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Electrically stimulated rapid gene expression in the brain: ornithine decarboxylase and *c-fos*

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A single electroconvulsive shock (ECS) resulted in a major induction of cerebral ornithine decarboxylase (ODC) mRNA and a rapid and transient elevation of ODC enzyme activity. The proto-oncogene *c-fos* was also transiently induced under the same conditions. Following a rapid rise in mRNA levels, the messages for these proteins take different courses. The *c-fos* mRNA fell to below control levels by 1 h, while the ODC mRNA remained elevated beyond 24 h. The ECS-induced elevation of ODC enzyme activity was not abolished by adrenalectomy but was attenuated significantly by the anti-convulsant MK-801. These results imply that the induction of cerebral ODC may be neuronal activity dependent, and suggest that the ODC/polyamine system may be linked to the proposed third messenger cascade, involving *c-fos*, which couples cell stimulation to gene expression, resulting in long-term adaptive responses.

INTRODUCTION

Ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine synthetic pathway²³, and the proto-oncogene *c-fos* are known to be involved in growth- and division-related phenomena. However, a multitude of chemical, thermal and physical stimuli, unrelated to proliferative events, result in the induction of ODC and *c-fos*^{8,11,12,14}. For every case of *c-fos* induction, a corollary in ODC stimulation can be found. Therefore, it appears that the polyamines and Fos may have specialized and related functions in the adult central nervous system (CNS).

A cell in the nervous system can react to incoming signals in several ways, including an immediate short-term response which is mediated by second messenger systems, and a long-term response that requires gene expression⁵. A number of gene products have recently been implicated in coupling cell-surface stimulation to gene transcription. The best studied member of this family, termed 'third messengers' is *c-fos*, which has been shown to be transiently induced in reaction to neuronal activation²⁰. The nuclear localization of Fos²⁰, its DNA-binding properties and the ability of Fos to activate the transcription of genes *in vitro*, is compatible with the role of Fos as a 'third messenger'^{3,10}.

ODC activity has also been reported to be induced by neuronal stimulation^{1,22}. The polyamines, whose synthe-

sis is dependent on ODC, are cationic in nature²⁶ and have long been shown to exert an effect on DNA replication and stability, RNA expression and protein synthesis. ODC, with both nuclear and cytoplasmic localizations², has one of the fastest turn-over rates of any known protein (<10 min), and can undergo rapid and drastic elevations in activity, primarily through the *de novo* synthesis of enzyme molecules²³.

The ODC/polyamine system and the nuclear proto-oncogenes thus share many similar properties. In this study, we demonstrate that electroconvulsive shock (ECS) results in the induction of ODC mRNA and enzyme activity as well as *c-fos* mRNA. We also propose that ODC is an early gene that is activated by neuronal stimulation and hence may participate in *c-fos* and other nuclear proto-oncogene mediated events²¹.

MATERIALS AND METHODS

Animals and treatment

Male Fischer CD rats, 6–10 weeks old were used. The animals were housed at 22 °C with free access to food and water, on a 12 h light–dark cycle. Electroconvulsive shock (ECS) was administered via ear electrodes, with saline-moistened contact pads, using a constant-current apparatus. Shock was applied to normal, adrenalectomized and sham operated rats at the following intensities: 85 mA for 1 s with 1 ms pulses at a frequency of 50 Hz. Control animals were treated similarly without the application of a current. These shock intensities have been demonstrated to be without detectable morphological damage to the brain⁶.

Some animals were dosed by interperitoneal injections of MK-801

(1 mg/kg), dissolved in saline. Animals were decapitated and their corresponding brain regions were dissected out, quickly placed in dry ice and kept at -70°C until the time of use.

Ornithine decarboxylase assay

ODC activity was determined by measurement of evolved $^{14}\text{CO}_2$ from carboxy- ^{14}C ornithine (55.9 mCi/nmol, New England Nuclear, Boston, MA). Tissue was homogenized in 19 volumes of 0.04 M Tris-HCl. After centrifugation (26,000 g, 20 min), 0.9 ml of various tissue preparations was added to 50 μl pyridoxal phosphate solution (1 nM) and 50 μl ^{14}C ornithine, in the presence of 0.045 M dithiothreitol. The final ornithine concentration was 2.5 μM . Incubations were carried out at 37°C for 45 min in a sealed tube and terminated by injection of 1 ml of 2 M acetic acid into the reaction mixture²⁵. Evolved $^{14}\text{CO}_2$ was trapped on a paper wick containing hyamine suspended above the reaction mixture. The decarboxylation process is linear for up to 1.5 h under these conditions. Decarboxylation, not attributable to ODC, was determined by running a parallel incubation in the presence of 5 mM difluoromethylornithine, the specific ODC inhibitor¹⁹.

RNA analysis

Total cellular RNA was isolated from brain regions of control and ECS-treated animals using the phenol-SDS method^{16,27}, with some modifications. The RNA was fractionated according to size in formaldehyde-agarose gels⁹ and transferred onto nitrocellulose filter paper. The filters were then probed with a ^{32}P -labelled ODC cDNA plasmid¹⁷ (labelled by nick-translation), washed under stringent conditions and exposed to X-ray film at -70°C for 5–7 days²⁹. Similarly, RNA obtained from control and treated animals was probed with a β -actin cDNA plasmid under the same conditions. The *EcoRI*BamHI 2.2 kbp *fos* insert fragment of pSP65 was also used to generate nick-translated probes. mRNA content was estimated by scanning the autoradiograms with a laser densitometer (LKB, Pharmacia), interfaced with a computer integrator.

RESULTS

ECS-induced seizures were used as an experimental paradigm to stimulate both ODC and *c-fos*. Following the administration of ECS all animals exhibited a 30 s clonic-tonic seizure which resulted in the subsequent

Fig. 1 The effect of a single ECS on ODC enzyme activity. ODC was determined by the measurement of evolved $^{14}\text{CO}_2$ from ^{14}C ornithine. The temporal profile of the mean ODC enzyme activity ($\pm$ SEM) following ECS from 3 to 4 determinations is shown. * Values differ significantly from basal levels ($P < 0.05$) using a two-tailed *t*-test, following analysis of variance.

Fig. 2 The effect of a single ECS on ODC mRNA and β -actin mRNA. Total RNA was isolated, fractionated on agarose gels and transferred onto nitrocellulose filter paper. Filters were then probed with ^{32}P -nick-translated cDNAs for both ODC and β -actin. The hybridized filters were exposed to X-ray film for 1 week. Control (C) ODC and β -actin mRNA levels are compared to ECS (E)-treated rats, 5 h post-ECS. The position of the ribosomal RNA bands is shown on the vertical axis as determined by ethidium bromide staining of the agarose gels.

elevation of ODC enzyme activity. This elevation reached over 2-fold above controls by 5 h, and was transient, returning to basal values by 10 h (Fig. 1).

The induction of ODC enzyme activity in the neocortex was accompanied by a rapid and significant elevation of the ODC mRNA (Figs. 2 and 3). The levels of an mRNA coding for a structural protein, β -actin, were not changed as a result of electrical stimulation (Fig. 2). In contrast to the transient rise of ODC enzyme activity,

Fig. 3 ODC and *c-fos* gene expression in response to electrical stimulation. A temporal plot of the mean ODC and *c-fos* mRNA content ($\pm$ SEM) following ECS is shown. Values shown are from 4 to 6 determinations, each consisting of pooled RNA from 2 animals. The broken line indicates the basal levels of both mRNA species.

Fig. 4. The effect of a single ECS on the levels of *c-fos* mRNA. An autoradiogram of *c-fos* mRNA levels at various time points is shown. *c-fos* mRNA analysis was performed in the same manner as for ODC using a cDNA fragment as a hybridization probe.

levels of the induced ODC mRNA peaked around the same time as the activity, but remained high for 24 h (Fig. 3).

Upon electrical stimulation, there ensued a rapid and transient elevation in the levels of expression for cerebral *c-fos* mRNA (Figs. 3 and 4). Peak *c-fos* mRNA induction reached about double that of basal levels by 30 min post-ECS, but fell below control values by 2 h. This sequence is consistent with previously reported findings²⁰.

In order to determine whether the induction of ODC was related to neuronal activity or to the altered endocrine state that follows seizures¹, we administered ECS to adrenalectomized rats. ECS increased ODC enzyme activity of both sham and adrenalectomized rats to the same extent. Thus adrenalectomy did not produce

Fig. 5 The modulation of ECS-induced ODC enzyme activity by adrenalectomy and MK-801. Shock was applied to normal, adrenalectomized and sham-operated male rats as described in the methods section. Some animals were injected interperitoneally with MK-801 (1 mg/kg) 15 min prior to the administration of ECS. Animals were sacrificed 5 h later and their neocortical ODC enzyme activity was assayed. Values shown are for the mean $\pm$ S.E.M. from 6 to 8 determinations. * Value differs significantly from basal levels ($P < 0.001$); † Significant difference between ECS only and MK-801-pretreated groups ($P < 0.01$). Student's two-tailed *t*-test was used, after analysis of variance.

any significant effect on the ECS-induced enzyme activity (Fig. 5).

To ascertain the dependence of ODC induction on seizure activity, rats were treated with the anti-convulsant MK-801 prior to the application of ECS. Such treatment, which potently and competitively blocks the NMDA-type of glutamate receptor in the brain³⁰, abolished the tonic-clonic seizures produced by electrical shock. There was approximately a 40% attenuation in the ECS-induced enzyme activity following the administration of this agent (Fig. 5).

DISCUSSION

These data suggest that the previously reported induction of ODC activity in the neocortex in response to ECS^{1,22}, may be partly due to the activation of the ODC gene (Fig. 2). There is good agreement between the induction of ODC enzyme activity in the neocortex and the elevation of the ODC mRNA. Intracerebral injections of actinomycin D prior to ECS, reduced ECS-stimulated increases in enzyme activity (data not presented), further showing that ODC induction is at least partially due to genomic derepression. This derepression is a somewhat selective process because the levels of β -actin mRNA were not altered by ECS treatment.

The ODC mRNA level, followed a different temporal profile to that of the enzyme activity. The mRNA peaked at similar times as the activity did, but remained elevated for over 24 h (Fig. 3). This phenomenon is not unique to ECS-induced ODC, but has been observed in interleukin-2 stimulation of lymphocytic ODC¹⁵. The fall in enzyme activity, while the mRNA remains elevated, suggests that the build-up in polyamine levels, as a consequence of heightened enzyme activity, may produce a feed-back inhibition of the translation of the mRNA. The ability of the polyamines, putrescine and spermidine, to inhibit the translation of the ODC mRNA has been demonstrated in tissue culture systems^{13,24}. Therefore, the polyamines can exert control over ODC activity by regulating the rate of translation of the ODC mRNA.

The proto-oncogene *c-fos* was also stimulated by ECS (Figs. 3 and 4). This *c-fos* induction, recently demonstrated to occur in response to a single electrical shock⁷, was very short lived. In contrast to the ODC mRNA which remained elevated for a longer time, *c-fos* mRNA rapidly fell to below basal levels (Figs. 3 and 4). This major difference in mRNA stability may have some physiological relevance. The persistent ODC mRNA, if open for translation, might enable ODC levels to respond rapidly to subsequent metabolic demands.

Animals given ECS experience a period of behavioral depression which occurs simultaneously with an increase

in cerebral ODC activity. Furthermore, ECS leads to changes in the levels of several humoral factors which can affect ODC activity²⁸. In this study, adrenalectomy had no significant effect on ECS-induced enzyme activity in the neocortex (Fig. 5), indicating that the ODC response to ECS is not modulated by adrenal glucocorticoid action.

The fact that adrenalectomy made no difference to ODC induction, suggests that, the elevation of ODC by ECS may be neuronal-activity dependent, and not due to the altered endocrine state that follows such treatment. Mitigation of the response of ODC levels to ECS by MK-801 pretreatment, implies neuronal mediation of ODC induction, following ECS. MK-801 has been previously reported to block ECS-induced seizures¹⁸.

We have shown that a stimulus modulating the cellular environment, can result in the parallel activation of the nuclear proto-oncogenes and the ODC/polyamine system. The sequence of activation of these two systems, by ECS, is overlapping, thus it is not known if either of them is responsible for the induction of the other. It is more

likely that the interactions between the ODC/polyamine system and the nuclear proto-oncogenes, is manifested at the level of their target sites.

While both systems can be activated by a wide variety of stimuli, the specificity of the cellular response must depend on the differentiated state of the cell. The polyamines may potentiate the effects of the proto-oncogenes, and may also have their own direct effects on genomic events. These two systems in the brain, may then subserve the long-term adaptive responses following intense or repeated neuronal activation. Such responses may underlie the initiation of repair mechanisms, the onset of maladaptive neural changes, or complex events relating to the deposition of memory.

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