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Short- and Long-Term Alterations of Gene Expression in Limbic Structures by Repeated Electroconvulsive-Induced Seizures

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Abstract: Rats were submitted to a series of 10 daily electroconvulsive shocks (ECS). A first group of animals was killed 1 day after the last seizure and a second group 30 days later. Tyrosine hydroxylase (TH) activity was measured using an in vitro assay in the nucleus caudatus, anterior cortex, amygdala, substantia nigra, ventral tegmental area, and locus ceruleus. The mRNA corresponding to this enzyme (TH-mRNA) was evaluated using a cDNA probe at the cellular level in the ventral tegmental area, substantia nigra, and locus ceruleus. Met-enkephalin (MET)-immunoreactivity and the mRNA coding for the preproenkephalin (PPE-mRNA) were assayed in striatum and the central nucleus of the amygdala. The day after the last ECS an increase of TH activity was observed in the ventral tegmental area, locus ceruleus, and substantia nigra in parallel with a similar increase in the amygdala and striatum; in the anterior cortex TH activity remained unchanged. TH-mRNA was increased in the locus ceruleus, evidencing the presence in this structure of a genomic activation. The amounts of MET and PPE-mRNA were unaffected in the striatum but increased in the amygdala. Thirty days after the last ECS we observed a decrease of TH activity in the amygdala and of TH-mRNA amount in the ventral tegmental area. In the locus ceruleus TH-mRNA remained higher in treated animals than in controls whereas TH activity returned to control levels. These results demonstrate that a series of ECS induces an initial increase of the activity of mesoamygdaloid catecholaminergic neurons followed by a sustained decrease through alterations of TH gene expression which could mediate the clinical effect of the treatment. **Key Words:** Electroconvulsive shocks—Limbic structures—Tyrosine hydroxylase mRNA—Preproenkephalin mRNA—Gene expression. **Leviel V. et al.** Short- and long-term alterations of gene expression in limbic structures by repeated electroconvulsive-induced seizures. *J. Neurochem.* **54,** 899–904 (1990).

Sysmotherapy has long been used as a treatment for human depression. Although it is used less frequently as a result of the development of antidepressive drugs, this therapy remains essential for some types of depression and presents the advantage that it is free of side effects. The biochemical modifications induced by this treatment could explain the mechanism of the depressive illness and numerous studies have been devoted to the consequences of repeated electroconvulsive shock (ECS) (Musacchio et al., 1969; Green et al., 1978; Hong et al., 1979; Bergstrom and Kellar, 1979). These observations generally have concerned the short-term effects of ECS (from minutes to hours) in spite of the fact that the clinical effects of electroconvulsive therapy are long lasting. Indeed it is several days before improvement of mood occurs after a series of 7–10 ECS and it may last for several weeks (Crow and Johnstone, 1986). As proposed earlier, such a duration most probably involves transcriptional modifications (Cupello et al., 1980; Yamagami et al., 1987). In this article we have considered this hypothesis in evaluating changes in mRNA encoding for tyrosine hydroxylase (TH-mRNA) and preproenkephalin (PPE-mRNA) 1 and 30 days after a series of 10 ECS. Indeed the activity of TH (a key enzyme in the synthesis of catecholamines) is greatly modified by ECS treatment as is the level of Met-enkephalin (MET) (Masserano et al., 1981; Green et al., 1978). Data presented here are consistent with

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Abbreviations used: AC, anterior cortex; AMY, central nucleus of the amygdala; DA, dopamine; DOPA, 3,4-dihydroxyphenylalanine; ECS, electroconvulsive shock(s); LC, locus ceruleus; MET, Met-enkephalin; NC, nucleus caudatus; PPE, preproenkephalin; SN, substantia nigra; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

the hypothesis of transcriptional modifications because after a series of 10 ECS we observed variations of TH activity and MET amount correlated with modifications of the corresponding mRNA. In addition, these results underline that the mesoamygdaloid system is preferentially affected after ECS treatment.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–280 g were used. Each test animal was housed with its control in a cage with free access to food and water in a light (12-h light/dark cycle)- and temperature (22°C)-controlled room.

ECS and dissection procedure

Rats were shocked through earclip electrodes by application of a 50-mA sinusoidal current for a duration of 2 s. All shocked rats experienced generalized tonic–clonic seizures that lasted approximately 30 s. Shocks were administered once daily for 10 consecutive days. Either 24 hours or 30 days after the last shock, rats were killed by decapitation. The brains were removed, frozen on dry ice, and stored at −80°C until dissection. Locus ceruleus (LC), ventral tegmental area (VTA), substantia nigra (SN), nucleus caudatus (NC), central nucleus of the amygdala (AMY), and anterior cortex (AC) were punched out of frontal sections (500 μm thick brain slices, made with a freezing microtome Leitz). The inside diameter of the micropunch instrument was 1.4 mm. The punches, right and left side pooled together (0.5–0.7 mg), were kept frozen and stored at −80°C until assayed.

Biochemical assays

Each sample was homogenized in 60 μl of 5 m*M* potassium phosphate buffer (pH 6.0), containing 0.2% Triton X-100. Aliquots of 40 μl were used for RNA quantification, 5 μl for TH activity, and MET was evaluated on 10-μl samples. Proteins were analyzed in all the samples (5 μl) by the method of Lowry et al. (1951) with bovin serum albumin used as the standard.

RNA preparation and assays

RNA was extracted from tissue homogenates as described by Faucon Biguet et al. (1986). Total RNA was quantified by densitometric scanning of agarose gels stained with ethidium bromide (1 μg/ml), fractionated by electrophoresis in denaturating conditions (formamide 50%, formaldehyde 6%) on agarose gel (Northern blotting), and transferred to a nitrocellulose filter. Treated and control samples were run on the same gel.

TH cDNA probe. A 1.5-kb *Pst*I fragment of PTH-51 cDNA clone complementary to TH mRNA from a PC12 rat pheochromocytoma cell line was used (Grima et al., 1985). The probe was ^{32}P labelled with a specific activity of 2×10^8 cpm/μg using the BRL nick-translation kit system.

PPE cDNA probe. The pRPE2 clone (Comb et al., 1982) containing a 1,281-bp insert that corresponded to the entire coding and 3′-untranslated region as well as most of the 5′-untranslated region of rat PPE was kindly furnished by S. L. Sabol. A 513-bp *Pst*I fragment was isolated, nick-translated (^{32}P with a sp act of 2×10^8 cpm/μg) using the BRL nick-translation kit system and used for hybridization.

Hybridization. The TH cDNA probe was hybridized predominantly to a RNA species that exhibits a mobility of 1.9 kb (1.3 kb for PPE cDNA). Hybridization and washing conditions were similar to those previously described by Faucon-Biguet et al. (1986).

Specific mRNAs were quantified by densitometric scanning of the autoradiograms and related to the corresponding value for total RNA. Comparison between structures was made by giving an arbitrary value of 100% to the control rats.

TH activity

TH activity was assayed by measuring the amount of exogenous tyrosine converted to 3,4-dihydroxyphenylalanine (DOPA) using HPLC and electrochemical detection (Buda et al., 1975; Nagatsu et al., 1979). Five microliters of the phosphate buffer homogenate was extended to 55 μl with the same buffer and centrifuged at 15,000 *g* for 15 min. Fifty microliters of the supernatant were transferred to 5-ml glass tubes, and the reaction was initiated by addition of 50 μl of a solution containing 10 μl of 1 *M* phosphate buffer (pH 5.5), 5 μl of 10 m*M* NADPH (Boerhinger), 10 μl of 10 m*M* 6-methyl-5,6,7,8-tetrahydropterine (6-MPH_4, Sigma), freshly prepared solution in 5 m*M* HCl, 10 μl (2,600 units) of catalase (Boerhinger), 5 μl of 20 m*M* $FeSO_4 \cdot 7H_2O$ (Merck), and 10 μl of 0.4 m*M* L-tyrosine (Sigma). Blanks were prepared by omitting tissue.

The incubation was carried out at 37°C for 15 min in air, then stopped by addition of 50 μl 1 *M* $HClO_4$ containing 5 m*M* sodium metabisulfite and 100 m*M* EDTA-Na_2. α-Methyl-DOPA (10 ng) was added to each sample as an internal standard. After centrifugation (15 min, 3,000 *g*), the supernatant was collected and its pH adjusted to 8.6 with 50 μl of a 3 *M* Tris-acetate buffer (pH 8.7). This supernatant was poured onto a column containing 50 mg of alumina (acid wash purified, Baker) equilibrated in pH with 0.1 *M* Tris-acetate buffer (pH 8.6). The columns were first washed with 150 μl of a 0.1 *M* Tris buffer, then with 150 μl of distilled water and finally the catechols were eluted with 150 μl of 0.3 *M* $HClO_4$ containing 5 m*M* sodium metabisulfite. Recovery from alumina was 68–82%.

To determine DOPA quantities, 50 or 100 μl of the eluate was injected onto the HPLC column maintained at 30°C (reverse-phase 5 μm C18 Nucleosil, 4.6 × 250 mm, SFCC). The mobile phase was composed of 100 m*M* potassium monophosphate, 0.15 m*M* EDTA-Na_2 adjusted to pH 3.2 and pumped at a flow rate of 1 ml/min. The working glassy carbon electrode of the electrochemical detector (Metrohm) was maintained at a potential of 0.8 V versus Ag/AgCl reference electrode, and the sensitivity of the detector was 1 nA full scale. The amounts of compounds analyzed were calculated by measuring peak areas and comparing them directly with the external standard freshly prepared. The quantities of DOPA analyzed for each sample were corrected by the internal standard.

MET assay

An enzymatic digestion was used prior to the assay to liberate MET from longer precursor peptides which show little or no cross-reactivity within the antibodies. Samples were digested with trypsin (10 μg/ml) for 16 h at 37°C followed by carboxypeptidase B (0.1 μg/ml) for 1 h at 37°C. The digestion buffer was 50 m*M* Tris-HCl, pH 8.4, containing 2 m*M* $CaCl_2$.

The MET assay was performed as described by Patey et al. (1985) using antiserum 2158 at a final dilution of 1:60,000. This assay had a sensitivity of <1 fmol and a IC_{50} of 28 fmol for each 100-μl sample.

RESULTS

Alterations of TH activity and TH-mRNA by ECS (Fig. 1)

A series of 10 ECS promotes important modifications of both TH activity and the amount of TH-mRNA. Markedly enhanced in the LC (192%) and VTA (172%), TH activity was also increased in AMY (142%), NC (131%), and SN (132%). The AC was the only region where no modifications of TH activity could be detected. In the animals killed 1 month after the last ECS, in all the regions studied TH activity did not differ from controls except in VTA and AMY in which the enzymatic activity was respectively lowered to 54% and 69% of the control animals.

The amount of TH-mRNA was evaluated in SN, VTA, and LC. In the animals killed the day after the last ECS, TH-mRNA amount was increased only in LC (387%). In the animals killed 1 month after the treatment the value in LC remained higher than in controls (186%) but was lower in VTA (55%) and unchanged in SN. For SN, the amount of TH-mRNA was affected neither 24 h nor 30 days after the series of ECS.

As presented in Fig. 2, a correlation between TH activity and TH-mRNA amount appears in LC the day after the last ECS. In addition, a lowering of TH activity can be correlated with a similar decrease of TH-mRNA amount in VTA in animals killed 1 month after the treatment.

Modifications of MET and PPE-mRNA (Fig. 3)

MET and the mRNA encoding the precursor of MET were evaluated in AMY and NC 1 day and 1 month after the cessation of daily ECS treatment. The day following the last ECS, a large increase of MET in AMY was observed (3.06 ± 0.41 versus 1.37 ± 0.15 fmol/mg) and correlated with an increase of PPE-mRNA quantities (313%). Simultaneously, a slight increase of MET (1.02 ± 0.14 versus 0.803 ± 0.15 fmol/mg) and PPE-mRNA (24%) was detected in the striatum. There were no significant differences between control and treated animals 30 days after the last ECS in either AMY or NC.

DISCUSSION

The present investigation was undertaken to evaluate the hypothesis of a sustained modification by ECS of transcriptional activity. Indeed, ECS treatment promotes long-lasting simultaneous modifications of TH activity and TH-mRNA levels. An increase of the MET level was also accompanied by an elevated level of PPE-mRNA. The modifications of transcriptional events thus appears to be a real mechanism of regulation. However, TH activity and MET amount are not necessarily linked, showing that the consequences of ECS do not concern only gene expression.

The present results are in accordance with the previous hypothesis that ascending catecholaminergic pathways are activated by ECS treatment. Indeed, it has already been reported that norepinephrine turnover (Schildkraut et al., 1971; Hendley and Welch, 1975), the number of receptors (Bergstrom and Kellar, 1979; Pandey et al., 1979; Biegon and Israeli, 1986; Blendy et al., 1988), and the activity of TH (Masserano et al., 1981) are increased as a short-term effect of a series of ECS. In the LC, the increase of the TH activity can be correlated to an increased amount of TH-mRNA, suggesting an activated TH gene transcription. In the noradrenergic cells of the LC, induction of the TH enzyme has previously been reported following reserpine treat-

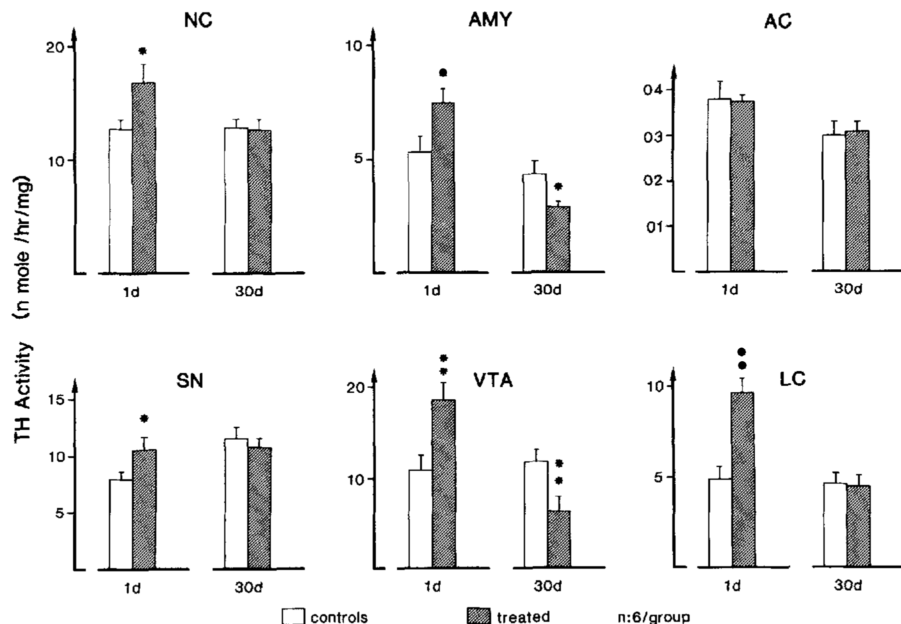

FIG. 1. Effects of repeated ECS on TH activity. TH activity was measured in NC, AMY, AC, SN, VTA, and LC 1 day (1 d) or 30 days (30 d) after the last of a series of 10 daily ECS. Each group of treated animals was associated with a control group of the same age housed together. Controls (□) and treated groups (▨) were compared using Student's t test with the following confidence: $^{*}p < 0.05$; $^{**}p < 0.01$.

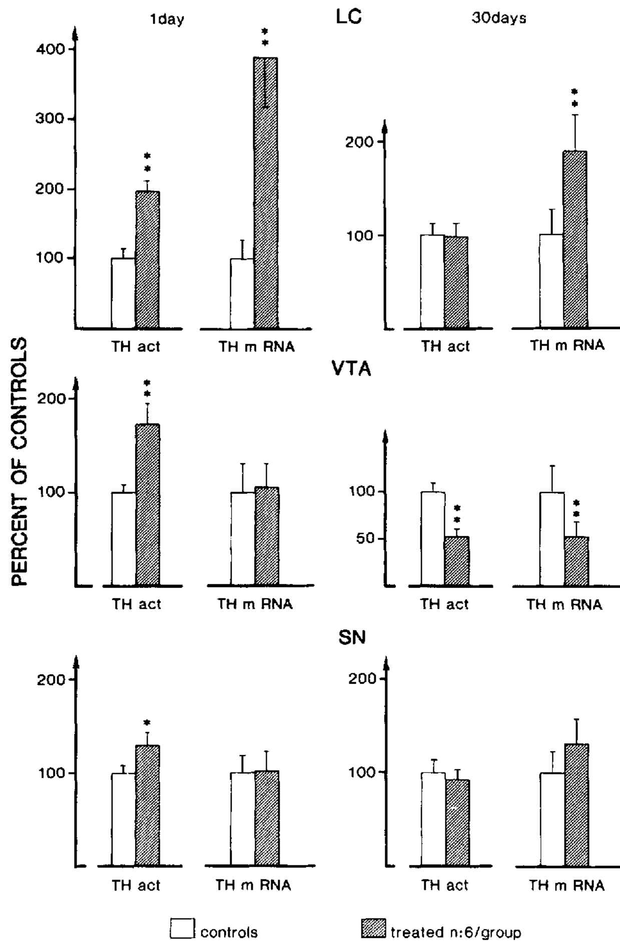

FIG. 2. Correlation between TH activity and TH-mRNA. Values are standardized as a percent with an arbitrary value of 100% for controls. The same animals presented in Fig. 1 were used. TH-mRNA, assayed in LC, VTA, and SN are compared with TH activity measured in vitro in the same structure. The same statistical analyses as in Fig. 1 were realized.

ment (Tank et al., 1985; Faucon Biguet et al., 1986) or stressful environmental conditions (Tank et al., 1985; Richard et al., 1988) and simultaneously with an increased TH-mRNA level. An estrogen-induced increase of TH-mRNA in hypothalamic cells was also recently observed by Blum et al. (1987) and in that instance it was clearly established that increased TH activity was a consequence of an increased gene transcription. These treatments, like ECS, remain, however, ineffective in the SN and VTA, evidence that a sustained activation of catecholaminergic neuronal pathway does not necessarily elevate the level of gene transcription. However, in the present study, in the absence of a direct measurement of the transcription, it cannot be excluded that an increased "turnover" of the TH-mRNA occurs without modification of the level of TH-mRNA.

Concerning the MET neurotransmission, in the present study we confirmed the observation of Yoshikawa et al. (1985) that MET is not induced by ECS in striatum. Studies have shown that the expression of PPE-mRNA is inhibited by the action of dopaminergic afference to caudate nucleus (Blanc et al., 1985; Scott Young et al., 1986; Normand et al., 1988; Vernier et al., 1988), and as noted above, the nigrostriatal DA pathway is slightly activated by ECS treatment. The absence of induction of the MET could be the consequence of the increased TH activity in the same structure. In contrast, we observed a simultaneous increase of TH activity in AMY and an enhancement of PPE expression. This fact appears paradoxical, but we recently reported an absence of short-term control of MET release by monoaminergic afferents in AMY (Fayada et al., 1987). In fact, local application of amphetamine which enhances dopamine (DA) release or haloperidol (a DA receptor blocker) was shown to be ineffective in modifying MET release measured in vivo in the central nucleus of the AMY. These observations and the present results underscore that DA in AMY does not simply inhibit MET cells as is the case in striatum. Furthermore, the increased PPE-mRNA in AMY could be the consequence of an induction of the peptide in this structure. Initial reports had already shown that repeated ECS promotes, on the one hand,

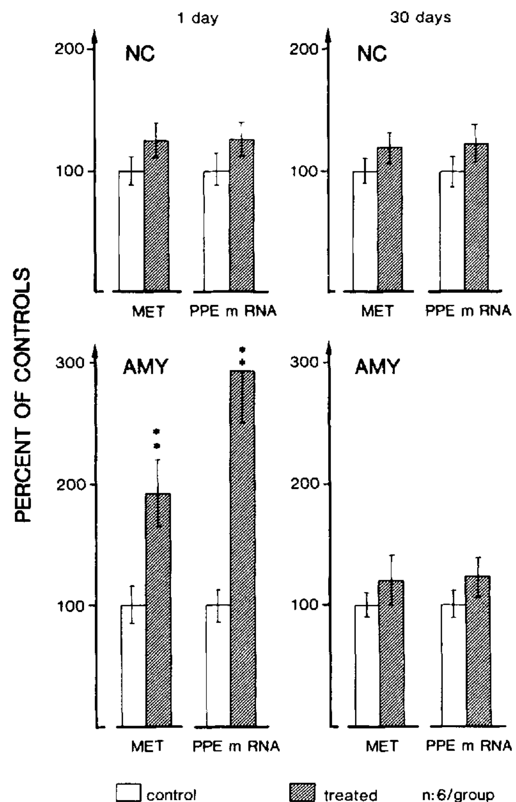

FIG. 3. Effects of repeated ECS on MET-like immunoreactivity and PPE-mRNA. For comparison purposes the values were standardized to 100% for control groups. The assays were realized in NC and AMY 1 and 30 days after the last of series of 10 daily ECS as described in the text. The statistical analysis was the same as in Fig. 1.

the release of endogenous opioids (Green et al., 1978; Hong et al., 1979) and on the other, a decrease of the corresponding binding sites (Nakata et al., 1985). Our results complement these observations, suggesting an increased transcription of the PPE gene.

The long-term consequences of ECS treatment were rather surprising. Indeed, 1 month after the last ECS the TH activity was reduced in AMY and VTA, contrasting with the initial increase. This secondary decrease of TH activity, well correlated with a decrease of TH-mRNA amount, suggests that TH activity is regulated over the long term through an alteration of TH gene transcription. As an explanation, a negative feedback mechanism could be engaged by the initial hyperactivity of the monoaminergic system and, in turn, alter the TH gene expression in the cells of the mesoamygdaloid pathway. It remains to be determined if the increased MET metabolism in AMY possesses a synergic action on this feedback which could be mediated through direct or indirect afferences to VTA and originate in forebrain structures (AMY or hippocampus).

Thirty days after the last ECS, TH activity had returned to the baseline level in LC whereas TH-mRNA remained elevated. Because a differential splicing for TH-mRNA failed to be demonstrated in the rat (Coker et al., 1988) as it has been shown in humans (Grima et al., 1987), it cannot be suggested that a synthesis of an enzyme with a lower specific activity could occur (Horellou et al., 1988). This discrepancy between TH activity and TH-mRNA is still unclear and underlines the complexity of the regulatory processes of the enzymatic function in noradrenergic cells.

It is of particular interest that TH activity is modified mainly in VTA and LC, the two structures of origin of the mesoamygdaloid catecholaminergic pathways. The AMY is often associated with the motivational function of the brain, and alterations of monoaminergic afferents could mediate the antidepressant effect of ECS. If the variations in the aminergic system are involved in the antidepressant action of repeated ECS, it is necessary to determine whether the initial increase or the secondary inhibition of the monoaminergic metabolism is the more relevant to the long-term effect of ECS. It should be noted that it takes several days for the effects of both ECS and other types of antidepressant treatment of mood to occur, thus suggesting the existence of an indirect mechanism of action. For a better understanding of the role of aminergic ascending pathways in the depressive syndrome it will be necessary to compare the ECS-induced alterations of catecholaminergic metabolism with the pharmacological models of depression constituted by chronic reserpine or amphetamine treatments.

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