Journal of Neurochemistry
Lippincott-Raven Publishers, Philadelphia

Rapid Communication

Electroconvulsive Seizure Increases the Expression of CREM (Cyclic AMP Response Element Modulator) and ICER (Inducible Cyclic AMP Early Repressor) in Rat Brain

Laura Rydelek Fitzgerald, Vidita A. Vaidya, Rosemarie Z. Terwilliger, and Ronald S. Duman

Laboratory of Molecular Psychiatry, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, Connecticut Mental Health Center, New Haven, Connecticut, U.S.A.

Abstract: Rapid expression of ICER (inducible cyclic AMP early repressor), an inducible member of the CREM (cyclic AMP response element modulator) family of transcription factors, has been reported in neuroendocrine tissues and cell lines, but not in brain. In the present study, we demonstrate that acute electroconvulsive seizure (ECS) increases the expression of ICER in several rat brain regions. RNase protection analysis demonstrated that 1–2 h after administration of ECS, levels of mRNA for ICER and a splice variant, ICERγ, were significantly increased in hippocampus, frontal cortex, and cerebellum. It is surprising that ECS also increased levels of mRNA for several CREM isoforms that previous studies have reported were not rapidly inducible. In situ hybridization analysis confirmed these findings and demonstrated that ECS induction of ICER was most obvious in the dentate gyrus granule cell layer of hippocampus and deep layers of cerebral cortex. Induction of ICER and CREM was accompanied by increased expression of two small CRE-binding complexes. Gel supershift analysis with CREM/ICER antisera confirmed that the inducible CRE-binding complexes contain CREM/ICER. Induction of CREM and ICER may contribute to negative feedback regulation of gene transcription that is increased by acute seizure and activation of CREB (cyclic AMP response element-binding protein). **Key Words:** Hippocampus—Cerebellum—Cortex—Cyclic AMP response element-binding protein—Gene transcription—Transcription factor.
J. Neurochem. **66,** 429–432 (1996).

The cyclic AMP response element modulator (CREM) transcription factors act as modulators of cyclic AMP (cAMP)-mediated transcription. The multiexonic CREM gene produces both activators and repressors of transcription that interact with the cAMP response element (CRE) (Foulkes et al., 1993). The isoform CREMτ includes a phosphorylation site and two glutamine-rich domains. Similar to cAMP response element-binding protein (CREB), CREMτ can be phosphorylated to render it a transcriptional activator. The CREM repressors, CREMα, β, and γ, contain a phosphorylation site but lack the two glutamine-rich domains. Thus, these products act primarily as inhibitors of cAMP-induced transcription.

Inducible cAMP early repressor (ICER), a recently described member of the CREM family (Stehle et al., 1993), is regulated by an alternative intronic promoter that contains several cAMP autoregulatory elements (CAREs) (Molina et al., 1993). Increased cAMP production is reported to increase ICER expression in cells of neuroendocrine origin (Molina et al., 1993; Stehle et al., 1993). Unlike the other CREM isoforms, ICER does not contain a kinase-inducible domain. Thus, ICER is believed to be regulated by changes in its expression level.

Expression of CREM and ICER has been characterized in neuroendocrine tissues and cell lines, but not in brain. The present study examined the influence of acute electroconvulsive seizure (ECS) on expression of CREM and ICER levels. Acute ECS has been shown to increase the levels of other transcription factors in the leucine zipper family (Sonnenberg et al., 1989; Winston et al., 1990). We have found that acute ECS increases the levels of CREM and ICER mRNA in hippocampus, frontal cortex, and cerebellum. The changes in mRNA levels were accompanied by an increase in the expression of two CRE-binding complexes containing CREM/ICER.

MATERIALS AND METHODS

Animals and treatment paradigms

Male Sprague-Dawley rats (150–200 g) (CAMM, Wayne, NJ, U.S.A.) were group housed and maintained on a 12-h light/dark cycle with food and water freely available. Rats were administered ECS via ear-clip electrodes (50 mA, 0.3 s) or received sham treatment (handled identically as those

Resubmitted manuscript received October 4, 1995; accepted October 6, 1995.

Address correspondence and reprint requests to Dr. R. S. Duman at Connecticut Mental Health Center, 34 Park St., New Haven, CT 06508, U.S.A.

Abbreviations used: cAMP, cyclic AMP; CARE, cAMP autoregulatory element; CRE, cAMP response element; CREB, CRE-binding protein; CREM, CRE modulator; DTT, dithiothreitol; ECS, electroconvulsive seizure; ICER, inducible cAMP early repressor; SSC, standard saline citrate.

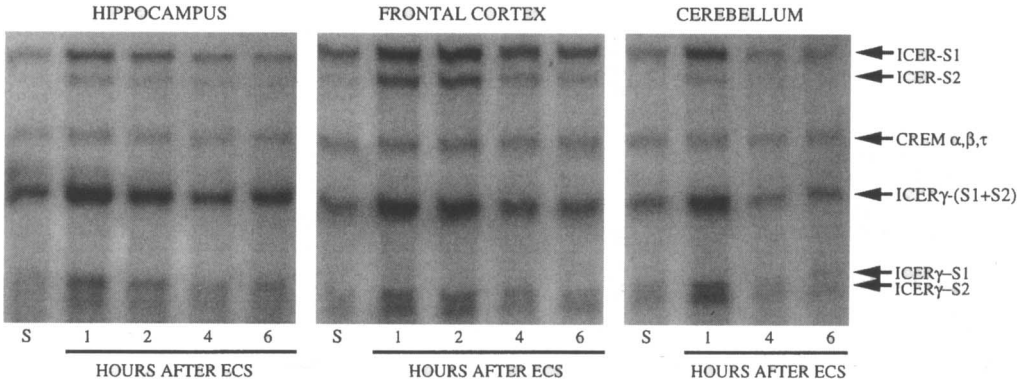

FIG. 1. Acute ECS increases levels of CREM and ICER mRNA in hippocampus, frontal cortex, and cerebellum. Rats were administered ECS or sham treatment and killed at different times after ECS, as indicated. Total RNA was isolated from sections of hippocampus, frontal cortex, or cerebellum and levels of CREM and ICER mRNA were determined by RNase protection analysis. A representative autoradiogram is shown. Similar results were observed in three separate animals.

that received ECS, but without electrical stimulation). Animals were killed at different times after ECS, as indicated. All animal use procedures were in strict accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Yale Animal Care Committee.

Riboprobe synthesis and RNase protection analysis

A 229-bp riboprobe template for CREM (−56 to 173) was generated as described previously (Fitzgerald and Duman, 1994). The plasmid was linearized by *Sep*I digestion and T7 RNA polymerase was used to synthesize antisense riboprobe. RNase protection analysis was performed as described previously (Hosoda et al., 1994). In brief, 20 μg of total RNA was incubated with ^{32}P-labeled riboprobe (10^5 cpm/sample) at 63°C for 16–20 h in hybridization buffer [80% formamide, 40 m*M* piperazine-*N,N'*-bis(2-ethanesulfonic acid), pH 6.4, 0.4 *M* NaCl, 1 m*M* EDTA]. The samples were treated with RNase A and T1. RNase-resistant hybrids were fractionated on a 7% polyacrylamide/8 *M* urea denaturing gel.

In situ hybridization analysis

Coronal sections of frozen brain (16 μm) were cut on a cryostat, mounted on RNase-free Probon slides (Fisher), and stored at −80°C. The labeled CREM/ICER riboprobe was hybridized with brain sections (10^6 cpm/section) for 18 h at 55°C in hybridization buffer [50% formamide, 0.6 *M* NaCl, 10 m*M* Tris, 1× Denhardt's solution, 2 m*M* EDTA, 10 m*M* dithiothreitol (DTT), 10% dextran sulfate, 50 μg/ml salmon sperm DNA, and 250 mg/ml tRNA]. Sections were washed in 2× standard saline citrate (SSC) at 25°C and then treated with 20 μg/ml RNase A for 45 min in 0.5 *M* NaCl, 10 m*M* Tris, and 1 m*M* EDTA. Sections were then washed twice for 30 min in 0.2× SSC at 55°C. The sections were dried and exposed to Hyperfilm (Amersham).

Gel mobility shift assays

Gel mobility shift assays were performed as described previously (Widnell et al., 1994). In brief, brain regions were dissected and homogenized in 20 m*M* HEPES, pH 7.9, 0.4 *M* NaCl, 5 m*M* $MgCl_2$, 0.5 m*M* EDTA, 0.1 m*M* EGTA, 5 m*M* DTT, 1 m*M* phenylmethylsulfonyl fluoride, 0.1 m*M* *p*-aminobenzamidine, 10 μg/ml leupeptin, 1 μg/ml pepstatin, 20% glycerol, and 1% Nonidet P-40 with a dounce homogenizer (12 strokes). Samples were incubated on ice for 20 min and centrifuged at 15,000 *g* for 20 min at 4°C. Supernatant protein was measured by Bradford analysis (Bio-Rad). CRE probe was derived from the sequence of the somatostatin promoter (Andrisiani et al., 1989). The probe was labeled with [α-^{32}P]dTTP and [α-^{32}P]dGTP using Klenow DNA polymerase. Crude nuclear extracts from cerebellum (20 μg), hippocampus (25 μg), or frontal cortex (25 μg) were incubated at room temperature for 20 min with 1 μg poly(dI-dC)·poly(dI-dC), 40 μg bovine serum albumin, 10 m*M* Tris-HCl, pH 7.9, 10 m*M* KCl, 1 m*M* EDTA, 4% glycerol, and 1 ng ^{32}P-labeled CRE probe. Samples were electrophoresed at 150 V for 4 h in a nondenaturing 6% acrylamide/0.14% *N,N'*-methylenebisacrylamide gel containing 25 m*M* Tris-borate buffer, pH 8.5, 1 m*M* EDTA, and 1.6% glycerol. Gels were dried and exposed to film. For supershift experiments, CREM1 antibody (Santa Cruz) or CREB antibody (UBI) was incubated with sample and gel mobility shift components for 18 h at 4°C before addition of the labeled probe.

RESULTS AND DISCUSSION

Previous studies have shown that CREM and ICER are regulated in a variety of cells of neuroendocrine origin (Foulkes et al., 1993; Molina et al., 1993; Stehle et al., 1993). We have recently reported that ICER is regulated in the C6 glioma cell by adrenergic stimulation, suggesting that the CNS may be another important area in which to investigate the regulation of the CREM gene (Fitzgerald and Duman, 1994). The present study demonstrates that acute ECS increases the expression of CREM and ICER mRNA in several brain regions. CREM and ICER mRNA levels were measured by RNase protection assays using a 229-bp riboprobe. This probe hybridizes with all the CREM and ICER isoforms, resulting in different lengths of RNase-resistant hybrids that can be distinguished by gel electrophoresis (see L. R. Fitzgerald et al., submitted). Acute ECS increased the expression of CREM and ICER mRNA levels in cerebellum, hippocampus, and frontal cortex (Fig. 1). Maximal induction of the CREM isoforms occurred at 1 h after ECS treatment.

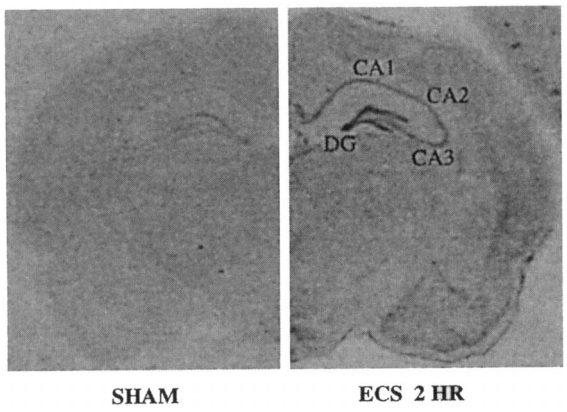

FIG. 2. Acute ECS increases levels of CREM/ICER mRNA determined by in situ hybridization. Rats were administered ECS (50 mA, 0.3 s) or sham treatment and killed 2 h later. CREM/ICER mRNA was analyzed by in situ hybridization. Dentate gyrus (DG), CA1, CA2, and CA3 regions are indicated. A representative autoradiogram is shown. Similar results were observed in three separate animals.

Transcription of ICER mRNA from an alternative start site, termed S2 (Molina et al., 1993), was more prevalent in frontal cortex. In situ hybridization was performed to determine more specifically the localization of these effects. CREM and ICER mRNA levels were increased 2 h after ECS in the dentate gyrus granule cell layer and CA1, CA2, and CA3 pyramidal cell layers of hippocampus (Fig. 2). The deep layers of cerebral cortex also showed a significant increase in CREM and ICER mRNA levels.

Differential regulation of the various CREM isoforms could provide a mechanism for controlling the expression of CREM. The CREM gene contains two promoters that allow for differential regulation of the CREM transcripts (Molina et al., 1993). The P1 promoter, which controls the transcription of CREMα, β, γ, and τ mRNA, does not contain any known consensus sequences and is believed to be constitutively regulated. These CREMs contain kinase-inducible domains that may be phosphorylated to regulate their ability to repress transcription (Laoide et al., 1993; Loriaux et al., 1994). The ICER isoforms are under the control of the intronic P2 promoter, which contains four CAREs (cAMP autoregulatory elements), making it highly responsive to cAMP stimulation. However, they do not contain the kinase-inducible domains that would make them sensitive to phosphorylation. Although the many isoforms are regulated in different manners, they all interact specifically with the CRE and all, except CREMτ, have been found to repress transcription. Although studies to date have only demonstrated regulation of ICER gene transcription, it is possible that increased mRNA stability also contributes to regulation of ICER, as well as CREM, mRNA levels in brain.

To determine if CREM and ICER mRNA induction was associated with induction of their corresponding proteins that bind to the CRE site, gel mobility shift analysis was performed. As shown in Fig. 3, assays with a CRE probe result in several CRE/protein complexes. The major band in each region represents CREB-binding activity; this has been confirmed by supershift analysis with a CREB-specific antibody (not shown) and is consistent with previous reports on CREB binding in brain (Konradi et al., 1994; Widnell et al., 1994). Levels of CREB CRE binding were not induced by acute ECS treatments. In fact, the CREB band tended to be decreased 4 h after ECS; this could result from dimerization of CREB with newly synthesized ICER or CREM. Two protein complexes were induced in cerebellar samples 4 h after ECS treatment. The higher molecular weight protein complex was also induced in hippocampus and frontal cortex at the 4 h time point, although the degree of induction was lower than that observed in cerebellum. Lower levels of the induced band in frontal cortex and hippocampus are surprising because the induction of ICER mRNA in these brain regions was equal to, or greater than, that observed in cerebellum (Fig. 1). This discrepancy could be due to different time courses for ECS induction of ICER in the three brain regions (e.g., levels of ICER mRNA may have already peaked in cerebellum). Alternatively, the translation efficiency of ICER may be higher in cerebellum than in the other two brain regions.

To study the composition of the induced CRE/protein complexes, gel mobility supershift experiments were per-

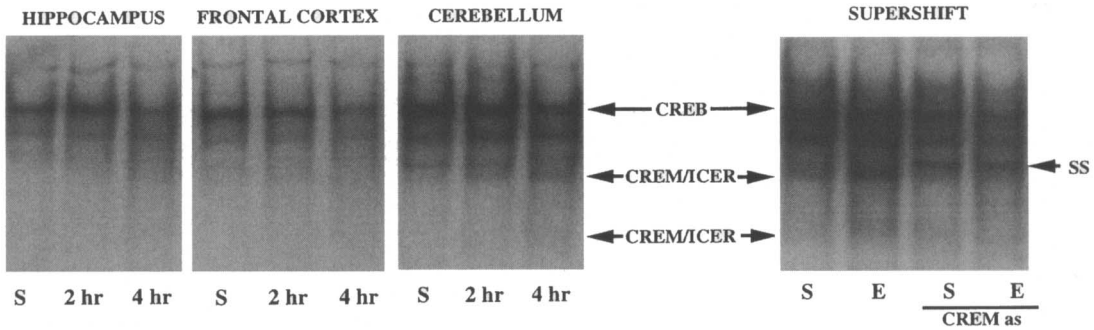

FIG. 3. Acute ECS induces CRE-binding complexes that contain ICER/CREM. Rats were administered ECS or sham treatment and killed 2 or 4 h after ECS, as indicated. CRE binding was measured by gel mobility shift analysis using crude nuclear extracts prepared from hippocampus, frontal cortex, or cerebellum. The locations of the CREM/ICER complexes induced by ECS treatment, as well as CREB, are indicated. For supershift analysis, nuclear extracts from cerebellum (4 h after ECS) were preincubated with antisera against CREM. CREM antisera (as) abolishes the complexes induced after ECS treatment and produces a slower migrating supershifted complex (SS). Representative autoradiograms are shown. Similar results were obtained in three separate animals for gel shift and two separate animals for the supershift experiments.

formed with a CREM polyclonal antibody that detects numerous CREM-immunoreactive proteins. The CREM antibody disrupted the CRE-binding activity of the two ECS-induced bands and resulted in the formation of a new supershifted band that migrated just above the larger of the two induced bands (Fig. 3). The other CRE/protein complexes were not affected. These data indicate that the complexes induced after ECS contain a CREM isoform. Due to the ability of this antibody to interact with several CREM isoforms, the supershift does not identify which isoforms are present. Supershift analysis with CREB antibody also disrupted the induced bands, as well as the CREB-binding band, suggesting that the induced bands are heterodimers that contain CREB and ICER or CREM (not shown).

Acute seizures are reported to increase the expression of many different types of proteins including neuropeptides, neurotrophins, and immediate early gene transcription factions (Sonnenberg et al., 1989; Winston et al., 1990; Nibuya et al., 1995). Induction of CREM and ICER may contribute to feedback inhibition of the transcriptional activation of these genes. Indeed, the time course for induction of CREM and ICER is consistent with the time course for inactivation of many of these genes; i.e., transcriptional activity is rapidly induced and then returns to basal by 4–6 h later. Thus, the regulation of CREM isoforms may represent a novel and unexplored mechanism in the CNS whereby cAMP-mediated signals can be modified or turned off at the level of gene expression.

Acknowledgment: This study was supported by USPHS grants MH 45481, MH51399, and 2 PO1 MH25642 and by a Veterans Administration National Center Grant for PTSD, VA Hospital in West Haven, Connecticut.

REFERENCES

Andrisiani O. M., Zhu Z. N., Pot D. A., and Dixon J. E. (1989) In vitro transcription directed from the somatostatin promoter is dependent upon a purified 43 kDa DNA-binding protein. *Proc. Natl. Acad. Sci. USA* **86,** 2181–2185.

Fitzgerald L. R. and Duman R. S. (1994) Adrenergic regulation of β-adrenergic receptor and ICER (inducible cAMP early repressor) mRNA levels in C6 glioma cells. *Soc. Neurosci. Abstr.* **20,** 931.

Foulkes N. S., Schlotter F., Pevet P., and Sassone-Corsi P. (1993) Pituitary hormone FSH directs the CREM functional switch during spermatogenesis. *Nature* **362,** 264–267.

Hosoda K. Feussner G. K., Rydelek-Fitzgerald L., Fishman P. H., and Duman R. S. (1994) Agonist and cyclic AMP–mediated regulation of β_1-adrenergic receptor mRNA and gene transcription in rat C6 glioma cells. *J. Neurochem.* **63,** 1635–1645.

Konradi C., Cole R. L., Heckers S., and Hyman S. E. (1994) Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *J. Neurosci.* **14,** 5623–5634.

Laoide B. M., Foulkes N. S., Schlotter F., and Sassone-Corsi P. (1993) The functional versatility of CREM is determined by its modular structure. *EMBO J.* **12,** 1179–1191.

Loriaux M. M., Brennan R. G., and Goodman R. H. (1994) Modulatory function of CREB-CREMα heterodimers depends upon CREMα phosphorylation. *J. Biol. Chem.* **269,** 28839–28843.

Molina C. A., Foulkes N. S., Lalli E., and Sassone-Corsi P. (1993) Inducibility and negative autoregulation of CREM: an alternative promoter directs the expression of ICER, an early response repressor. *Cell* **75,** 875–886.

Nibuya M., Morinobu S., and Duman R. S. (1995) Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J. Neurosci.* **15,** 7534–7547.

Sonnenberg J. L., Macgregor-Leon P. F., Curran T., and Morgan J. I. (1989) Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. *Neuron* **3,** 359–365.

Stehle J. H., Foulkes N. S. Molina C. A., Simmonneaus V., Pevet P., and Sassone-Corsi P. (1993) Adrenergic signals direct rhythmic expression of transcriptional repressor CREM in the pineal gland. *Nature* **365,** 314–320.

Widnell K. L., Russell D. S., and Nestler E. J. (1994) Regulation of expression of cyclic AMP response element-binding protein in the locus coeruleus in vivo and in a locus coeruleus-like cell line in vitro. *Proc. Natl. Acad. Sci. USA* **91,** 10947–10951.

Winston S. M., Hayward M. D., Nestler E. J., and Duman R. S. (1990) Chronic electroconvulsive seizures down-regulate expression of the immediate-early genes c-*fos* and c-*jun* in rat cerebral cortex. *J. Neurochem.* **54,** 1920–1925.