating gene expression through interaction with specific regulatory sequences located in promoter and enhancer gene regions. This paper reviews recent data suggesting that L-glu can selectively stimulate genes encoding transcription factors like zif/268 and fos and jun gene families, as well as the factors themselves both in neurons and astroglia cultured in vitro and brain cells in vivo.

Keywords: Amino acids – AP-1 – c-fos – c-jun – zif/268 – NGF-IA – egr-1 – krox-24 – NMDA – Neurons – Glia – Neural plasticity – Excitatory amino acids

1. Glutamate and long term cellular responses

L-glutamate (L-glu), (more broadly – excitatory amino acids) exert their effects on the nervous system through interaction with specific cell surface receptors. These receptors could be classified into ionotropic: non-NMDA (kainate or AMPA), NMDA, and metabotropic receptor subtypes. Over the last years it has been increasingly clear that L-glu besides being an excitatory neurotransmitter acts also as a neuromodulator. In this latter role, L-glu can produce long lasting modifications of functioning of nervous tissue, including e.g. plastic changes or neurodegeneration.

Also during the last few years, the very basic mechanisms of long term cellular responses to different stimuli have been elucidated. For instance several authors (Goelet et al., 1986; Kaczmarek, 1986; Curran and Morgan, 1987; Kaczmarek and Kaminska, 1989; Sheng and Greenberg, 1990) have proposed that protein

products of certain genes, now well recognized as transcription factors (TFs), regulating gene expression, are acting as third messengers linking extracellular stimuli (acting through cell surface receptors, second messengers and kinases) with long term phenotypic changes. An apparent unspecificity of activation of certain TFs in different kinds of long term cellular responses – proliferation, differentiation, execution of function of terminally differentiated cells (Kaczmarek, 1986; Kaczmarek and Kaminska, 1989) – clearly shows that these TFs are not sufficient to produce aforementioned effects. Nevertheless, they could be necessary, provided that they act in concert with other regulatory factors, as previously suggested (Kaczmarek, 1986). Indeed, at least for such disparate cellular activities like proliferation of fibroblasts (Karin and Angel, 1991; Riabowol et al., 1992), programmed cell death (apoptosis) (Colotta et al., 1992) and forming a neuronal basis for cocaine-dependent modulation of locomotor behavior (Heilig et al., 1993) an involvement of *c-fos* gene has been experimentally proven.

In conclusion, it has been reasonable to ask whether L-glu dependent modulation of neuronal activity could also follow similar pathways. As a prerequisite to answer this question it was necessary to document that indeed L-glu may stimulate gene expression. With this reasoning in mind several research groups focused their attention on genes encoding transcription factors as well as their protein products and finally on the TFs themselves. This approach proved indeed to be very productive. Most of the research dealt with transcription factor named activator protein 1 (AP-1), or being more precise, rather with genes encoding its components. The AP-1 is a protein dimer which can be composed of proteins belonging to *fos* and *jun* gene families. There are at least 4 *fos* genes (*c-fos*, *fos B*, *fra-1*, *fra-2*) coding for proteins designated as: c-Fos, Fos B, Fra-l, Fra-2. In the *jun* family there are 3 genes: *c-jun*, *jun B* and *jun D*, coding for, respectively, c-Jun, Jun B and Jun D proteins (for review see: Morgan and Curran, 1991a). At present it remains unclear what are specific, differential functions carried out by AP-1 made of different components. Another gene encoding transcription factor widely investigated in the brain is *zif/268* (also known as *egr-1*, *NGF-IA*, *krox 24*, *TIS 8*).

In this paper I would like to review an evidence that excitatory amino acids (L-glu and its homologues) are able to activate expression of the aforementioned genes, as well as transcription factors themselves, in brain cells in vivo and in vitro. It is worthy to note that such genes are of particular interest in analyzing gene expression phenomena. The fact that they are usually expressed at a very low level in nonstimulated cells and then induced dramatically at the transcription, mRNA and protein levels, makes them relatively easy to study. Moreover, the fact that they code for regulatory elements like TFs raises intriguing questions about processes they might control. Altogether they seem to be a model to investigate of utmost importance.

2. L-glu and gene expression in cultured brain cells

2.1. Neurons

It is obvious that in vitro cell culture offers particularly convenient tool to study effects of various treatments on gene expression. Neuronal cultures are no

exception, however, there is one limitation important to note. Usually it is assumed that adding a chemical under study to the cultured cells permits to investigate solely its effect. It is though not necessarily true as cell-to-cell interactions should also be taken into account. This is of particular importance in investigation of neurons. Any neurotransmitter or its agonist can alter not only the immediate target cells but can provoke transsynaptic effects as well. This raises a necessity to evaluate effects of both agonists and antagonists on the observed phenomena.

The first reports on cultured neurons and L-glu driven gene expression dealt with granule cells of the postnatal rat cerebellum. Szekely et al. (1987, 1989) initially found that 10 μM L-glu and 50 μM NMDA can provoke *c-fos* mRNA accumulation in these cultures and 100 μM APV, an antagonist of NMDA receptors, completely blocked the response to L-glu. In further studies the same authors (Szekely et al., 1990) reported that 10 μM L-glu activates also *c-jun, jun B* and *zif/268* mRNA accumulation and all of these increases were almost completely abolished by NMDA receptor antagonists: 1 μM PCP or 10 μM CPP. On the other hand, 2 μM CNQX (a non-NMDA L-glu receptor antagonist) did not have significant effects on L-glu driven *c-fos* and *c-jun* mRNA accumulation. An interesting difference between the patterns of expression of *c-jun* and other genes was revealed. The latter were characterized by only one, early and transient, peak of activity, while the former displayed biphasic expression with the first transient wave of mRNA levels peaking at 0.5 h after the L-glu stimulation, and second, prolonged wave of mRNA elevation starting from 4 h and lasting at least up to 12 h after the treatment. The AP-1 DNA binding activity was also elevated at 1 to 6 h following the L-glu stimulation. Apparently the fos-related antigens (Fra) could take part in the AP-1 complexes at later times as indicated by the Western analysis (Szekely et al., 1990).

The AP-1 formation was also reported (Sakurai et al., 1992) for granule cells of cerebellum after either 100 μM NMDA or 100 μM kainate treatment. Specific antagonists, APV for NMDA and CNQX for kainate reversed these increases. The NMDA stimulatory effect was abolished in the either absence of Ca^{2+} or presence of Mg^{2+}. On the other hand, kainate effects required calcium to be present, but magnesium did not revert it. Parallel increases were also noted for the CRE (cAMP responsive element, another regulatory sequence activated by CRE-binding, or CREB transcription factor, see: Montminy et al., 1990) DNA binding activity, and the authors suggested that the same protein complexes may cross react with both AP-l and CRE regulatory sequences (Sakurai et al., 1992).

Didier et al. (1989; 1992) reported that in the granule cerebellar cells c-Fos immunoreactivity could be correlated with maturation of these cells and remained persistent if the cells were grown under depolarizing conditions dependent e.g. on the presence of 100 μM NMDA.

The studies on cerebellar cells show that L-glu activates AP-1 and its components and the NMDA receptors provide major but not the only input in this regard. Similar conclusions could be drawn from investigations of other types of neuronal cultures. Murphy et al. (1991a,b) investigated *c-fos, fos B, c-jun, jun-B* and *zif/268* mRNA levels as well as immunoreactivity in primary cortical cultures derived from rat fetuses and cultured for long time (up to 3 weeks) in order

do develop spontaneous electrical activity. In those cells basal gene and protein expression were observed and then disappeared in the presence of NMDA receptor antagonists: 10 μM MK-801 and 300 μM APV. The gene expression blocked by MK-801 could be restored by kainate and this latter effect was dependent on voltage sensitive calcium channels (L-type channels) (Murphy et al., 1991b).

Vaccarino et al. (1992) found that also in cortico-striatal neurons in culture 10 μM L-glu induced mRNA levels for *c-fos*, *c-jun*, *zif/268* and (to a lesser extent) *jun B*. Even 30 sec pulse treatment with L-glu was enough to elicit these effects. MK-801 and to a lesser extent CNQX decreased these elevations. The role of non-NMDA L-glu receptors was further underlined by the finding that 1 μM quisqualate was also able to provoke *c-fos*, *jun B* and *zif/268* mRNA accumulation. Similar results were obtained by Condorelli et al. (submitted) who found that in cultures of fetal cerebral hemispheres L-glu, NMDA, kainate, quisqualate, AMPA, and to a much lesser extent t-ACPD (an agonist of L-glu metabotropic receptors)evoked *c-fos*, *c-jun* and *zif/268* mRNA accumulation. L-glu led also to an increase of fos B mRNA levels. Again the critical role of NMDA receptors was underlined by use of MK-801, as well as studies carried out in the presence or absence of magnesium. Nevertheless, only combination of MK-801 and DNQX (a non-NMDA L-Glu receptor antagonist) was fully successful in abolishing the *c-fos* activation induced by L-glu. Similarly, the AP-1 DNA binding activity was found to be increased following the L-glu and NMDA receptor activation. The effect of the former was partially, and of the latter completely, abolished by the MK-801. Contrary to the AP-1, the CRE and NFκB (another TF, see: Baeuerle, 1991) DNA binding activities were not elevated by the L-glu treatment in neuronal cultures (Lukasiuk et al., submitted). Interestingly, NMDA receptors antagonists blocked not only L-glu driven *c-fos* mRNA and protein accumulation but also translation dependent on accumulation of mRNA driven by other agents including basic fibroblast growth factor (Hisanaga et al., 1992).

Having established the role of L-glu and NMDA receptors in gene activation opened a way to study their effects in more detail. Following this lead, Lerea et al. (1992; 1993) and Bading et al. (1993) found that in hippocampal neurons in culture calcium acts as the main second messenger involved in *c-fos* gene activation. Interestingly, there are two main routes of Ca^{2+} influx into the neuron – either through NMDA receptor channel or through voltage sensitive calcium channels, opened as a response to non-NMDA L-glu receptor driven depolarization. Downstream to the calcium elevation there are also divergent pathways. The NMDA driven c-fos activation was abolished by inhibitors of phospholipase A2 and of cyclooxygenase, implicating prostaglandins to be involved. No effect of calmodulin antagonist, calmidazolium was, however, noted. On the contrary, kainate evoked *c-fos* mRNA accumulation was markedly inhibited by calmidazolium (Lerea et al., 1993). Bading et al. (1993) further confirmed an ability of two kinds of calcium channels (NMDA and L-type) to transmit signals to the nucleus and regulate c-fos expression through two distinct Ca^{2+} signalling pathways, acting in consequence on separate regulatory sequences contained within the *c-fos* gene.

2.1. Astroglia

Besides neurons, also astroglia are endowed with L-glu receptors in the brain. It has been shown that except for the NMDA receptor, both ionotropic and metabotropic receptor subtypes responsive to excitatory amino acids are present on these cells. Following initial finding that ibotenate, an agonist of L-glu metabotropic receptors is able to stimulate *c-fos* mRNA accumulation (Condorelli et al., 1989), further studies proved that in fact all kinds of L-glu receptors could be involved.

McNaughton and Hunt (1993) found that *c-fos*, *c-jun*, *jun B* and *zif/268* mRNA levels were increased upon treatment of astroglia with 100 μM kainate, quisqualate and AMPA, while NMDA was without any effect. The effect of quisqualate stimulation was not inhibited by CNQX or withdrawal of external Ca^{2+}. In contrast, the kainate effects was abolished by CNQX but not by the removal of external Ca^{2+}.

Condorelli et al. (1992) also showed that, besides L-glu, kainate, AMPA and tACPD can transmit the signal activating the *c-fos*, *c-jun*, *jun B*, *fos B* and *zif/268* gene expression, while NMDA treatment did not produce any effect. Studies with antagonist (DNQX, MK-801 and kynurenate, general L-glu antagonist) further proved the role of various receptors in eliciting increased gene expression following L-glu treatment of astroglia. Moreover, L-glu was also found to stimulate formation of AP-1 DNA binding complexes in these cells, but did not elevate CRE and NFκB DNA binding activities (Lukasiuk et al., submitted).

3. L-glu and gene expression in the brain *in vivo*

3.1. Injections of agonists and antagonists

Among the first clues that L-glu may activate gene expression were results of effects of injection of 60 μg of L-glu beneath the hippocampal formation in rats (Kaczmarek et al., 1988). This treatment resulted in dramatic and transient accumulation of *c-fos* mRNA in the hippocampus. However, injection of physiological saline produced similar, albeit less pronounced effect. Though encouraging, this result also questioned the feasibility of the direct intrabrain injections as an approach to study the effects of the injected compounds.

Another approach i.e. systemic injections is, unfortunately, not without its own pitfalls. Even without mentioning the blood-brain barrier, peripheral delivery of the L-glu agonists still leads to problems with interpretations of the data. These compounds can obviously excite neurons and, therefore, secondary transsynaptic neuronal activation has to be considered. Even more importantly, L-glu agonists can provoke seizures as well as neurodegeneration, further complicating dissection of all components of the observed gene expression responses.

Considering the aforementioned limitations it is of note that acute dose (225 mg/kg) of NMDA resulted in very regional induction of *c-fos* mRNA, mostly in the dentate gyrus of the hippocampus and in the piriform cortex (Morgan and Linnoila, 1991). NMDA driven activation of c-fos expression in the hippocampus was also reported by Sonnenberg et al. (1989).

It is also interesting, though not very well understood that MK-801 by itself or in combination with other treatments can elevate expression of *c-fos* and/or other similar genes or proteins (Dragunow and Faull, 1990; Dragunow et al., 1993; Guthrie et al., 1993; Gass et al., 1993).

Injections of convulsant doses of kainate (KA) to rats and mice, either peripherally or intraventricularly, caused a dramatic increase of mRNA level of *c-fos* (Sonnenberg et al., 1989b; Morgan and Curran, 1991a,b). This elevation was well visible in neurons of the hippocampus as well as entorhinal cortex. An increase of the mRNA level was followed by elevated c-Fos protein immunoreactivity (Jensen et al., 1993; Le Gal La Salle, 1988; Popovici, et al., 1990; Pennypacker et al., 1993; Sakurai-Yamashita et al., 1991). Smeyne et al. (1992; 1993), using *c-fos-lacZ* transgenic mice, expressing β-galactosidase under control of *c-fos* regulatory elements, observed that β-gal levels were increased transiently within hours after the KA treatment, then declined and raised again at several days following KA injection. Other genes encoding transcription factors, induced by the kainate injections were *c-jun*, *fos B*, *zif/268* and *CREM* (CRE modulating factor) (Nedivi et al., 1993).

Also AP-1 DNA binding activity was found to be elevated within hours after the kainate treatment (Sonnenberg et al., 1989b; Pennypacker et al., 1993). More detailed scrutiny of this last phenomenon revealed that there are two waves of AP-1 responsiveness to the kainate – first within a few hours and second 3 days after the injection. Moreover these effects were somewhat specific as CRE and OCT (another regulatory sequence) DNA binding activities were not influenced by kainate (Kaminska et al., submitted).

3.2. Other forms of stimulation involving L-glu receptors

Several different neuronal responses are known to be mediated by L-glu receptors, and even if they are of great complexity they may provide a circumstantial evidence that observed effects on gene expression could be driven by these receptors. Below there is a list of some of those, for which activation of transcription factors and/or their genes has been well documented (reviewed in: Greenberg and Sheng, 1990; Morgan and Curran, 1991a,b; Kaczmarek 1992, 1993a,b)

- regulation of circadian rhythms;
- electrical stimulation;
- neuronal plasticity phenomena including cortical development, LTP, learning and memory formation;
- responses to brain injury;
- various forms of seizures;
- ischemia;
- different forms of sensory stimulations;
- spreading depression.

However, as all of them are based on a vast array of interactions between several neurotransmitter systems, final proof that indeed the L-glu receptors are involved is very difficult to obtain. A good lead is provided by the antagonist studies, however, the aforementioned transsynaptic effects have to be kept in

mind. For instance Dragunow et al., (1990) provided an evidence that haloperidol and YM 09151-2, both binding to D2 dopamine receptors can activate c-Fos expression in striatal neurons, but apparently their effects were not direct, as effects of both ligands were reversed by MK-801.

3.3. L-glu antagonists in vivo

In different models of brain injury, elevated c-Fos immunoreactivity was reported and then shown to be dependent on NMDA receptors. Herrera and Roberston (1990) found that this increase was blocked by ketamine (100 mg/kg, NMDA receptor antagonist) and MK-801 (1 and 3 mg/kg). Sharp et al., (1990) observed that CPP was effective in preventing the increase. Similarly, systemic injection of MK-801 prior to and after lesioning of the entorhinal cortex prevented appearance of c-Fos immunoreactivity in neurons and glia in the hippocampus (Nitsch and Frotscher, 1992).

MK-801 (3 mg/kg) was also effective in blocking enhancement of c-Fos immunolabelling produced by application of KCl to the brain surface (which induces spreading depression) (Herrera and Robertson, 1990).

Ketamine (100 mg/kg) reduced the c-Fos induction resulting from ischemic cerebral devascularization (Herrera and Robertson, 1989). In another ischemic model, based on unilateral carotid ligation, MK-801 treatment prevented *c-fos* induction in the non-ligated hemisphere while was associated with increased *c-fos* expression in hippocampal neurons from the ligated side (Gunn et al., 1990). Kynurenine, a precursor of endogenous brain kynurenic acid prevented ischemia-driven c-Fos immunoreactivity in the rat cerebral cortex (Nozaki and Beal, 1992).

MK-801 (down to 0.3 mg/kg) as well as CGS-19755 (10mg/kg, NMDA receptor antagonist) reduced the basal levels of *c-fos* and *zif/268* mRNA levels in the visual cortex of rats (Worley et al., 1990, 1991). In addition, MK-801 reduced also basal DNA binding activities of AP-1 and ZIF/268 as well as light-induced *zif/268* mRNA accumulation in the rat visual cortex (Worley et al., 1990, 1991). NMDA and non-NMDA receptor antagonists were found to be able to inhibit photic induction of Fos protein in the hamster suprachiasmatic nucleus (Abe et al., 1992, 1993).

Accumulation of *zif/268* mRNA, provoked by induction of LTP (Bliss and Lomo, 1973) was blocked by MK-801 (Cole et al., 1989). Similarly LTP-induced c-Fos, cJun, Jun B and Fras expression were all blocked by APV, CPP or MK-801 (Dragunow et al., 1989; Demmer et al., 1993; Wisden et al., 1990).

Concluding remarks

Taken together the data presented in this paper, while not exhausting all the existing literature, provide nevertheless compelling evidence that L-glu is a potent activator of gene expression in the brain cells, and different subtypes of L-glu receptors are involved. The literature is particularly abundant with a critical role of stimulation of NMDA receptors in induction of neuronal gene expression. It has to be also mentioned that, while beyond the scope of this

article, the data about involvement of other than L-glu receptors in gene activation are much more limited. It remains as a challenge for the studies to come, whether these findings indeed reflect apparently dominant role of L-glu and NMDA receptors in particular, in modulation of long term functioning of nervous tissue (Collingridge and Singer, 1990).

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