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Systemic Injection of Lidocaine Induce Expression of c-fos mRNA and Protein in Adult Rat Brain

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Both direct and indirect environmental stress to brain were increase the expression of transcription factor c-fos in various populations of neurons. In this study, we examined whether the intraperitoneal injections of lidocaine at doses inducing convulsion within 10 min increased the level of c-fos mRNA and protein in forebrain areas. In situ hybridization using [^{35}S]UTP-labeled antisense c-fos, cRNA increased c-fos mRNA levels though hippocampal formation, piriform cortex, septum, caudate-putamen, neostriatum, and amygdala within 2 hr. In parallel with the mRNA expression, c-FOS protein immunoreactivity was also observed in the same forebrain areas. In contrast to the seizure activity and widespread neuronal degeneration following a kainate treatment, injections of lidocaine did not produce neuronal death within 3 days. The present study indicates that lidocaine induces convulsion and c-fos expression without causing neurotoxicity.

Key Words: Transcription factor, c-fos mRNA and protein, Neurotoxicity

INTRODUCTION

Local anesthetics such as procaine and lidocaine are known to depress nerve conduction by blocking voltage-gated Na^+ channels in the peripheral nervous system (Strichartz & Ritchie, 1987). When local anesthetics are absorbed into the brain following systemic injections, convulsions and even death can occur within 20 min (Covino, 1987). It has been proposed that selective disinhibition of inhibitory neurons by local anesthetics causes increase in excitatory neurotransmission (Tanaka & Yamasaki, 1966; Kim et al, 1991). Supporting this argument is the fact that cocaine treatment decreases current threshold and duration for afterdischarges (Lesse, 1980) and induces persistent rhythmic slow waves in limbic brain areas (Matsuzaki & Misra, 1978). Moreover, antagonists of NMDA or AMPA/kainate receptors decrease incidence of seizure activities following a treatment with cocaine or lidocaine (Barat & Abdel-Rahman, 1997; Ushijima et al, 1998).

Expression pattern of immediate early genes appear to reflect changes in neuronal activities following the onset of a seizure (Morgan et al, 1987; Willoughby et al, 1997). For example, administration of kainate induces expression of c-fos mRNA and protein in cortical and subcortical areas which are associated with the propagation of kainate-induced seizure behavior such as staring, sniffing, wet dog shakes, nodding and chewing. Furthermore, other seizure-inducing agents or electrical stimulation also increase the levels of c-fos mRNA and protein in relevant brain areas (Shehab et al, 1992; Chen et al, 1995; Erdtmann-Vourliotis et al, 1997). Treatment with lignocaine induces convulsion without generating c-fos protein in rat hippocampus (Nakao et al, 1994). In the present study, we examined expression profiles of c-fos and neurodegenerative changes following the systemic injections of lidocaine. We observed that marked expression of c-fos first appeared in the granule cells of dentate gyrus and extended to the pyramidal cells in hippocampus, amygdala, and piriform cortex unlike in the previous reports, and that neuro-

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nal cell death did not occur.

METHODS

Animals and brain tissue preparation

This study was approved by the Animal Research Committee of the Wonkwang University Faculty of Dentistry. Male Sprague-Dawley rats weighing 230~250 g were allowed free access to food and water. Spontaneously breathing and conscious rats were randomly assigned to four groups and received intraperitoneal injection of lidocaine (120 mg/kg). A control group received vehicle only. The animals were killed at 20 min, 2, 4, 8, 12, 24, and 72 hr, after the lidocaine injection. The rats were anesthetized by the intraperitoneal injection of pentobarbital (50 mg/kg) and perfused transcardially with 100 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.4) containing 10,000 IU/L heparin until blood was washed out. Perfusion was continued for 15 min, with 4% paraformaldehyde and dissolved in 0.01 M phosphate buffer (pH 7.4). Brains were removed, postfixed overnight in the same fixative, and placed for another day in a cryoprotective solution of 30% sucrose.

Immunohistochemistry

Frozen sections were cut into slices and immersed in 0.01 M PBS. The sections were then incubated with anti-c-Fos antibody in a 1/1,000 diluted solution of 0.1 M PBS containing 0.3% Triton X-100 (PBST) at 4°C for 24 hr. The sections were washed three times with PBST for 30 min and incubated with biotinylated anti-rabbit antibody (Vector Labs) at room temperature for 1 hr. After three washes, the sections were incubated with the Vectastain Elit ABC solution containing 0.1 M PBS at temperature for 1 hr and were visualized using a solution containing 0.02% 3,3'-diaminobenzidine, 0.3% nickel ammonium sulfate, and 0.0045% H_2O_2 in 0.05 M Tris-HCl (pH 7.6) for 5 min.

In situ hybridization

Brains were removed and stored at −20°C until use. Serial sections of coronal cryostat were cut, collected onto reaction vials, and prewarmed at 48°C until further processing. The sections were placed in a hybridization buffer containing 2×saline-sodium citrate (SSC), Denhardt's solution, dithiotheitol, salmon sperm DNA, formamide and dextran sulphate at

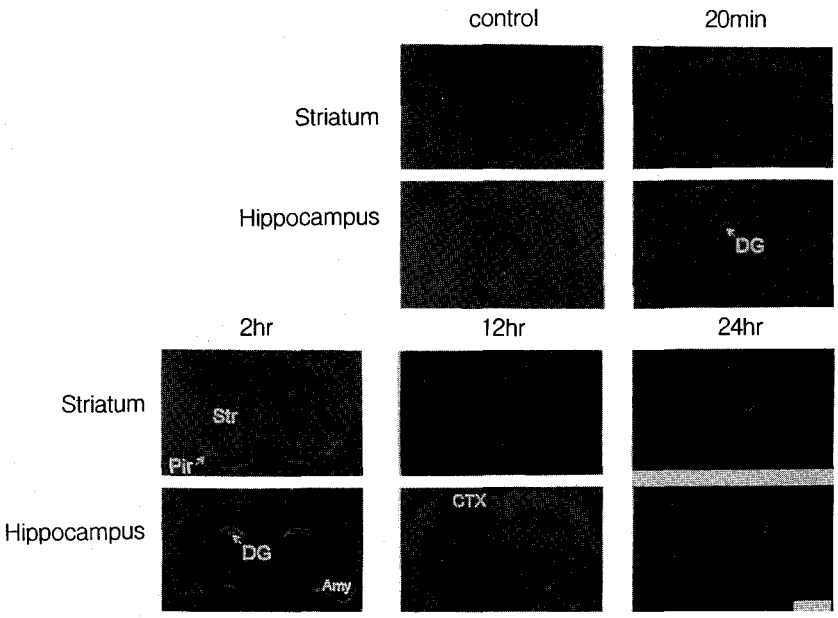

Fig. 1. Regional order of appearance of lidocaine-induced convulsion increases in hybridization to c-fos mRNA. Dark-field photomicrographs showing the autoradiographic localization of hybridization of the ^{35}S-cRNA probe (evident as white in this and subsequent illustrations) in tissue sections though the striatum and hippocampus of a control rat and rats sacrificed after lidocaine-induced convulsion. Bar=3.4 mm. Amy: amygdala, CTX: cerebral cortex, DG: dentate gyrus, Pir: piriform cortex, Str: striatum

48°C for 2 hr. The antisense c-fos cRNA was labeled with [^{35}S]UTP using terminal deoxynucleotidyl transferase to a specific activity of 2.0×10^{19} d.p.m/mol at 48°C in a hybridization buffer for 24 hr. Subsequently, the sections were washed in decreasing concentrations of SSC (2 to 0.1 times) and then mounted onto gelatin-coated slides. Slides were then exposed to X-ray film for 6 days.

RESULTS

The systemic injections of lidocaine produced preconvulsive behavior in adult rat including staring, immobilization, facial and mouth movement, wet-dog shakes, and loss of postural control within 10 min. The animals progressively developed convulsions from 15 to 20 min after the lidocaine injection. In situ hybridization, histochemistry using ^{35}S-labeled sense or antisense riboprobes was performed to examine expression profiles of c-fos mRNA. Compared to sham-operated or normal control, lidocaine treatment significantly increased levels of c-fos mRNA in dentate gyrus and piriform cortex within 20 min (Fig. 1, 2). The c-fos hybridization signal in dentate gyrus and piriform cortex was further increased by 5-fold 2 hr later. By that time, c-fos mRNA levels were also increased thoughout hippocampal layers, neostriatum, and amygdala. The lidocaine-induced expression of c-fos mRNA in those limbic areas returned to control levels by 12 hr. 12~24 hr after the lidocaine treatment, the superficial and deep layers of neocortex showed increase in c-fos mRNA expression. Brain sections hybridized with labeled antisense probes after RNase treatment or sense probes did not show specific hybridization signals in the experimental groups (data not shown).

Immunohistochemistry using a polyclonal c-FOS antibody showed that c-FOS protein was also increased following the lidocaine treatment. In the hippocampal formation, c-FOS immunoreactivity increased in stratum granulosum of the dentate gyrus for 2 hr after the lidocaine treatment, and then started to decline by 4 hr. Within 12 hr, it completely disappeared (Fig. 3A). The hilus and CA3-CA1 neurons also showed slight increase in the c-FOS expression. The c-FOS immunoreactivity was increased slightly in the piriform cortex within 20 min (Fig. 3B). The highest expression of c-FOS in the piriform cortex and amygdala was observed 2~4 hr after the lido-

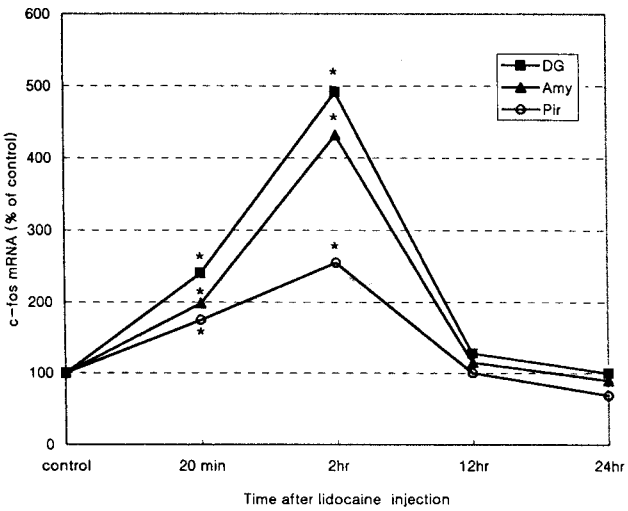

Fig. 2. Time course of changes in c-fos mRNA expression in the dentate gyrus (DG), amygdala (Amy), and piriform cortex (Pir) after lidocaine treatment as quantified by computerized image analysis of X-ray films. Values are expressed relative to control animals, mean± S.E.M (n=3 animals), *$p<0.05$ using analysis of variance and Student-Newman-Keuls's test

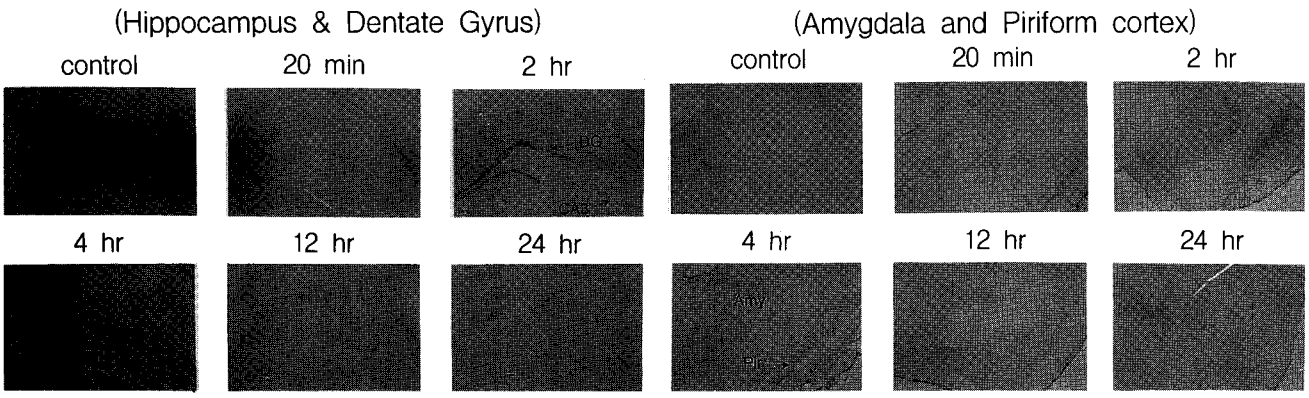

Fig. 3. Time course of c-Fos expression in amygdala, piriform cortex and hippocampus after lidocaine-induced convulsion. Rats received lidocaine (120 mg/kg, i.p.) at time 0, and were killed at the times indicated. The tissues were then processed for c-Fos immunohistochemical staining.

caine treatment.

Finally, we examined whether the lidocaine-induced convulsion involved concomitant neuronal loss in limbic structures. Unlike the selective neuronal death in the hilus and CA3 following systemic injections of kainate in rat, administration of lidocaine did not produce neuronal death in the hippocampal formation and other limbic areas (data not shown).

DISCUSSION

The present study showed that c-fos mRNA and protein were expressed in limbic structures following the parenteral administration of lidocaine. Our results confirmed and extended earlier studies in which c-fos-like proteins were activated by various epileptic procedures (Dragunow & Robertson, 1987a; Sonnenberg et al, 1989; Shin et al, 1990; Willoughby et al, 1997). The expression of Fos has been well-documented as a useful marker for the neuronal activation induced by pharmacological, electrical, and physiological stimuli to the central nervous system (Morgan et al, 1987; Saffen et al, 1988; Sager et al, 1988; Erdtmann-Vourlitis et al, 1997). In the CNS, the expression of c-fos mRNA and protein is rapidly stimulated in response to a neuronal activity. Seizure has been shown to generate a rapid elevation of Fos expression under a number of experimental conditions. The expression of c-fos in the brain after a single or multiple seizure follows a specific time course. Levels of c-fos mRNA are significantly increased within minutes after a seizure, continue to increase until reaching a peak by 2 hr, and then return to control levels by 12 hr (Dragunow & Robertson, 1987b; Morgan et al, 1987). These reports are consistent with our results that expression of c-fos mRNA and protein was transiently increased between 20 min and 4 hr after the lidocaine injection.

Numerous studies have shown that the regional patterns of c-fos mRNA and protein expression are grossly similar following the multiple seizures induced by kainic acid, picrotoxin, electroshock, and forebrain injections of convulsant agents (Dragunow & Robertson, 1987a; Dragunow & Robertson, 1987b; Morgan et al, 1987; Saffen et al, 1988; Sager et al, 1988; Sonnenberg et al, 1989; Shin et al, 1990; Hiscock et al, 1997). Fos is induced in primary cultures of cerebellum by glutamate, in the dorsal horn of the spinal cord by physiological stimulation of primary sensory neurons, and in motor and sensory thalamus by the stimulation of sensory cortex (Dragunow & Robertson, 1987b; Hunt et al, 1987; Morgan et al, 1987). The mRNA and protein of c-fos are detected in cerebral cortex and limbic system for specific periods after a convulsion and other stimuli including hormones, neurotransmitters, injury, and growth factors (Hoffman et al, 1990; Sharp et al, 1990). Agents which produce seizure activities are known to induce the expression of AP-1 transcription factor (Morgan et al, 1987; White & Gall, 1987; Simonato et al, 1991; Kaminska et al, 1994). Convulsant doses of kainate also induce c-fos expression in the mouse brain. 30 to 90 min after a kainate administration, entorhinal cortex and dentate gyrus are the first brain regions to exhibit changes in the expression of c-fos. The presence of c-fos in CA1-CA4 fields of hippocampus is not discernible until 3 hr after a kainate administration. Our lidocaine-induced c-fos expression was different from the results of kainate experiment. This study showed a transient increase in c-fos expression in granular cells of dentate gyrus and in CA1-CA4 pyramidal neurons from 20 min to 4 hr after a lidocaine administration. Most of the pyramidal neurons in CA3 to CA4, amygdala, piriform cortex, and dentate gyrus granule cells showed intense nuclear staining, whereas only a few pyramidal neurons in CA1 regions did so 2 hr after the lidocaine-induced convulsion. In dentate gyrus and CA3-CA4 region, Fos protein first appeared 20 min after the lidocaine injection, reached its maximum within 2~4 hr, and apparently disappeared within 12 hr. In other brain regions including amygdala, piriform cortex, and cerebral cortex, Fos expression was delayed and reached a peak within 2 to 4 hr. These results suggest that dentate gyrus and CA3-CA4 regions may play important roles regarding the spread of seizures and modification of the development of dramatic epileptic discharges.

The physiological significance of c-fos expression is still unknown. The c-fos is a family of inducible transcription factors which complex with members of the jun transcription factor family (Rauscher et al, 1988; Sambucetti & Curran, 1986). This complex recognizes AP-1 DNA elements in the promoter regions of target genes to modulate gene transcription, which presumably regulates gene expression involved in the control of cell growth, differentiation and regeneration (Sonnenberg et al, 1989; Herdegen et al, 1993; Lanaud et al, 1993).

In this study, we found a rapid and transient increase of c-fos expression in dentate gyrus, CA3-CA4 region of hippocampus, piriform cortex, and amygdala shortly after a lidocaine injection. Transient increase in c-Fos expression may be a common modification associated with long-lasting changes in neuro-

nal function, where its induction is one of the earliest genetic events to follow neuronal activation, such as kindling and epilepsy. In conclusion, elucidation of the regulatory mechanism and function of c-fos in the brain may contribute to the understanding of the physiological processes and neuronal circuits which underlie lidocaine-induced convulsion. We hereby suggest that c-fos plays a prominent role in the long-term plastic changes induced by a seizure activity.

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