Title
The in vivo proconvulsant effects of corticotropin releasing hormone in the developing rat are independent of ionotropic glutamate receptor activation

Journal
Brain Research, 111(1)

ISSN
1385-299X

Authors
Brunson, Kristen L
Schultz, Linda
Baram, Tallie Z

Publication Date
1998-11-01

DOI
10.1016/s0165-3806(98)00130-8

Published in final edited form as:
Brain Res Dev Brain Res. 1998 November 1; 111(1): 119–128.

The in vivo proconvulsant effects of corticotropin releasing hormone in the developing rat are independent of ionotropic glutamate receptor activation

Kristen L. Brunson[a], **Linda Schultz**[a], and **Tallie Z. Baram**[a,b,c,*]

[a]Department of Anatomy/Neurobiology, University of California, Irvine, CA 92697, USA

[b]Department of Pediatrics, ZOT 4475, University of California, Irvine, CA 92697-4475, USA

[c]Department of Neurology, University of California, Irvine, CA 92697, USA

Abstract

Corticotropin releasing hormone (CRH) produces age-dependent limbic seizures in the infant rat. Both the phenotype and the neuroanatomic matrix of CRH-induced seizures resemble the seizures induced by the rigid glutamate analogue, kainic acid (KA), and by rapid amygdala kindling. The experiments described in this study tested the hypothesis that the in vivo proconvulsant effects of CRH require activation of ionotropic glutamate receptors. Non-competitive (+MK-801) or competitive (CGP-39551) antagonists of *N*-methyl-D-aspartate (NMDA) receptors decreased or eliminated the motor effects of CRH, but electrographic CRH-induced seizures were unaffected. Administration of CRH antagonists did not affect the acquisition or the maintenance of rapid kindling, which are mediated by NMDA and α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) receptor activation, respectively. CRH receptor blockers failed to alter the latency or duration of seizures induced by activation of KA receptors, and threshold doses of CRH and KA had additive effects. CRH given repeatedly decreased the convulsant threshold dose of KA, probably via injury to hippocampal neurons. These results suggest that CRH and glutamate increase neuronal excitability via independent mechanisms. Because the proconvulsant effects of CRH are highly specific to the developmental period, glutamate-receptor-independent, CRH-receptor mediated excitation may account for some of the enhanced susceptibility to seizures during this period.

Keywords

Corticotropin releasing hormone; Glutamate receptors; Seizure; Rat

1. Introduction

Corticotropin releasing hormone (CRH) is the cardinal central nervous system transducer of stressful stimuli [43]. The limbic system, particularly the amygdala and hippocampus, are rich in neuronal populations that either synthesize CRH or possess CRH receptors [3,14,42]. In the majority of neuronal circuits studied, CRH functions as an excitatory neuromodulator [2,15]. During the second week of life in the rat, picomolar amounts of CRH induce severe and prolonged seizures within minutes [10]. Furthermore, repeated administration of CRH

*Corresponding author. Fax: + 1-949-824-1106; tallie@uci.edu.

doses (150×10^{-12} mole), which result in limbic status epilepticus, leads to excitotoxic injury in select hippocampal and amygdala neurons in the infant rat [9].

CRH induces phenotypic 'limbic seizures'. These consist of unstoppable oral automatisms, wet dog shakes (WDS) and clonus [10,15]. These seizures resemble limbic seizures induced by activation of the glutamate receptor family, for example, by the prototypical convulsant, kainic acid (KA) [28]. Using depth-electrode mapping, CRH-induced seizures in the immature rat were shown to originate in the amygdala and spread to the hippocampus and neocortex [8]. The precise excitatory mechanisms by which CRH results in seizures have not been fully determined. In particular, the requirement for activation of specific glutamate receptor types in CRH-induced seizures has not been elucidated.

The purpose of the series of experiments reported here was to investigate potential interactions between CRH and activation of discrete types of ionotropic glutamate receptors. Several experimental paradigms were used for this purpose: (a) we studied the effects of blocking *N*-methyl-D-aspartate (NMDA) type glutamate receptors on the behavioral and electrographic aspects of CRH induced seizures in the immature rat. (b) we examined whether blocking CRH receptors affected the latency or the duration of seizures induced by activation of ionotropic glutamate receptors, using the prototypical convulsant KA. (c) we determined potential interactions of glutamate and CRH receptor activation by using threshold doses of both agents. (d) we evaluated the effects of CRH antagonists on functions considered to require activation of NMDA and non-NMDA glutamate receptors, i.e., the acquisition and maintenance of kindling, respectively [25,32].

2. Materials and methods

2.1. Drugs and chemicals

CRH and the CRH antagonist (9–41)-α-helical-CRH were purchased from Bachem (Torrance, CA). KA and (+)MK-801 were purchased from Sigma (St. Louis, MO), and CGP-39551 was a gift from Dr. M. Baudry, University of Southern California. Because of the known variation in seizure severity and duration induced by different batches or dilutions of KA, the drug was dissolved in a stock solution of 10 mg/kg, which was aliquoted and kept frozen. Working solutions of 1 mg/ml were prepared freshly.

2.2. Animals

About 180 infant rats (postnatal days 10–12) were used. Pups were offspring of time-pregnant, Sprague–Dawley rats (Zivic-Miller, Zelienople, PA). Pups were born in our federally-approved animal facility, kept on a 12 h light/dark cycle and dams given access to unlimited food and water. Cages were monitored for presence of pups every 12 h, and the day of birth was considered day 0. Litters were culled to 12 pups and mixed among experimental groups, so that each experiment consisted of groups matched for age. Cages were maintained in a quiet, non-crowded room, and were undisturbed for 24 h prior to experiments. All experiments were conducted in the morning, to avoid potential diurnal variability in endogenous CRH content [45] or seizure susceptibility [35]. All experiments were carried out according to NIH guidelines for the care of experimental animals and were approved by the institutional animal care committee.

2.3. Surgical procedures and drug administration

For administration of CRH and the CRH antagonist, pups were implanted with lateral cerebral ventricle (i.c.v.) cannulae [10], since CRH and the peptide CRH antagonists do not cross the blood brain barrier. For electroencephalographic (EEG) recording and for kindling, pups were equipped, in addition, with depth electrodes 24 h prior to experiments. The

surgical procedures, including the age-specific stereotaxic coordinates for the amygdaloid complex and for the dorsal hippocampus developed for the Sprague–Dawley rat pup, have been described in detail elsewhere [6,7]. Briefly, electrodes were implanted under halothane anaesthesia, using an infant-rat stereotaxic apparatus (Kopf, Tujunga, CA). Bipolar twisted wire electrodes, (Plastics One, Roanoke, VA) with a wire diameter of 0.1–0.15 mm and vertical inter-tip distance of 0.5–1.0 mm were inserted through a burr-hole and aimed at the basolateral nucleus of the amygdala (for monitoring of CRH-induced seizures and kindling), hippocampus (for KA- and CRH-induced seizures), or cortex (for KA-induced seizures). Electrodes were anchored to the skull with an acrylic cement 'cap' attached to one or two screws. Cannulae and electrode positions were verified after the experiments in all animals. Brain growth during the 2 days of the experiments results in minimal (~ 0.08 mm) anterior–posterior shift of cannulae and electrodes relative to bregma [37], but this has not been a problem because both lateral ventricles and amygdala extend for > 1 mm on each side of the cannula in the anterior–posterior axis.

CRH and the CRH-antagonist were infused i.c.v. to freely moving pups maintained euthermic on a warming pad. The peptides were infused in a volume of 1–2 μl via the chronic cannula using a micro-infusion pump and a Hamilton syringe attached to flexible tubing. Cannula-carrying control animals were given a dye vehicle [8,9]. KA and the selective glutamate receptor antagonists, which are known to cross the blood brain barrier, were injected into the peritoneal cavity (i.p.) at a volume of 1.5–2 μl/g, and control animals received the same volume of vehicle.

2.4. Parameters of behavioral seizure evaluation

Subsequent to CRH or KA administration, seizure latency and duration were monitored: animals were scored for behavioral limbic seizures occurring during 5-min epochs for the 180 min subsequent to drug administration [10]. For kindling, a behavioral scale [7] has been modified from Haas et al. [18]. Significant behavioral overlap between the behavioral phenomena of CRH-induced seizures, rapid amygdala kindling and KA-induced seizures were evident, as shown in Table 1.

2.5. EEG monitoring

For each treatment, a group of rat pups ($n \geq 4$) was subjected to depth electrode implantation, and the presence and location of epileptic discharges associated with CRH-, KA- and kindling-induced seizures was monitored by EEG, as described in detail elsewhere [6,7,10].

2.6. Experimental design

2.6.1. Experiment I—effect of NMDA-type receptor antagonists on CRH-induced seizures—For this experiment, a moderate CRH dose (150×10^{-12} mole, about 0.05 μg/g body weight) was chosen, to result in seizures lasting for approximately 3 h [8]. The non-competitive NMDA receptor blocker, (+)MK-801 (0.2 and 1 mg/kg) and the competitive antagonist CGP-39551 (3–30 mg/kg) were administered 40 min prior to CRH infusion. The higher (+)MK-801 dose (1 mg/kg) resulted in very abnormal behavior (pups appeared ill and unable to ambulate, occasionally with stiff tails) and EEG pattern. Therefore, the data shown resulted from experiments in which the lower dose was used. For CGP-39551, the highest dose utilized (30 mg/kg), also resulted in abnormal behavior and EEG tracings. Therefore, a maximal dose of 10 mg/kg, shown to be effective as a neuroprotectant after KA administration [44], was used for the EEG recordings. Fifty infant rats were assigned to receive CRH alone or CRH after pre-treatment with the NMDA receptor antagonists. Control groups consisted of pups receiving an NMDA antagonist only, as well as cannula-carrying animals receiving vehicle. For all groups, the latency to onset

and the duration of CRH-induced behavioral seizures was recorded. An additional set of animals was monitored for the effects of the NMDA antagonists on CRH-induced EEG epileptiform discharges, as noted above.

2.6.2. Experiment II—establishment of the threshold convulsant dose of KA and validation of the EEG correlates of the behavioral seizures—Increasing doses of KA were administered to groups (n = 3 to 12) of 10-day-old rats via i.p. injections. KA doses ranged from 0.2 to 1 mg/kg, based on pilot data, to determine a threshold dose capable of inducing automatisms and limbic seizures. Controls were injected in the same mannner with equal volumes of vehicle. Following injections, the latency to onset and the duration of seizures were recorded.

2.6.3. Experiment III—effect of CRH antagonist on KA induced seizures—The competitive non-selective blocker of CRH receptors, (9–41)-α-helical CRH (1320×10^{-12} mole), was administered i.c.v. to the experimental group (n = 14) 30–40 min prior to KA administration. This timing was based on the previously established time course for the actions of this antagonist, and the antagonist dose was chosen based on its ability to attenuate or abolish seizures induced by moderate doses of CRH [4,10]. Both the control (n = 8) and experimental groups of infant rats received a moderate dose of KA for this age group (1 mg/kg). An additional control group (n = 5) received the CRH antagonist alone. For EEG, a separate group (n = 4) was implanted with bipolar electrodes aimed at the dorsal hippocampus and cortex, to correlate behavioral KA-induced seizures with epileptic discharges.

2.6.4. Experiment IV—does co-administration of threshold doses of CRH and KA produce additive or synergistic effects?—Based on the results of experiment II, a threshold i.p. dose of KA (0.2 mg/kg) was administered to the experimental group (n = 9) 30 min prior to i.c.v. infusion of a threshold CRH dose ($22.5–30 \times 10^{-12}$ mole) [10]. The duration and severity of the resulting seizures were compared to those produced by each agent alone in litter-mate controls (n = 8 each for KA and CRH).

2.6.5. Experiment V—effect of repeated CRH administration on the convulsant threshold dose of KA—Based on previous experiments showing that four infusions of CRH over 2 days led to excitotoxicity 16 h later [9], the effects of this regimen on the threshold dose of KA was determined. CRH (150×10^{-12} mole) was infused i.c.v. to the experimental group (n = 8) 4 times: at 0800 and 1600 h on postnatal days 10 and 11. Control groups consisted of cannula-implanted, sham-infused litter-mates (n = 3), and of a naive group (n = 8). A threshold dose of KA (0.2 mg/kg) was administered i.p. to all groups at 0800 h on postnatal day 12. The latency to onset of automatisms and motor seizures and the duration of both were determined [9,12].

2.7. Experiment VI—effect of CRH and the CRH antagonist on the acquisition of rapid amygdala kindling

The kindling paradigm was modified from Haas et al. [18] as described elsewhere [7]. Briefly, the kindling stimulus consisted of a 3 s train of 60 Hz biphasic 400 μA peak-to-peak current, generated by an isolated pulse stimulator (A-M model 2100; Everett, WA) and visualized using a Tektronix 5111a oscilloscope. Baseline EEGs were recorded for 5 min. Following each stimulus, EEG was recorded for 1–2 min. Pups were stimulated at 15 min intervals [7]. Since infant rats (7–12 days) displayed a unique sequence of kindling-induced behaviors, a kindling scale was generated for them, based on the one defined by Haas et al. for older pups [18] (Table 1). The rate of kindling development was assessed by measuring afterdischarge (AD) duration after each stimulation and by the number of stimulations

needed for the achievement of each kindling stage. Rats aged 10 days were infused with the CRH antagonist 15–20 min prior to initiation of kindling. The time of administration and the dose were appropriate for blocking CRH receptors as determined by prevention of CRH-induced seizures [10].

2.8. Data analysis

All statistical analyses were performed using Prism GraphPad (San Diego, CA). All values are given as means as ± S.E.M. Results were analyzed for statistical significance ($p < 0.05$) using a one-way analysis of variance (ANOVA), student's t test, or the non-parametric Mann–Whitney *U* test, as appropriate.

3. Results

3.1. Glutamate receptor antagonists alter motor but not EEG features of CRH-induced seizures

Both the competitive and non-competitive antagonists of the NMDA-type glutamate receptor altered parameters of CRH-induced seizures. Fig. 1 demonstrates that (+)MK-801 tended to increase the latency to the onset of the limbic automatisms induced by CRH, and also shortened seizure duration (CRH + MK-801: 93.2 ± 39 min; CRH alone: 190 ± 10 min; $p < 0.05$). Table 2 demonstrates a dose-dependent effect of CGP-39551 on the latency and duration of CRH-induced seizures.

However, the inhibitory effects of the glutamate receptor blockers on CRH-induced *behavioral* seizures were not due to direct interaction with CRH at the site of origin of the *electrophysiological* seizure manifestations: EEGs recorded from the dorsal hippocampus of pups pretreated with CGP-39551 prior to CRH administration revealed persistent epileptiform discharges produced by CRH administration (Fig. 2). Thus, while motor seizures produced by CRH were attenuated, likely by blocking the actions of endogenous glutamate on motor-neuron control, the epileptic EEG discharges induced by CRH were not inhibited. EEGs from pups given (+)MK-801 were quite abnormal (data not shown), consistent with abnormal behaviors and previous reports in the literature [24,40]. Therefore, the EEGs were not useful for examination of the effects of this agent on CRH-induced EEG changes.

3.2. Threshold dose and EEG characterization of KA seizures in the infant rat

KA, probably via activation of several ionotropic glutamate receptor types, leads to neuronal excitation and seizures in both the mature and developing brain. CRH administration leads to seizures primarily during development, via a mechanism requiring activation of CRH receptors. However, we tested the hypothesis that CRH mediated excitation utilized the 'universal' excitatory means of glutamate receptor activation. More specifically, to examine potential interactions between the excitatory actions of CRH and of KA, the threshold convulsant dose of the latter was established for 10–12 day old pups. As is evident from Table 3, a dose of 0.2 mg/kg led to short bouts of limbic automatisms and behavioral seizures in 6 of 8 rats, with a long latency. As KA doses increased, latency to onset of seizures decreased, and the duration of behavioral phenomena lengthened. To ascertain the epileptic nature of the observed KA-induced behaviors, EEGs were recorded from four 10-day-old rats injected i.p. with 1 mg/kg KA. Fig. 3 demonstrates bipolar cortical and dorsal hippocampal recordings from these pups 55–60 min after KA administration. While cortical leads (top tracings) consist of low-voltage non-rhythmic activity, hippocampal leads (bottom tracings) reveal spike, poly-spike and rhythmic epileptiform discharges. During these recordings, animals were either motionless, in a 'trance-like' state (B,D), or displayed oral and motor automatisms (A,C).

3.3. Interaction of CRH with KA

To examine for a potential synergistic interaction between CRH and KA, threshold doses of both agents were co-administered to infant rats. The threshold dose of KA (0.2 mg/kg), Table 3, and the threshold dose of CRH (chosen based on previously published findings [8]) were utilized. Table 4 demonstrates a complex interaction between the two agents: addition of CRH to threshold KA doses increased the duration and severity of seizures, but the duration of seizures produced by the combined treatment was not longer than when CRH was given alone. Behavioral manifestations of these seizures, however, were more severe with the combined treatment. Overall, these findings are consistent with either an additive or synergistic interaction of KA and CRH.

3.4. Effects of CRH antagonists on KA seizures

KA in moderate age-specific doses (1 mg/kg) produced consistent and reproducible limbic seizures when administered to infant rats. The latency to the onset of limbic automatisms averaged 20 min (Table 5), and seizure duration over the following 180 min was 145.7 ± 11.2 min. Pre-treatment with the CRH antagonist did not modify any qualitative (i.e., spectrum of behaviors) or quantitative parameters (latency and duration) of these seizures. EEG recordings from dorsal hippocampus (Fig. 4B) showed no suppression of the KA-induced seizures. A second experiment, in which a lower, threshold dose of KA was used (0.25 mg/kg) also did not reveal any attenuation of the seizures induced by this convulsant after CRH-antagonist administration (data not shown). The same dose of antagonist was clearly adequate for attenuation of seizures induced by CRH itself (Table 5).

3.5. Effects of repeated CRH administration on the threshold to KA

Based on evidence that repeated CRH administration has a dose-dependent excitotoxic effect on amygdala and hippocampal neurons [9], the peptide was given four times to infant rats, and KA threshold tested 16 h following the last dose. Under these conditions, a dramatic enhancement of the convulsant potency of KA was observed (Table 6). The duration and the severity of limbic seizures induced by 0.2 mg/kg of the drug in rats treated chronically with CRH were equivalent to the effects of a 1.0 mg/kg dose, yielding an effective five-fold enhancement of KA potency. No spontaneous seizures were noted in the CRH-treated pups prior to KA administration. The pups that had received four doses of CRH were not moribund and engaged in age-appropriate behaviors including suckling.

3.6. Effect of CRH antagonist on kindling acquisition

To examine the potential role of CRH in the acquisition of kindling, rats were injected with the CRH antagonist (9–41)-α-helical CRH prior to onset of kindling. No significant difference was observed for the number of cycles prior to AD onset between rat pups pretreated with CRH antagonist (4.0 ± 0.63) and pups that received vehicle (3.2 ± 0.2; Fig. 5A). There was also no significant difference between CRH- (5.0 ± 1.2) and vehicle- (4.8 ± 1.6) treated pups in the number of kindling cycles needed to achieve stage 3 seizures (Fig. 5B). As with the KA-induced seizures, the dose of CRH antagonist chosen was sufficient to block seizures induced by CRH.

4. Discussion

The major findings of this study are that CRH induces behavioral and electrographic in vivo limbic seizures via mechanisms that are independent from activation of the ionotropic glutamate receptors. In addition, CRH-dependent excitatory actions do not play a significant role in in vivo glutamate-receptor-mediated seizure paradigms such as those related to kindling and KA administration. However, chronic CRH administration leads to an excitable

state which heightens the proconvulsant potency of glutamate-receptor activators such as KA.

The excitatory effects of CRH have been demonstrated in several species, using both in vivo [10,15,31] and in vitro methods [2,21,39]. In the adult rat, Ehlers [15] described the long latency (hours) to the onset of seizures induced by CRH. Additionally, a single administration of CRH produced a sequence of behaviors similar to the behavioral stages of kindling [15,46]. Therefore, it was hypothesized that CRH could be a kindling stimulus for the development of limbic seizures [46,47]. Weiss et al. [47], using mature male Sprague–Dawley rats, studied the effect of CRH on the development of kindling. Pre-administration of CRH significantly accelerated the development of stage 3 seizures (after 8.2 stimulations versus 16.1 in vehicle-treated rats). AD duration throughout the kindling process was significantly longer in CRH pre-treated rats. The data were interpreted to suggest a role for endogenous CRH in limbic excitability, and a mechanistic interaction with the kindling process.

The acquisition or development of kindling is generally considered to depend on activation of NMDA receptors [33]. For example, Holmes et al. [25] have demonstrated that the selective NMDA receptor blockers 2-amino-phosphonovalerate (APV) and carboxypiperazine-phosphate (CPP) blocked the development of the kindled state. These agents had only small depressant effects on seizure expression in fully kindled rats. Conversely, selective blockers of the AMPA receptor attenuated the expression of seizures in previously kindled adult rats [32]. Thus, NMDA receptor activation is the major mechanism for kindling acquisition while expression of kindled seizures depends mainly on AMPA receptor function. Therefore, the acceleration of kindling acquisition in *adult* rats by CRH may indicate an interaction with NMDA receptor activation.

In the *infant* rat rapid kindling paradigm [7,18], the current study did not suggest a robust effect of CRH-mediated neurotransmission on the acquisition or the maintenance of the kindled state. The lack of significant effect of CRH or its antagonist on acquisition of kindling provides evidence for the NMDA type glutamate-receptor independent mechanism of action of CRH. Furthermore, the lack of effect on expression of kindling suggests a mechanism for CRH-mediated excitation that is independent of AMPA receptors as well.

The absence of significant interaction of CRH-mediated excitation with the kindling process is consistent also with the rapid onset of CRH-induced seizures in the infant rat (Table 5). In addition to producing seizures very rapidly, CRH has a much higher proconvulsant *potency* during the first two postnatal weeks in the rat, as compared with the adult [10]. Seizures occur with doses 200-fold lower than in adults (7.5×10^{-12} mole for infants vs. 1500×10^{-12} mole for adults) and are quite protracted [4]. The reason for this enhanced potency of CRH can be attributed, at least in part, to the high levels of CRH receptors in target limbic structures during this age [3,26]. Messenger RNA levels for the first member of the CRH receptor family, CRF_1, peak in the amygdala on postnatal day 9, and in the hippocampus on postnatal day 6 [3]. We, and others, have shown that seizures produced by CRH are abolished by treatment with competitive CRH receptor antagonists [10]. More recently, CRF_1 has been demonstrated to mediate the proconvulsant effects of CRH in vivo [4].

Potential interactions of CRH with glutamate-receptor mediated excitation and excitotoxicity have been postulated based on other lines of evidence as well. For example, excitotoxic cell death is generally considered a glutamate-mediated effect, and pretreatment with CRH antagonists has been shown to attenuate excitotoxic cell death induced by direct activation of glutamate receptors [41], ischemia [29,41], or by status epilepticus [30]. Moreover, the ability of CRH to cause selective injury of immature hippocampal and

amygdala neurons has been demonstrated [9]. However, the findings of the current study do not support a direct interaction between excitation and excitotoxicity induced by glutamate receptor activation and the actions of CRH.

The current study has established that blocking of CRH receptors does not attenuate KA-induced seizures. As is evident from Fig. 3, KA administration to immature rats results in limbic seizures with epileptiform discharges in the hippocampus, but not in the cortex, consistent with several previous studies on KA-induced seizures in infant rats [1,23]. Cherubini et al. [13] demonstrated apparent cortical discharges after KA administration to neonatal rats, but the true origin of the EEG discharges is difficult to ascertain [6]. Fig. 4 documents that administration of CRH antagonists either prior to, or following, KA injection does not alter the epileptiform EEG activity produced by KA. Thus, although the behavioral (Table 1) and electrographic seizures produced by CRH and glutamate receptor activation are quite similar, no evidence for direct interaction between these excitatory processes has been demonstrated in this study. Since KA-induced seizures are considered to involve activation of both NMDA and non-NMDA receptors [16,44], the failure of the CRH antagonist to attenuate these seizures suggests a lack of interaction between CRH and either of these types of glutamate receptors in this paradigm.

The current study also documented that NMDA receptor antagonists do not alter the electrographic seizures induced by CRH. Interestingly, the motor correlates of these seizures were abolished by blocking NMDA receptors. These findings may be interpreted to suggest that the antagonists may reach motor regions in sufficient concentrations to block behavioral seizures, yet may not reach limbic regions (e.g., amygdala and hippocampus) in levels which block the EEG correlates of CRH induced seizures. Contrary to this argument, MK-801 has been demonstrated to have profound effects on learning and memory in concentrations which do not affect motor activity appreciably [48]. Taken together, these findings suggest that excitation induced by CRH, at least as evidenced by in vivo seizures, may be additive to glutamate actions, but not directly related to them.

In both the developing and the mature CNS, glutamate, via activation mainly of ionotropic receptors, is the major excitatory neurotransmitter [36]. The role of glutamate receptor activation in hypoxic seizures during development has been established [27]. A potential role for NMDA-type glutamate receptor activation in febrile seizures has been postulated [34]. A number of distinctive properties of glutamate-mediated neurotransmission in the developing CNS have been described, which may account for increased proconvulsant potency of this agent in the immature brain. For example, increased levels of glutamate receptors in the limbic system, unique subunit distribution, splicing variants which permit increased calcium entry have all been documented in the developing brain [22]. However, the incidence of spontaneous seizures during development is not increased [5]. It is the *susceptibility* to seizures in response to pharmacological convulsants and stressful circumstances (fever, hypoxia), which is increased during this period [5,22,38]. Since these insults and stressors induce CRH release in a number of limbic brain regions [17], potential interaction of glutamate, an excitatory neurotransmitter during both early and adult life, with CRH, a proconvulsant with maximal potency during development, may account for the profile of seizure susceptibility observed in the immature human and rat.

The current study established that repeated administration of CRH leads to dramatic reduction in the convulsant threshold to KA. This phenomenon was evident 14–16 h after the last of four doses of CRH, each of which led to limbic status epilepticus lasting 4–6 h [9]. We have previously shown that a number of stressful conditions alter CRH levels in the hypothalamus, and have recently demonstrated increased amygdala levels of CRH in stressed neonatal rats [19]. Taken together, these findings suggest that stressful stimuli may

lead to increased CRH levels in key limbic structures (e.g., amygdala and hippocampus), and that repeated activation of CRH receptors may alter excitability in these structures. Hollrigel et al. [21] showed that in vitro application of CRH (0.15 μM) to hippocampal slices leads to hyperexcitability in CA3 pyramidal neurons, which can result in a net increase of glutamate release. It is conceded that direct comparison of exogenously administered and endogenously released CRH is difficult, due to the fact that microdialysis measurements of CRH in specific brain regions of the immature rat are technically daunting. In addition, determination of the actual portion of exogenous CRH that reaches the synaptic cleft is not possible. However, based on the effects of synthetic application, it is proposed that glutamate receptor activation under circumstances of increased CRH levels may lead to marked enhancement of the susceptibility to seizures. Thus, after stressors such as fever, trauma or hypoxia, CRH-mediated excitation may play a role in the sensitization of the developing brain to glutamatergic proconvulsant effects.

In conclusion, the significance of CRH as an excitatory neuromodulator may derive from the fact that CRH levels in limbic regions may be increased under conditions that promote seizures. The human infant and young child commonly develop seizures with onset of stressful circumstances such as fever [11], trauma [20], or hypoxia [27]. It is proposed that stress-induced rapid CRH release may be involved in these types of seizures. CRH would not be expected to contribute significantly to adult seizures, since the latency of CRH-induced seizures in the adult is much longer (hours), consistent with a reduction of receptor abundance in the adult amygdala and hippocampus. However in the developing brain, improvement in the understanding of CRH as an excitatory neuromodulator in the developing brain may provide a basis for developing pharmacological agents that are more efficient in treating age-specific seizures in the developing human.

Acknowledgments

This study was supported by National Institutes of Health grant NS-28912 to TZB. We thank Dr. Michel Baudry for providing the NMDA antagonist CGP-39551 and Dr. John Weiss and Greg Hollrigel for insightful comments on the manuscript.

References

1. Albala BJ, Moshe SL, Okada R. Kainic-acid-induced seizures: a developmental study. Brain Res. 1984; 315:139–148. [PubMed: 6722574]
2. Aldenhoff JB, Gruol DL, Rivier J, Vale W, Siggins GR. Corticotropin releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons. Science. 1983; 221:875–877. [PubMed: 6603658]
3. Avishai-Eliner S, Yi SJ, Baram TZ. Developmental profile of messenger RNA for the corticotropin-releasing hormone receptor in the rat limbic system. Dev. Brain Res. 1996; 91:159–163. [PubMed: 8852365]
4. Baram TZ, Chalmers DT, Chen C, Koutsoukos Y, De Souza EB. The CRF_1 receptor mediates the excitatory actions of corticotropin releasing factor in the developing rat brain: in vivo evidence using novel, selective, non-peptide CRF receptor antagonists. Brain Res. 1997; 770:89–95. [PubMed: 9372207]
5. Baram TZ, Hatalski CG. Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. Trends in Neurosci. 1998; 21:471–476.
6. Baram TZ, Hirsch E. EEG recording in neonatal and infant rats: some pitfalls and solutions. Dendron. 1992; 1:39–46.
7. Baram TZ, Hirsch E, Schultz L. Short-interval amygdala kindling in neonatal rats. Dev. Brain Res. 1993; 73:79–83. [PubMed: 8513558]
8. Baram TZ, Hirsch E, Snead OC, Schultz L. Corticotropin-releasing hormone-induced seizures in infant rats originate in the amygdala. Ann. Neurol. 1992; 31:488–494. [PubMed: 1596084]

9. Baram TZ, Ribak CE. Peptide-induced infant status epilepticus causes neuronal death and synaptic reorganization. NeuroReport. 1995; 6:277–280. [PubMed: 7756609]
10. Baram TZ, Schultz L. Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat. Dev. Brain Res. 1991; 61:97–101. [PubMed: 1914160]
11. Berg AT, Shinnar S, Shapiro ED, Salomon ME, Crain EF, Hauser WA. Risk factors for a first febrile seizure: a matched case-control study. Epilepsia. 1995; 36:334–341. [PubMed: 7541745]
12. Chang D, Baram TZ. Status epilepticus results in reversible neuronal injury in infant rat hippocampus: novel use of a marker. Dev. Brain Res. 1994; 77:133–136. [PubMed: 7510587]
13. Cherubini E, De Feo MR, Mecarelli O, Ricci GF. Behavioral and electrographic patterns induced by systemic administration of kainic acid in developing rats. Brain Res. 1983; 285:69–77. [PubMed: 6883128]
14. De Souza EB, Insel TR, Perrin MH, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors are widely distributed within the rat CNS: an autoradiographic study. J. Neurosci. 1985; 5:3189–3203. [PubMed: 3001239]
15. Ehlers CL, Henriksen SJ, Wang M, Rivier J, Vale W, Bloom FE. Corticotropin releasing factor produces increases in brain excitability and convulsive seizures in rats. Brain Res. 1983; 278:332–336. [PubMed: 6605787]
16. Fariello RG, Golden GT, Smith GG, Reyes PF. Potentiation of kainic acid epileptogenicity and sparing from neuronal damage by an NMDA receptor antagonist. Epilepsy Res. 1989; 3:206–213. [PubMed: 2543557]
17. Gray TS, Bingaman EW. The amygdala: corticotropin-releasing factor, steroids, and stress. Crit. Rev. Neurobiol. 1996; 10:155–168. [PubMed: 8971127]
18. Haas KZ, Sperber EF, Moshe SL. Kindling in developing animals: expression of severe seizures and enhanced development of bilateral foci. Dev. Brain Res. 1990; 56:275–280. [PubMed: 2261687]
19. Hatalski CG, Guirguis C, Baram TZ. Corticotropin releasing factor mRNA expression in the hypothalamic paraventricular nucleus and the central nucleus of the amygdala is modulated by repeated acute stress in the immature rat. J. Neuroendocrinol. 1998 in press.
20. Hauser WA. The prevalence and incidence of convulsive disorders in children. Epilepsia. 1994; 35 Suppl. 2:S1–S6. [PubMed: 8275976]
21. Hollrigel GS, Chen K, Baram TZ, Soltesz I. The pro-convulsant actions of corticotropin-releasing hormone in the hippocampus of infant rats. Neuroscience. 1998; 84:71–79. [PubMed: 9522363]
22. Holmes GL. Epilepsy in the developing brain: lessons from the laboratory and clinic. Epilepsia. 1997; 38:12–30. [PubMed: 9024181]
23. Holmes GL, Thompson JL. Effects of kainic acid on seizure susceptibility in the developing brain. Brain Res. 1988; 467:51–59. [PubMed: 3359330]
24. Holmes GL, Werner S, Liu Z, Carmant L, Mikati M. Adverse effects of excitatory amino acid antagonists on the developing brain. Ann. Neurol. 1994; 36:494.
25. Holmes KH, Bilkey DK, Laverty R, Goddard GV. The *N*-methyl-d-aspartate antagonists aminophosphonovalerate and carboxypiperazinephosphonate retard the development and expression of kindled seizures. Brain Res. 1990; 506:227–235. [PubMed: 1967965]
26. Insel TR, Battaglia G, Fairbanks DW, De Souza EB. The ontogeny of brain receptors for corticotropin-releasing factor and the development of their functional association with adenylate cyclase. J. Neurosci. 1988; 8:4151–4158. [PubMed: 2846796]
27. Jensen FE, Blume H, Alvarado S, Firkusny I, Geary C. NBQX blocks acute and late epileptogenic effects of perinatal hypoxia. Epilepsia. 1995; 36:966–972. [PubMed: 7555960]
28. Lothman EW, Collins RC. Kainic acid induced limbic seizures: metabolic, behavioral, electroencephalographic and neuropathologi- cal correlates. Brain Res. 1981; 218:299–318. [PubMed: 7272738]
29. Lyons MK, Anderson RE, Meyer FB. Corticotropin releasing factor antagonist reduces ischemic hippocampal neuronal injury. Brain Res. 1991; 545:339–342. [PubMed: 1860056]
30. Maecker H, Desai A, Dash R, Rivier J, Vale W, Sapolsky R. Astressin, a novel and potent CRF antagonist, is neuroprotective in the hippocampus when administered after a seizure. Brain Res. 1997; 744:166–170. [PubMed: 9030428]

31. Marrosu F, Fratta W, Carcangiu P, Giagheddu M, Gessa GL. Localized epileptiform activity induced by murine CRF in rats. Epilepsia. 1988; 29:369–373. [PubMed: 3260555]
32. Meldrum BS, Craggs MD, Durmuller N, Smith SE, Chapman AG. The effects of AMPA receptor antagonists on kindled seizures and on reflex epilepsy in rodents and primates. Epilepsy Res. 1992 Suppl. 9:307–311.
33. Morimoto K, Holmes KH, Goddard GV. Kindling-induced changes in EEG recorded during stimulation from the site of stimulation: III. Direct pharmacological manipulations of the kindled amygdala. Exp. Neurol. 1987; 97:17–34. [PubMed: 2884127]
34. Morimoto T, Kida K, Nagao H, Yoshida K, Fukuda M, Takashima S. The pathogenic role of the NMDA receptor in hyperthermia-induced seizures in developing rats. Dev. Brain Res. 1995; 84:204–207. [PubMed: 7743639]
35. Oliverio A, Castellano C, Puglisi-Allegra S, Renzi P. Diurnal variations in electroconvulsive shock-induced seizures: involvement of endogenous opioids. Neurosci. Lett. 1985; 57:237–240. [PubMed: 4041021]
36. Olney JW. Excitotoxic amino acids and neuropsychiatric disorders. Annu. Rev. Pharmacol. Toxicol. 1990; 30:47–71. [PubMed: 2188577]
37. Sherwood, NM.; Timiras, PS. A Stereotaxic Atlas of the Developing Rat Brain. Berkeley: University of California Press; 1970.
38. Shinnar, S.; Moshe, SL. Age specificity of seizure expression in genetic epilepsies. In: Anderson, VE.; Hauser, WA.; Leppik, IE.; Noebels, JL.; Rich, SS., editors. Genetic Strategies in Epilepsy Research, Epilepsy Res. Elsevier; 1991. p. 69-85.
39. Smith BN, Dudek FE. Age-related epileptogenic effects of corticotropin-releasing hormone in the isolated CA1 region of rat hippocampal slices. J. Neurophysiol. 1994; 72:2328–2333. [PubMed: 7884462]
40. Stafstrom CE, Tandon P, Hori A, Liu Z, Mikati MA, Holmes GL. Acute effects of MK-801 on kainic acid-induced seizures in neonatal rats. Epilepsy Res. 1997; 26:335–344. [PubMed: 9095395]
41. Strijbos PJ, Relton JK, Rothwell NJ. Corticotrophin-releasing factor antagonist inhibits neuronal damage induced by focal cerebral ischaemia or activation of NMDA receptors in the rat brain. Brain Res. 1994; 656:405–408. [PubMed: 7820601]
42. Swanson LW, Sawchenko PE, Rivier J, Vale WW. Organization of ovine corticotropin releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology. 1983; 36:165–186. [PubMed: 6601247]
43. Vale W, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science. 1981; 213:1394–1397. [PubMed: 6267699]
44. Virgili M, Migani P, Contestabile A, Barnabei O. Protection from kainic acid neuropathological syndrome by NMDA receptor antagonists: effect of MK-801 and CGP 39551 on neurotransmitter and glial markers. Neuropharmacology. 1992; 31:469–474. [PubMed: 1356249]
45. Watts AG, Swanson LW. Diurnal variations in the content of preprocorticotropin-releasing hormone messenger ribonucleic acids in the hypothalamic paraventricular nucleus of rats of both sexes as measured by in situ hybridization. Endocrinology. 1989; 125:1734–1738. [PubMed: 2788078]
46. Weiss GK, Castillo N, Fernandez M. Amygdala kindling rate is altered in rats with a deficit in the responsiveness of the hypothalamo–pituitary–adrenal axis. Neurosci. Lett. 1993; 157:91–94. [PubMed: 8233039]
47. Weiss SR, Post RM, Gold PW, Chrousos G, Sullivan TL, Walker D, Pert A. CRF-induced seizures and behavior: interaction with amygdala kindling. Brain Res. 1986; 372:345–351. [PubMed: 3486694]
48. Wozniak DF, Olney JW, Kettinger L III, Price M, Miller JP. Behavioral effects of MK-801 in the rat. Psychopharmacology. 1990; 101:47–56. [PubMed: 2188277]

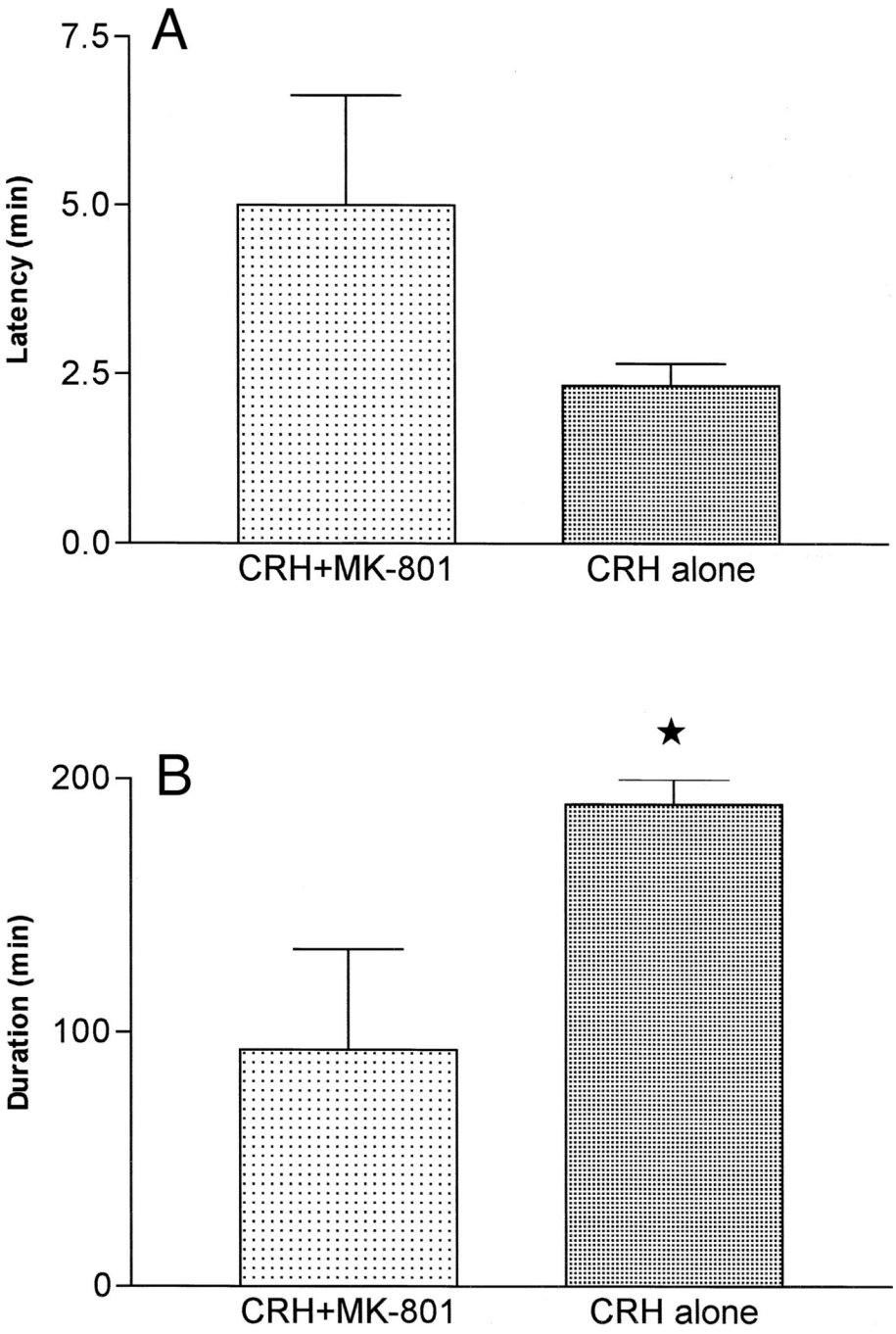

Fig. 1.

Effect of (+)MK-801 on CRH-induced seizures. The non-competitive NMDA antagonist (+)MK-801 (0.2 mg/kg) was administered intraperitoneally to 10 day old rats 15 min prior to intracerebroventricular infusion of CRH (150×10^{-12} mole). Latency to onset of oral automatisms (A) and duration of behavioral seizures (B) were recorded. All values are means ± S.E.M. *Indicates statistical significance ($p < 0.05$; Student's *t*-test). CRH = corticotropin releasing hormone.

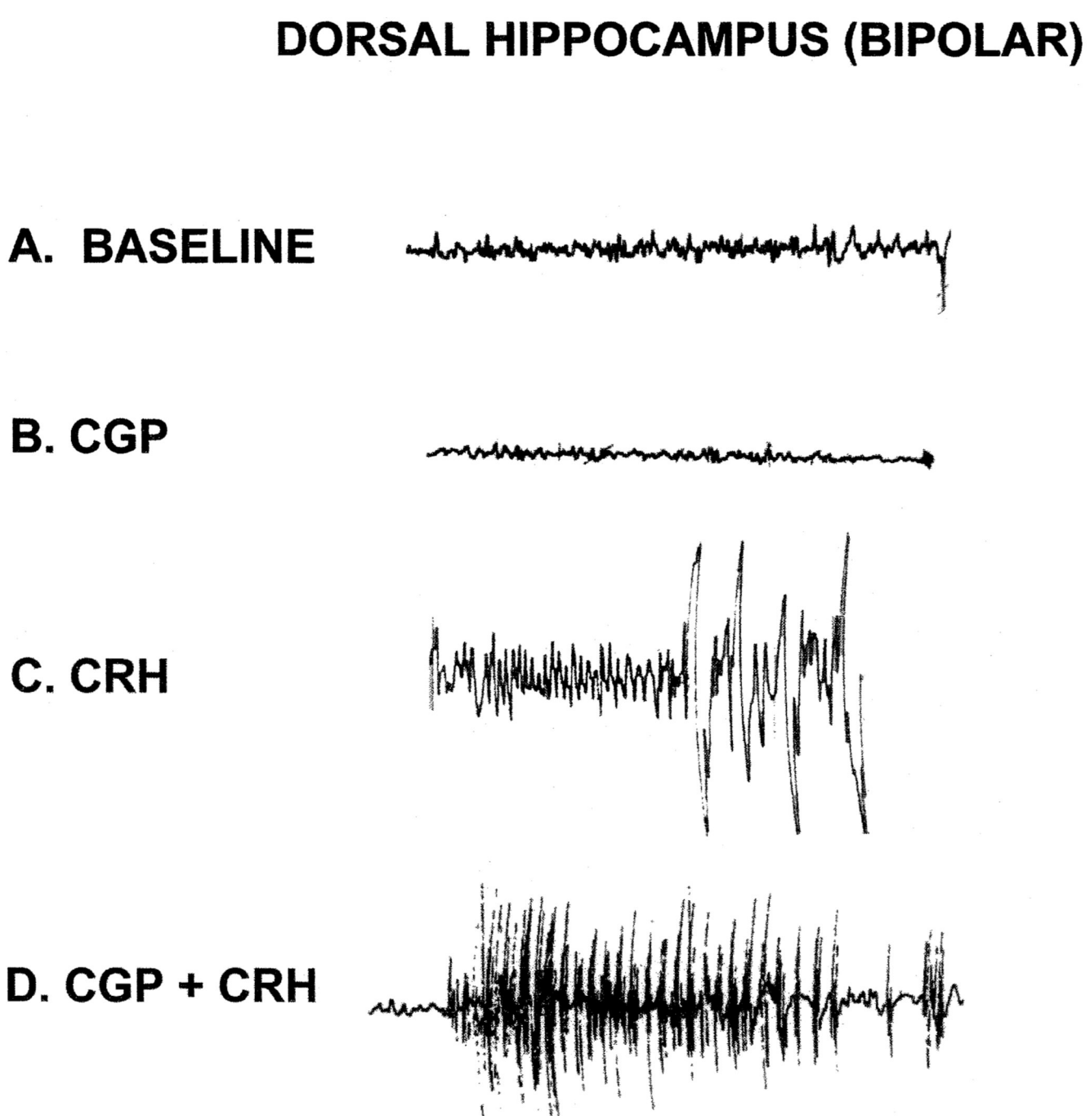

Fig. 2.

Effect of CGP-39551 on epileptic discharges induced by CRH. The tracings depict bipolar dorsal hippocampal recordings from 10 day old rats. (A) Baseline recordings 5 min prior to corticotropin releasing hormone (CRH) administration. (B) 40 min after intraperitoneal injection of the competitive NMDA antagonist CGP-39551 (10 mg/kg). (C) 20 min after intracerebroventricular (i.c.v.) infusion of CRH (150×10^{-12} mole). (D) Epileptiform discharges recorded 60 min following CRH administration in a rat pretreated with CGP-39551 (10 mg/kg) 40 min prior to i.c.v. infusion of CRH (150×10^{-12} mole) to permit absorption. Horizontal bar = 1 s; Vertical bar = 50 μV.

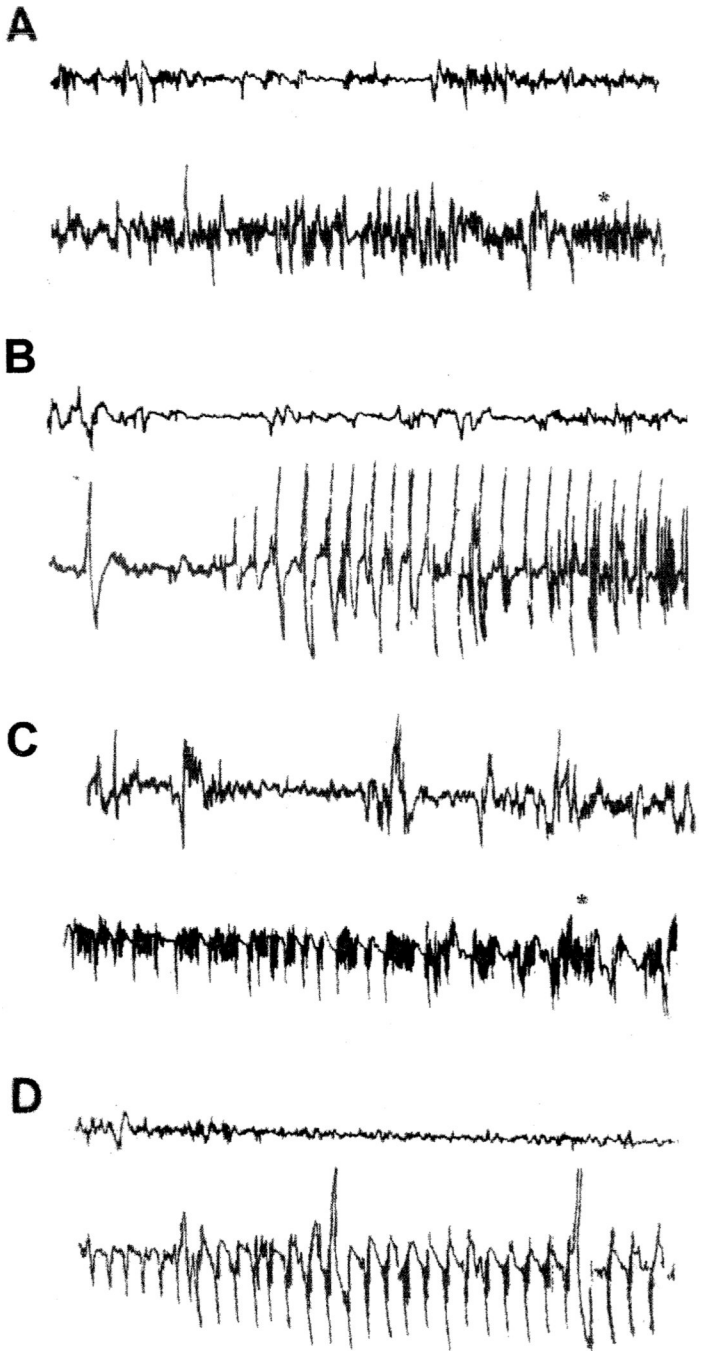

Fig. 3.

Effect of KA-induced seizures on EEG recordings. Bipolar cortical (top tracing) and hippocampal (bottom tracing) recordings in four 10 day old rats. Recordings were obtained 55–60 min after intraperitoneal administration of 1 mg/kg kainic acid (KA). Cortical recordings consist of low-voltage non-rhythmic activity while hippocampal leads show rhythmic epileptiform discharges. Behaviorally, animals A,C displayed oral and motor automatisms(*) and animals B,D were motionless, in a ‘trance-like’ state. Horizontal bar = 1 s; Vertical bar = 50 μV.

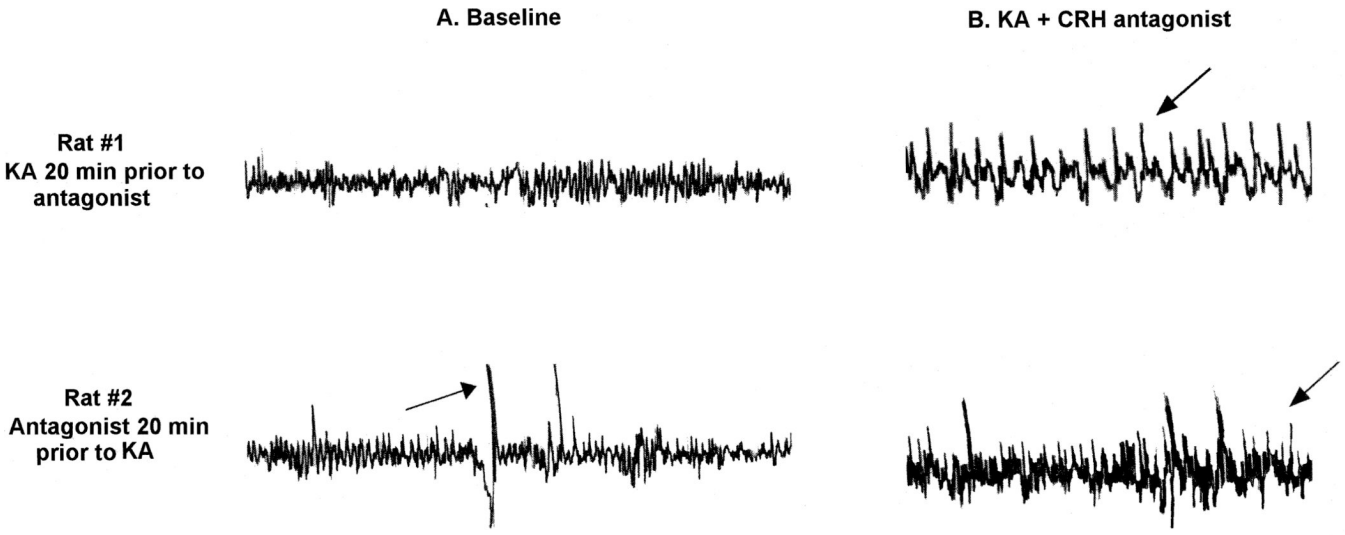

Fig. 4.

Effect of CRH antagonist on epileptic discharge of KA-induced seizures. The tracings represent bipolar dorsal hippocampal recordings from two 13-day-old rats. (A) Baseline recordings 3 min prior to kainic acid (KA) and corticotropin releasing hormone (CRH) antagonist administration show non-rhythmic, low-voltage activity. Note the motion artifact (arrow) (B) 45–50 min after administration of KA (1 mg/kg) and CRH antagonist (1320×10^{-12} mole). Rat #1 received KA intraperitoneally (i.p.) 20 min prior to intracerebroventricular (i.c.v.) infusion of CRH antagonist, and rat #2 received i.c.v. infusion of CRH antagonist 20 min prior to i.p. injection of KA. Hippocampal leads from both animals show rhythmic epileptiform discharges (arrows). Horizontal bar = 1 s; Vertical bar = 50 μV.

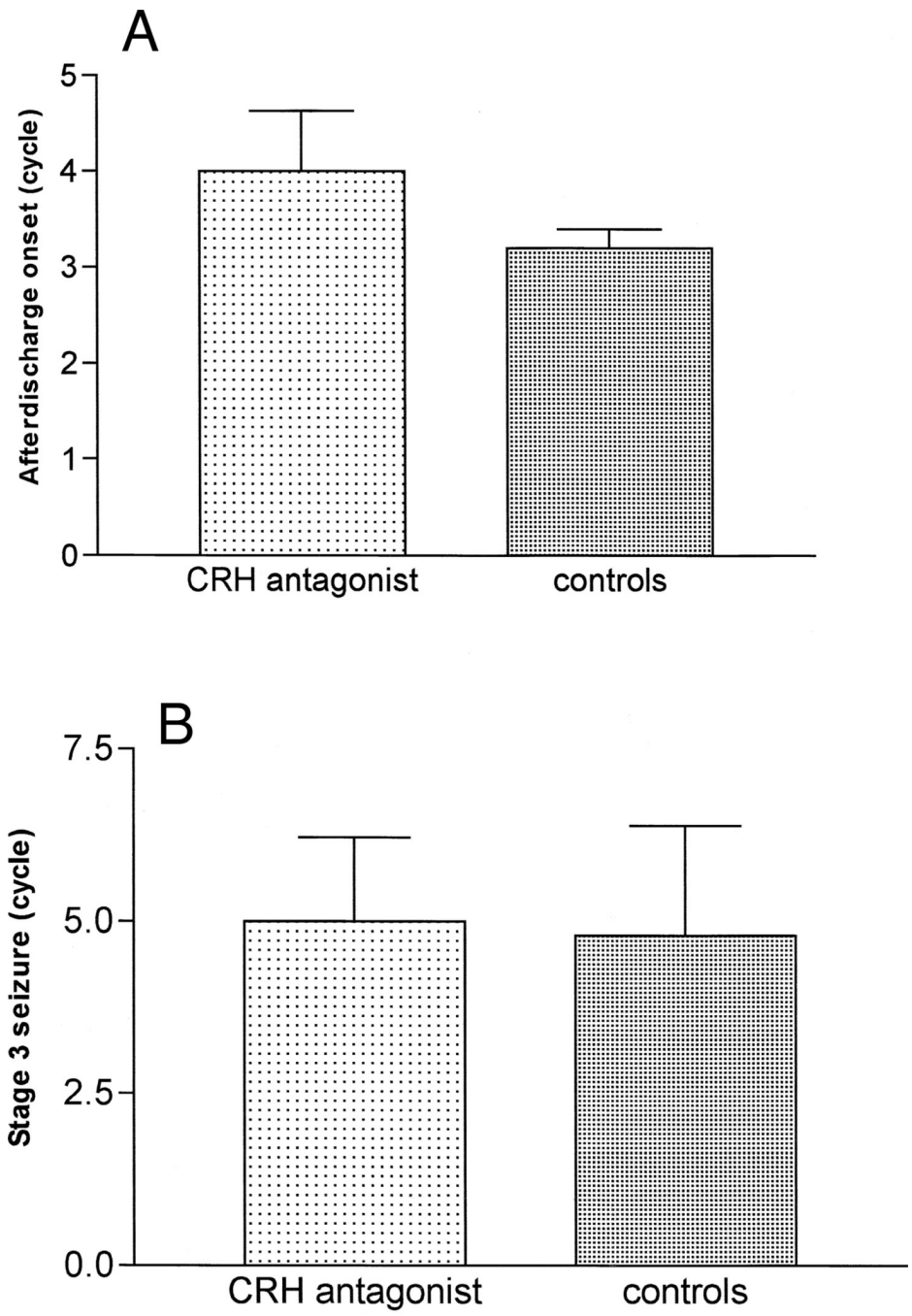

Fig. 5.

Effect of CRH antagonist on the acquisition of kindling. Prior to kindling, corticotropin releasing hormone (CRH) antagonist (1320×10^{-12} mole) or vehicle were given via intracerebroventricular infusion to infant rats. Number of kindling cycles to after discharge onset (A) and number of cycles to stage 3 seizure activity (B) were noted in each group. All values are means ± S.E.M. No statistically significant difference ($p > 0.05$; One-way analysis of variance) was detected between experimental and control groups for either parameter.

Table 1

Grading of KA-, CRH- and kindling-induced behaviors in infant rats

Stage	Kindling	CRH seizures	KA seizures
	Behavior	**Behavior**	**Behavior**
0	behavior arrest	–	behavior arrest
1	head bob, facial movement	jaw myoclonus	scratch, groom
2	'chewing'/neck flexion	licking, chewing	constant chew
3	unilateral clonus	forepaw clonus	clonus, tonic tail
3.5	alternating clonus	'swimming', WDS	WDS, alt. clonus
4	limb rotation, bilateral clonus	–	bilateral clonus
5	loss of balance, extension	loss of balance	loss of balance

The stages for kindling are modified from [7]; CRH- and KA-induced behaviors were based on observations in > 400 and 80 pups, respectively [8–10,12] and on unpublished observations. CRH = corticotropin releasing hormone; KA = kainic acid; WD = wet dog shakes; alt = alternating.

Table 2

Lack of effect of CGP-39551 on the behavioral manifestations of CRH-induced seizures

Treatment	*N*	Latency (min)	Duration (min)
CRH (150×10^{-12} mole)	16	10.1± 2.0	140.3 ± 13.0
CRH + CGP (30 mg/kg)	9	No seizures[a]	No seizures
CRH + CGP (15 mg/kg)	3	15, No seizures (2/3)[b]	5, No seizures (2/3)[b]
CRH + CGP (10 mg/kg)	3	6.6±2.8	14.0±1.60
CRH + CGP (3 mg/kg)	3	10.3±1.2	58.3±4.0

[a] Abnormal behavior.

[b] A single animal had seizures with a latency of 15 min. Two others did not have seizures.

Dose effect of administration of the competitive NMDA antagonist CGP-39551 (10 mg/kg) 40 min prior to infusion of CRH (150×10^{-12} mole) into the lateral cerebral ventricle of infant rats. Duration and latency to onset of limbic automatisms following infusion of CRH were monitored. All values are means ± S.E.M. CRH = corticotropin releasing hormone.

Table 3

Determination of threshold convulsant dose of KA in the 10 day old rat

Dose (mg/kg)	*N*	Latency (min)	Duration (min)
0.2	8	72.1 ± 5.5[a]	18.8 ± 3.2[a]
0.3	12	28.1 ± 2.5	43.3 ± 10.6
0.8	3	23.0 ± 0.6	120.0 ± 2.8
1.0	8	20.6 ± 3.3	160.2 ± 3.2

[a]Only 6 of 8 rats had seizures.

KA was administered intraperitoneally to infant rats, while controls (n = 8) received equal volumes of vehicle, to determine a threshold dose. Latency to the onset of limbic automatisms and the duration of seizures were recorded. All values are means ± S.E.M. KA = kainic acid.

Table 4

Interaction of threshold doses of CRH and KA

Treatment	*N*	Seizure duration (min)	Behavioral features
KA	8	28.1 ± 3.8[a]	oral automatisms, groom, scratch
CRH + KA	9	63.7 ± 8.2	WDS, severe automatisms
CRH	8	62.5 ± 6.0	oral automatisms, rare WDS

Ten day old rat pups were given KA (0.2 mg/kg) intraperitoneally alone or followed 30 min later by intracerebroventricular infusion of 22.5–30 × 10^{-12} mole of CRH (see Section 2). A third group received CRH only. Control groups carrying cannula alone or receiving vehicle only did not have any abnormal behavior.

[a]Indicates significant difference from the other two groups ($p < 0.05$) determined by one-way ANOVA analysis.

CRH = corticotropin releasing hormone; KA = kainic acid; WD = wet dog shakes.

Table 5

Effect of CRH antagonists on KA induced seizures

Treatment	*N*	Latency (min)	Duration (min)
KA	8	20.6 ± 3.3	160.2 ± 3.2
CRH antagonist + KA	14	20.0 ± 6.7	145.7 ± 11.2
CRH	11	7.1 ± 0.8	108.5 ± 13.5
CRH + CRH antagonist	12	21.4 ± 4.4[a]	52.2 ± 14.4[a]

[a]Only 8 of 12 rats had seizures.

The CRH antagonist, (9–41)-α-helical CRH (1320×10^{-12} mole), or vehicle, were administered by intracerebroventricular infusion 15–20 min prior to the intraperitoneal injection of KA. KA dose (1 mg/kg) was determined to be a moderate one for the infant rat. An additional group of five animals received the CRH antagonist alone, and did not display behavioral abnormalities. The experimental (KA) and the control groups (KA + CRH antagonist) do not statistically differ in the latency to or the duration of kainic acid induced seizures ($p > 0.05$). CRH antagonist does block or significantly suppress CRH-induced seizures ($p < 0.05$; Student's *t*-test). CRH = corticotropin releasing hormone; KA = kainic acid.

Table 6

Effect of four CRH infusions on threshold for KA seizures

Treatment	*N*	Automatisms		Motor seizures	
		Latency (min)	Duration (min)	Latency (min)	Duration (min)
KA only[a]	8	69.7 ± 7.0	10.8 ± 1.2	No seizures	No seizures
Cannulated + KA	3	43.3 ± 6.0	27.0 ± 18.0	No seizures	No seizures
CRH + KA	8	30.1 ± 4.0	114.0 ± 26.0	35.9 ± 3.0	114.0 ± 26.0[b]

[a] Two pups had no automatisms and were excluded from calculations.

[b] Mixed with automatisms.

A threshold dose of KA (0.2 mg/kg) was administered intraperitoneally to 12 day old rats after pretreatment with four intracerebroventricular infusions of CRH (150×10^{-12} mole). Latency and duration of automatisms and motor seizures were recorded after injection of KA. All values are means ± S.E.M. CRH = corticotropin releasing hormone; KA = kainic acid.