Molecular Brain Research, 7 (1990) 243–247
Elsevier

BRESM 70193

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

N.H. Zawia and S.C. Bondy

Departments of Pharmacology, Community and Environmental Medicine and The Southern Occupational Health Center, University of California, Irvine, CA 92717 (U.S.A.)

(Accepted 12 September 1989)

Key words Ornithine decarboxylase; c-*fos*, mRNA; Electroconvulsive shock, Gene expression, Neocortex; Adaptation

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 pathway[23], 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 expression[5]. 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 activation[20]. The nuclear localization of Fos[20], 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 synthesis is dependent on ODC, are cationic in nature[26] 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 localizations[2], 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 molecules[23].

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 events[21].

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 brain[6].

Some animals were dosed by interperitoneal injections of MK-801

Correspondence S.C. Bondy, Dept. of C & E Medicine, University of California, Irvine, CA 92717, U.S.A.

(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 $^{14}CO_2$ 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 mixture[25] Evolved $^{14}CO_2$ 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 inhibitor[19].

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 gels[9] and transferred onto nitrocellulose filter paper The filters were then probed with a ^{32}P-labelled ODC cDNA plasmid[17] (labelled by nick-translation), washed under stringent conditions and exposed to X-ray film at −70 °C for 5–7 days[29] Similarly, RNA obtained from control and treated animals was probed with a β-actin cDNA plasmid under the same conditions The *Eco*RI*Bam*HI 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

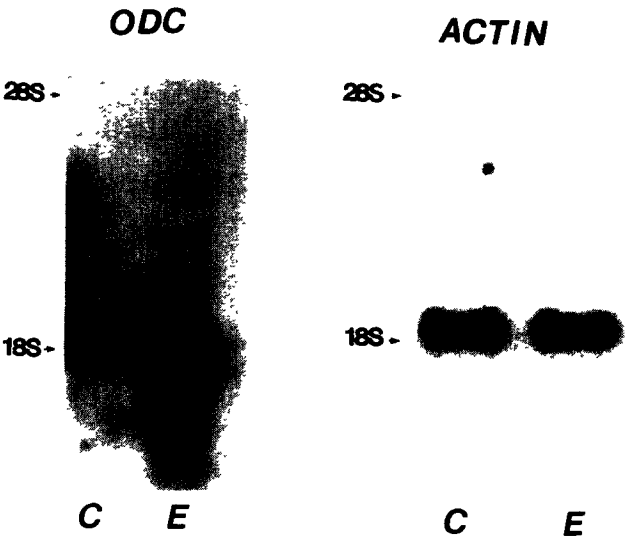

Fig. 2 The effect of a single ECS on ODC mRNA and β-actin mRNA Total RNA was isolated fractionated on agarose gels and transferred onto nitrocellulose filter paper Filters were then probed with ^{32}P-nick-translated cDNAs for both ODC and β-actin The hybridized filters were exposed to X-ray film for 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,

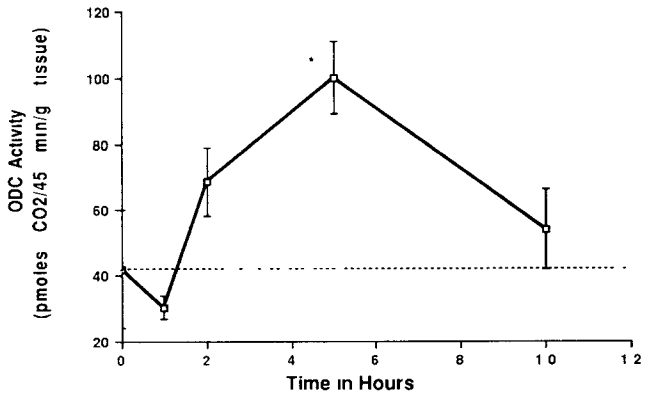

Fig 1 The effect of a single ECS on ODC enzyme activity ODC was determined by the measurement of evolved $^{14}CO_2$ from [^{14}C]ornithine The temporal profile of the mean ODC enzyme activity (±S E M) following ECS from 3 to 4 determinations is shown * Values differ significantly from basal levels ($P < 0\ 05$) using a two-tailed *t*-test, following analysis of variance

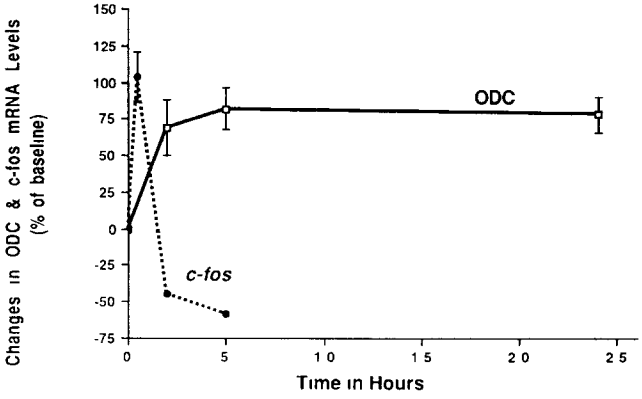

Fig 3 ODC and c-*fos* gene expression in response to electrical stimulation A temporal plot of the mean ODC and c-*fos* mRNA content (±S E M) following ECS is shown Values shown are from 4 to 6 determinations, each consisting of pooled RNA from 2 animals The broken line indicates the basal levels of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

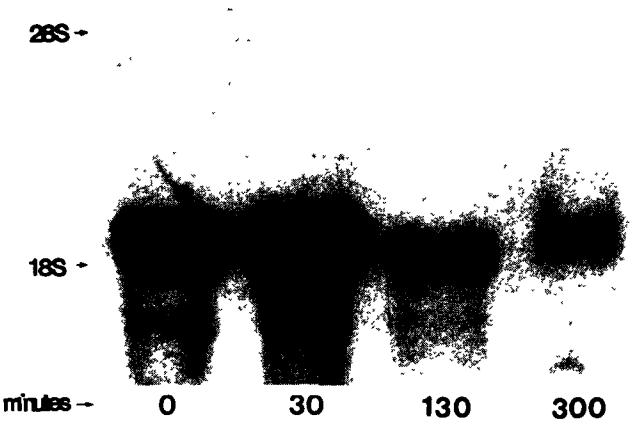

Fig. 4. The effect of a single ECS on the levels of c-*fos* mRNA An autoradiogram of c-*fos* mRNA levels at various time points is shown. c-*fos* mRNA analysis was performed in the same manner as for ODC using a cDNA fragment as a hybridization probe.

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 findings[20].

In order to determine whether the induction of ODC was related to neuronal activity or to the altered endocrine state that follows seizures[1], 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

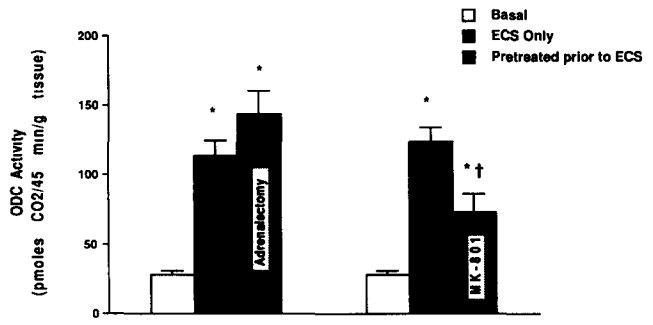

Fig 5 The modulation of ECS-induced ODC enzyme activity by adrenalectomy and MK-801. Shock was applied to normal, adrenalectomized and sham-operated male rats as described in the methods section. Some animals were injected interperitoneally with MK-801 (1 mg/kg) 15 min prior to the administration of ECS Animals were sacrificed 5 h later and their neocortical ODC enzyme activity was assayed. Values shown are for the mean ± 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 brain[30], 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 ODC[15]. 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 shock[7], 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 activity[28]. 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 seizures[18].

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.

Acknowledgements This work was supported by Grant ES04071 from NIH and N H Z is a recipient of an A I D fellowship through the University of Sana'a, Yemen We would like to thank Dr Phillip Coffino for providing us with the ODC cDNA plasmid and Tom Curran and James Morgan for kindly donating the c-*fos* probe The technical assistance of Cara Mattia and Clayton Huntington is greatly appreciated

REFERENCES

1 Bondy, S.C , Mitchell, C , Rahmaan, S and Mason, G , Regional variation in the response of cerebral ornithine decarboxylase to electroconvulsive shock, *Neurochem Pathol* , 7 (1987) 129–141.
2 Cintra, A , Fuxe, K , Agnati, L F , Persson, L , Harfstrand, A , Zoli, M , Eneroth, P and Zini, I , Evidence for the existence of ornithine decarboxylase-immunoreactive neurons in the rat brain, *Neurosci Lett* , 76 (1987) 269–274
3 Chiu, R , William, J B , Meek, J , Smeal, T. and Hunter, T , The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes, *Cell*, 54 (1988) 541–552
4 Cleveland, D W , Lopata, M A , MacDonald, R K , Cowan, N J , Rutter, W J and Kirschner, M W , Number and evolutionary conservation of alpha-actin and β-tubulin and β- and δ-actin genes using specific cloned cDNA probes, *Cell*, 20 (1980) 95–105.
5 Curran, T. and Morgan, J I , Memories of *fos*, *Bioessays*, 7 (1987) 255–258
6 Dam, A.M , Hippocampal neuron loss in epilepsy and after experimental seizures, *Acta Neurol Scand* , 66 (1982) 606–642
7 Daval, J L , Nakajima, T , Gleiter, C H , Post, R M and Marangos, P J , Mouse brain c-fos mRNA distribution following a single electroconvulsvie shock, *J Neurochem* , 52 (1989) 1954–1957
8 Dienel, G A and Cruz, N F., Induction of ornithine decarboxylase during recovery from metabolic, mechanical, thermal or chemical injury, *J. Neurochem* , 42 (1984) 1053–1061
9 Fourney, R , Miyakoshi, J., Day, R and Paterson, M , Northern blotting efficient RNA staining and transfer, *Focus*, 10 (1988) 5–7
10 Franza, B.R , Jr , Rauscher III, F J , Josephs, S F and Curran, T , The Fos complex and Fos-related antigens recognize sequences that contain AP-1 binding sites, *Science*, 239 (1988) 1150–1153
11 Gubits, R M and Fairhurst, J L , *c-fos* mRNA levels are increased by cellular stressors hear shock and sodium arsenite, *Oncogene*, 3 (1988) 163–169.
12 Hollander, M C and Fornace, A J , Jr , Induction of *fos* RNA by DNA-damaging agents, *Cancer Res* , 49 (1989) 1687–1692
13 Holtta, E and Pohjanpelto, P , Control of ornithine decarboxylase in Chinese ovary cells by polyamines, *J Biol Chem* , 261 (1986) 9502–9508
14 Joregensen, M B , Decket, J , Wright, D C and Gehlert, D.R , Delayed c-fos proto-oncogene expression in rat hippocampus induced by transient global cerebral ischemia an in situ hybridization study, *Brain Res* , 484 (1989) 393–398
15 Legraverend, C , Potter, A , Holta, E Alitalo, K and Andersson, L C , Interleukin-2 induces a rapid increase in ornithine decarboxylase mRNA in a cloned murine T lymphocytic cell line, *Exp Cell Res* , 181 (1989) 273–281
16 Maniatis, T , Fritsch, E and Sambrook, J , *Molecular Cloning A Laboratory Manual*, Cold Spring Harbor Lab, New York, 1982
17 McConlogue, L , Gupta, M , Wu, L and Coffino, P , Molecular cloning and expression of the mouse ornithine decarboxylase gene, *Proc Natl Acad. Sci U S A* , 81 (1984) 540–544
18 McNamara, J O , Russel, R D , Rigsbee, L and Bonhaus, D W , Anticonvulsant and antiepileptogenic actions of MK-801 in the kindling and electroshock models, *Neuropharmacology*, 27 (1988) 563–568
19 Metcalf, B W , Bey, P , Danzin, C , Jung, M J , Casara, P and Vevert, J P , Catalytic irreversible inhibition of mammalian ornithine decarboxylase by substrate and product analogues, *J Am Chem Soc* , 100 (1978) 2551–2552
20 Morgan, J I , Cohen, D R , Hempstead, J L and Curran, T , Mapping patterns of c-fos expression in the central nervous system after seizure, *Science*, 237 (1987) 192–196
21 Morgan, J I and Curran, T , Calcium as a modulator of the immediate-early gene cascade in neurons, *Cell Calcium*, 9 (1988) 303–311
22 Pajunen, A , Hietala, O , Virransalo, E and Piha, S , Ornithine decarboxylase and adenosylmethionine decarboxylase in mouse brain-effect of electrical stimulation, *J Neurochem* , 30 (1978) 281–283
23 Pegg, A E , Recent advances in the biochemistry of polyamines in eukaryotes, *Biochem J* , 234 (1986) 249–262
24 Persson, L and Heby, I , Regulation of ornithine decarboxylase mRNA translation by polyamines studies using a cell-free system and a cell line amplified ornithine decarboxylase gene, *J Biol Chem* , 263 (1988) 3528–3533

25 Russell, D.H. and Snyder, S H., Amine synthesis in regenerating rat liver — extremely rapid turnover of ornithine decarboxylase, *Proc Natl Acad. Sci. U.S.A.*, 60 (1969) 1420–1427
26 Russell, D H., Ornithine decarboxylase as a biological and pharmacological tool, *Pharmacology*, 20 (1980) 117–129.
27 Soreq, H , Safran, A. and Eliyahu, D., Modified composition of major ontogenetically regulated mRNAs and proteins in the cerebellum of old and of staggered mice, *Dev. Brain Res* , 10 (1983) 73–82.
28 Thiagarajan, A B , Gleiter, C H., Mefford, I N , Eskay, R.L and Nutt, D J , Effect of a single and repeated electroconvulsive shock on the hypothalamic-pituitary-adrenal axis and plasma catecholamines in rats, *Psychopharmacology*, 97 (1989) 548–552.
29 Thomas, P., Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose, *Proc Natl Acad. Sci U.S.A.*, 77 (1980) 5201–5205.
30 Wong, E.H.F., Kemp, J A , Preistley, T , Knight, A.R , Woodruff, G N. and Iversen, L L., The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist, *Proc Natl Acad. Sci U S A.*, 83 (1986) 7104–7108