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Chapter 13

Mechanisms of non-genetic, provoked seizures in the neonatal and infant brain

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Summary

The immature human, particularly during the neonatal and infancy periods, has a high propensity to develop seizures due to fever, trauma, hypoxia and other adverse circumstances. The mechanisms by which these instigators lead to enhanced neuronal excitability and seizures are not fully understood. We proposed that the common denominator of fever, trauma and other pro-convulsant 'stressors' was their activation of the peptide corticotropin releasing hormone (CRH), the key mediator of the limbic-neuroendocrine stress response.

We tested the hypothesis that CRH acts at specific limbic receptors to increase neuronal excitability, and that this effect is limited to the developing brain. In the infant rat, CRH was shown to be a potent convulsant: administration of picomolar doses caused limbic status epilepticus. CRH gene expression was found to increase after a variety of stressors in both the immature and adult rat, but the peptide's potency was maximal during infancy because hippocampal and amygdala CRH-receptors were present in maximal concentrations during this developmental period.

Thus, activation of the stress neurohormone CRH during fever, trauma and other stressors may increase limbic neuronal excitability and lead to seizures. The developmental regulation of the CRH receptor involved may determine convulsant potency of CRH, and limit the induction of 'reactive' seizures by proconvulsant stressful signals to specific developmental periods. The structure and regulation of CRH are highly conserved across species. Therefore, increased activity of this excitatory neuromodulator may contribute to the generation of age-specific seizures in the human neonate and infant.

Introduction

The immature brain is more excitable than the fully mature brain (see Holmes, 1997 for a recent review). This concept is manifest in the human by a much higher incidence of seizures in the infant and child, as compared with the adult (Hauser, 1995). Enhanced excitability and a higher propensity to develop seizures have also been demonstrated in immature experimental animals including rodents (primarily rats), cats (Purpura *et al.,* 1968) and monkeys (Kubova & Moshé, 1994). A number of characteristics of the developing neuronal circuitry which may account for this enhanced excitability have been documented, and are discussed in detail in other chapters of this book. For example, gamma-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the mature central nervous system (CNS), has depolarizing and excitatory properties during the first postnatal week in the rat (Ben-Ari *et al.,* 1994). In addition, excitatory amino acid receptors are both more abundant in the immature brain (Johnston, 1996) and possess a subunit-makeup which promotes

neuronal depolarization (Monyer *et al.,* 1994). These factors are thought to promote an altered excitation–inhibition balance during development. At the circuit level, these properties may provide the mechanism for the increased neuronal interaction evident from the robust long-term potentiation observed during the second postnatal week in both hippocampal and cortical synapses (McDonald & Johnston, 1990; Crair & Malenka, 1995). Furthermore, the number of cortical and hippocampal excitatory synapses is increased during the second postnatal week (Swann *et al.,* 1993) and the regulation of the propagation of convulsant discharges by the substantia nigra is immature (Moshé *et al.,* 1994). All of these factors favour enhanced excitation and a susceptibility for seizure generation and propagation.

Despite its enhanced excitability and seizure susceptibility, the immature brain is mainly engaged in normal neuronal activity, and most immature humans and rodents do not have spontaneous seizures. Furthermore, the onset of spontaneous seizures which are determined by the presence of an abnormal genetic makeup, i.e. genetic epilepsies, typically occurs beyond the neonatal and infancy periods in both the human and rat (Noebels, 1996). During these early developmental periods, seizures are most commonly triggered, i.e. they are induced by abnormal alterations of either the internal or external environment. Thus, trauma, anoxia, fever or hypoglycemia are all stressful signals which rapidly lead to seizures during early postnatal development. In fact, in the case of fever (Baram *et al.,* 1997b) and anoxia (Jensen *et al.,* 1991), seizures are induced exclusively and uniquely during a restricted vulnerable period in the rat, limited to the second postnatal week. Human febrile convulsions, the most common type of seizures, are restricted to infancy and early childhood (Berg *et al.,* 1995). Anoxia-related seizures occur primarily in the full term neonate (Volpe, 1981) . Other non-genetic seizures such as infantile spasms, which have been linked to a large number of central nervous system injuries, malformations, infections and stressors, are highly age-specific and primarily restricted to the first year of life (Baram *et al.,* 1993; Dulac *et al.,* 1993).

Thus, although the immature brain is susceptible to seizures, the propensity to generate them is selective: spontaneous intrinsic and genetic seizures are relatively uncommon, while 'reactive' seizures induced by a large variety of adverse environmental events are prevalent. These facts raise several questions:

- How do abnormal excitation and seizures arise after external or internal perturbation of the normal neuronal environment?
- Why are many of these 'reactive' seizures limited to the early developmental periods?

The focus of this chapter is on the mechanisms by which adverse external stimuli are integrated to result in altered neuronal excitability. Specifically, this chapter describes studies of the neuronal and neuroendocrine modulators which are upregulated by stressful signals and alter neuronal excitability during the developmental period. The molecular events by which stress increases neurotransmission mediated by the stress neurohormone corticotropin releasing hormone (CRH) are discussed, as well as the excitatory effects of this neuropeptide on limbic neurons in the amygdala and hippocampus. Finally, the putative causes for the age-selectivity of CRH-mediated excitation are presented.

Methods

Animals

For the purpose of these studies in the rat, 'neonatal' denotes postnatal days 0–7, while 'infant' refers to postnatal days 7–14. The pertinent developmental correlations between human and rat brain are addressed in the discussion.

Pups were offsprings of time-pregnant Sprague–Dawley derived rats. Mothers were housed singly, kept on a 12 h light/dark schedule and given access to unlimited lab chow and water (Yi *et al.,* 1993; Yi & Baram, 1994). Time of birth of pups was determined every 12 h, and the day of birth was

considered day 0. Litters were culled to 12 pups on the first postnatal day and kept in quiet, uncrowded AALAC-approved facilities at a room temperature of 21–22 °C.

Unless indicated otherwise, all determinations of CRH gene expression and of the peptide's receptors were performed on 'stress free' animals, sacrificed within 45 s of disturbance (Yi & Baram, 1994). Detailed discussion of these procedures, and of the standardized, age-appropriate stress, acute cold-separation, are found elsewhere (Yi & Baram, 1994; Avishai-Eliner *et al.,* 1995).

Surgical procedures, CRH administration and EEG recordings

CRH and the CRH receptor antagonists were administered, using a micro-infusion pump, into the lateral cerebral ventricle via an indwelling cannula, while the pups were freely moving in a heated Plexiglas chamber (Baram & Schultz, 1991, 1995; Baram *et al.,* 1992). Pups were implanted with cannulae 24 h prior to experiments, and the position of the cannulae was verified in all cases. Briefly, stainless steel cannulae were implanted into the lateral ventricles under halothane anaesthesia, using an infant-rat stereotaxic apparatus (Baram & Schultz, 1991; Yi & Baram, 1993). CRH doses were 22 to 750 pmoler, delivered in 0.5–1 μl. Subsequent to the infusion of CRH the latency to seizure onset and the duration of the resulting seizures were monitored for a minimum of 180 min. Animals were scored for behavioural limbic seizures every 5 min so that seizure duration is expressed in 5-min epochs (Baram & Schultz, 1991, 1995).

To establish the concordance of the limbic automatisms and motor behaviours that were induced by CRH with epileptic discharges, electroencephalographic (EEG) recording was obtained from relevant brain regions, i.e. dorsal and ventral hippocampus, the amygdala and frontal and parietal cortex (Baram *et al.,* 1992). Separate groups of rats were implanted (in addition to cannulae) with bipolar electrodes directed to the amygdala, hippocampus, or both using coordinates established previously (Baram *et al.,* 1992). EEGs were recorded using a GRASS 78E polygraph, connected via long, flexible wires to freely moving animals. All infusions were carried out at 8–10 a.m., to avoid the effects of circadian variability in endogenous CRH (Watts & Swanson, 1989).

Tissue processing and in situ hybridization histochemistry

For all experiments, brains were rapidly removed onto powdered dry ice and stored at –80 °C. Brains were cut into 20 μm coronal sections with a cryostat and mounted on gelatin-coated slides. Preparation of oligonucleotide probes for CRH and CRF_1, and details of the *in situ* hybridization histochemistry (ISH) and image analysis, have been described elsewhere (Yi *et al.,* 1993, 1994). Briefly, sections were brought to room temperature, air-dried and fixed for 20 min in fresh 4 per cent buffered paraformaldehyde. After a graded ethanol treatment, sections were exposed to acetic anhydride–triethanolamine and dehydrated through 100 per cent ethanol. Sections were prehybridized for 1 h, then hybridized using 0.5×10^6 cpm of the appropriate probe for 20 h at 40 °C in a humidity chamber, using a buffer containing 50 per cent formamide (Yi *et al.,* 1993). Sections were washed in 2 × saline–sodium-citrate buffer (SSC) for 15 min four times at 40 °C, followed by 1 × and 0.3 × SSC for 30 min each at room temperature. The sections were dehydrated through graded ethanol solutions, air-dried and apposed to film (Hyperfilm B-Max, Amersham, IL) for 5–7 days.

Quantitative image analysis of CRH and the CRF_1 receptor mRNAs was achieved using the MCID software image analysis system (Imaging Research, St. Catherine, Ontario, Canada). For CRH, optical density was determined over the hypothalamic paraventricular nucleus (PVN) and the central nucleus of the amygdala; for CRF_1 , optical density was determined over the CA1 and CA3 hippocampal regions, the dentate gyrus, the frontal and piriform cortex and the lateral nucleus of the amygdala. Each point was derived from a minimum four sections from two or three individual rats. Brain-paste standardized values and the ratio of structure/background were both obtained for quantitation (Avishai-Eliner *et al.,* 1996).

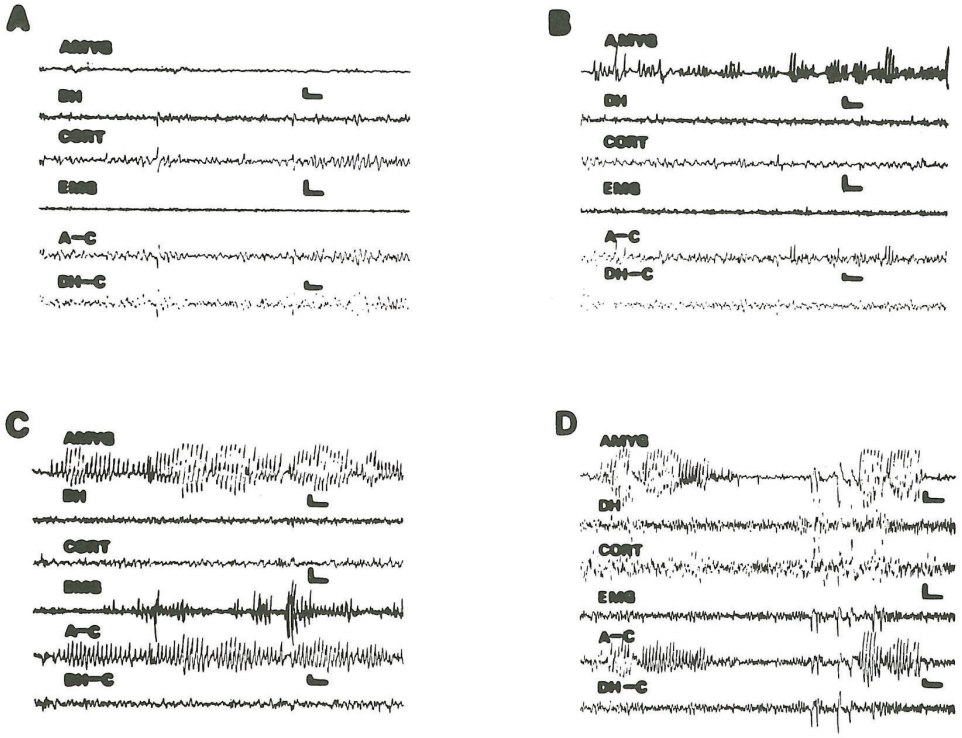

Fig. 1. EEG of a 13-day-old rat prior to (A) and 2, 35 and 174 min following administration of 150 picomole of CRH into the cerebral ventricle (B, C and D, respectively). Epileptic discharges are evident within 2 min in the bipolar amygdala lead (AMYG) and propagate to the amygdala-cortex (A-C) leads, but not, in this animal, to the contralateral dorsal hippocampus (DH). EMG is a motion-detecting electrode. Vertical bar = 50 μV; horizontal bar = 1 s (modified from Baram et al, (1992) and printed with permission).

Immunocytochemistry (ICC) for CRH

In the hippocampus, the anatomical relationships and synaptic interactions among neurons are critical for their functional integration (Freund & Buzsaki, 1996). Therefore, immuno-cytochemistry which permits visualization of individual neuronal cell bodies and their processes was used.

The ICC was a modification of the standard VECTOR ABC protocol, using a CRH antiserum generously provided by Dr W.W. Vale (Salk Inst., La Jolla, CA). Briefly, perfused, sucrose-cryoprotected 20 μm coronal sections were postfixed for 10 min in 4 per cent para-formaldehyde, rinsed twice in Tris-buffered saline and blocked using 0.5 per cent BSA. The sections were incubated overnight with the antiserum to CRH, rinsed and subjected to the appropriate biotinylated second antibody for 1 h. The signal was amplified (Vectastain ABC Elite, Vector), and CRH-immunoreactive cells were visualized using diamino-benzidine with Nickel ion enhancement (Yan *et al.*, 1996).

Statistical analysis was performed using non-parametric tests (Mann–Whitney unpaired two tailed comparison, INSTAT software) without assumptions regarding the distribution of values.

Results

The neuro-excitatory effects of CRH are far more pronounced in the immature brain

In the neonatal and infant rat (first and second postnatal weeks, respectively), CRH given into the cerebral ventricles produced severe seizures. The latency to seizure onset depended on the dose of the peptide, but was as short as 1–2 min. Both the electrographic and behavioural seizures caused by CRH persisted for several hours (Baram & Schultz, 1991). The seizures occurred in the developing rats with doses 200-fold lower than those required for seizure generation in adults (7.5×10^{-12} mol). Furthermore, these doses of CRH did not result in neuroendocrine effects such as elevation of plasma corticosterone (Baram & Schultz, 1991). Once seizures commenced, however, the stress associated

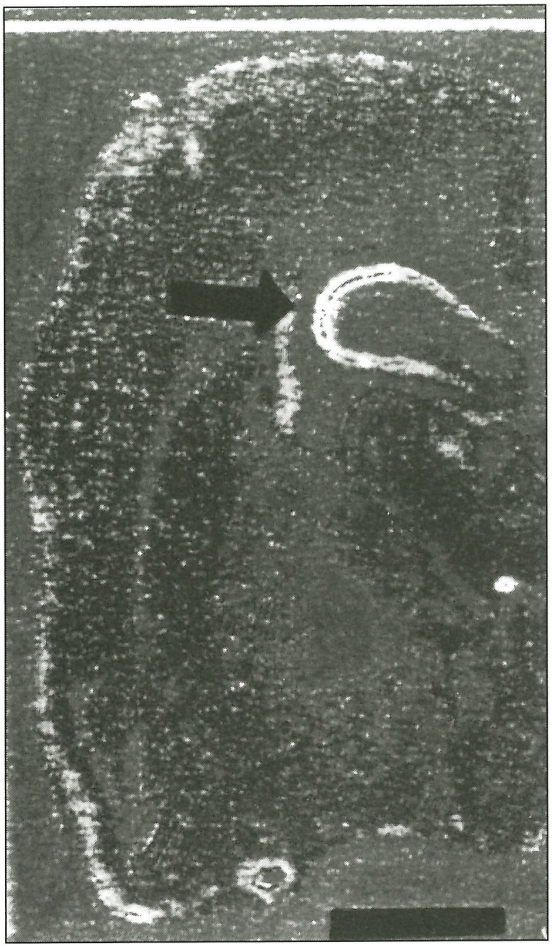

Fig. 2. A coronal hemi-section at the level of the diencephalon from a nine-day-old rat. The section was subjected to in situ hybridization using an ^{35}S-labelled deoxynucleotide probe complementary to the coding sequence of CRF_1-mRNA. This computer-generated false-colour image reveals high levels (pink-white) of the receptor-mRNA in the CA3 region of the dorsal hippocampus. Bar = 0.2 mm (from Avishai-Eliner et al. (1996), with permission).

with the ongoing electrographic and behavioural ictus led to marked elevations of plasma corticosterone (Baram & Schultz, 1991 and unpublished observations). The behavioural aspects of CRH-induced seizures conformed to the pattern observed in seizures with a limbic origin. The origin of CRH-induced epileptiform discharges was defined using multiple bipolar depth electrodes directed to limbic structures, i.e. the amygdala and the dorsal and ventral hippocampus, in addition to cortical electrodes. This approach permitted localization of the onset of CRH-induced epileptiform discharges to the amygdala (Fig. 1).

The mechanisms by which CRH activates limbic neurons have been investigated at a cellular level. In the *in vitro* hippocampal slice preparation, Smith & Dudek (1994) found an increase in the amplitude of population spikes in the CA1 region. Using single cell-patch clamp recording, Hollrigel *et al.* (1996, 1998) have demonstrated that CRH dramatically increased the frequency of spontaneous firing in CA3 pyramidal neurons.

The high potency of CRH in the developing amygdala and hippocampus may be due to the developmental profile of the CRH receptors

In situ hybridization histochemistry analysis demonstrated that mRNA levels of the first member of the CRH-receptor family, CRF_1, were high throughout development (Avishai-Eliner *et al.,* 1996). In the hippocampal CA3, CRF_1 -mRNA levels peaked during the second postnatal week (Fig. 2). Since recent work has documented that the CRF_1 receptor mediates the excitatory effects of CRH (Baram *et al.*, 1997a), high levels of this receptor in target neurons in the hippocampus may predispose the developing brain to the proconvulsant actions of CRH.

Levels of CRF_1 -mRNA in the amygdala were also maximal in the immature rat, consistent with our electrophysiological studies which localized the origin of the CRH-induced seizures to this region (Baram *et al.,* 1992) (Fig. 3).

Age-specific stressors increase the levels of CRH in the hypothalamus and amygdala, and induce apparent release of the peptide in hippocampus

Cold exposure, a prototypical age-appropriate environmental stress, increases CRH synthesis in the hypothalamus

Cold-stress has been found to be a powerful, age-specific stimulus in the developing rat, due to the lack of fur and immature thermoregulation during the first two postnatal weeks (Yi & Baram, 1994). The paradigm was precisely defined and shown to result in significant augmentation of hypothalamic CRH-mediated neurotransmission in the hypothalamic–pituitary–adrenal axis (Yi & Baram, 1994). In the infant rat, i.e. during the second postnatal week, cold-stress caused a rapid secretion of CRH

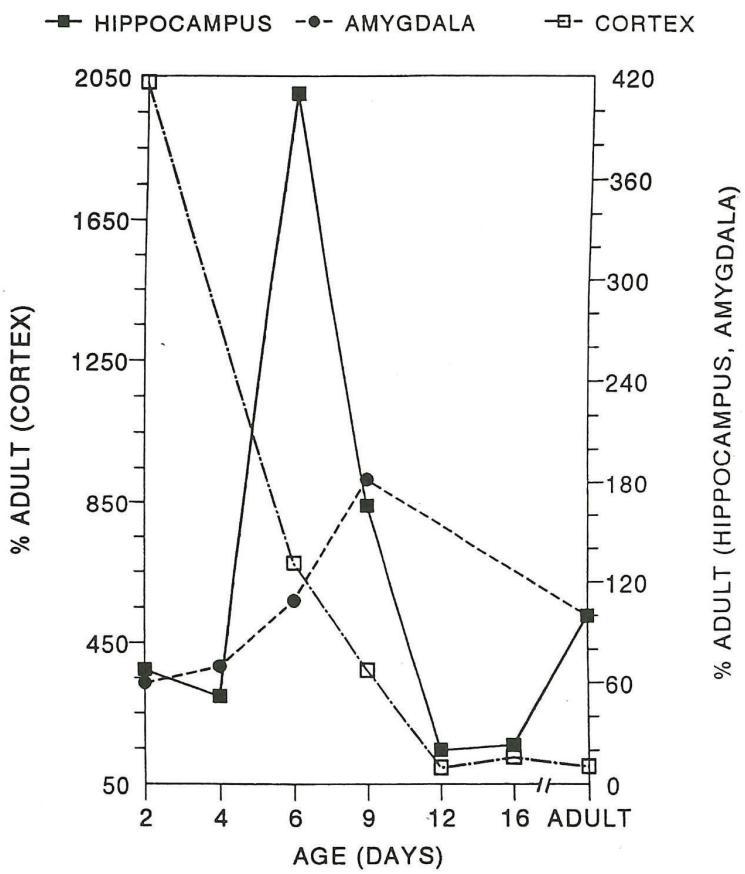

Fig. 3. Schematic quantitative representation of the developmental profile of CRF1-mRNA in the hippocampus, amygdala and cortex of the rat. Values are described as per cent of adult levels. The dramatic peak of hippocampal and amygdala CRF1-mRNA at the onset of the second postnatal week is evident (from Avishai-Eliner et al. (1996), with permission).

from peptidergic neurons, leading to elevation of plasma glucocorticoids. Within 4 h after the stress, an upregulation of CRH gene expression resulted in significantly increased CRH-mRNA levels in the hypothalamic PVN (Fig. 4).

A number of stressful conditions increase CRH production in the amygdala of infant rats

Synthetic CRH administered i.c.v. induces seizures which involve a limbic circuit including amygdala and hippocampus. The neuroanatomical origin of endogenous CRH which could mediate these limbic seizures has not been fully elucidated. The central nucleus of the amygdala (ACE) is a major site of CRH containing neurons and terminals (Sawchenko *et al.*, 1993; Gray & Bingaman, 1996). About 1500 ACE neurons produce CRH, and many are interneurons impinging on cell bodies within the nucleus (Uryu *et al.*, 1992; Gray & Bingaman, 1996). CRH receptors are found in the ACE and in the lateral/basolateral amygdaloid nucleus, which projects to the ACE (Chalmers *et al.*, 1995; Avishai-Eliner *et al.*, 1996). The preferential increase in CRH gene expression in the ACE after stress has been demonstrated in adult animals (Makino *et al.*, 1994). Table 1 shows that CRH-mRNA levels in the ACE are increased under stressful conditions such as hypothermia in 9-day-old rats. A similar increase was documented after kainic acid-induced seizures (and stress) in the infant rat.

Table 1. Effect of the age-specific acute cold stress on steady-state messenger RNA for CRH in the central nucleus of the amygdala in nine-day old rats

Treatment group	(*n*)	CRH-mRNA in ACE	
Controls	(6)	0.316 ± 0.08	
Cold-stressed	(5)	0.546 ± 0.038	$p < 0.05$

CRH-mRNA was analysed in the central nucleus of the amygdala (ACE) of infant rats subjected to cold-separation stress, and sacrificed 4 h later. The experimental paradigm and methods of ISH have been published (Yi & Baram, 1994). N denotes the number of animals per group. One to three sections from each brain were analysed blindly using the MCID image analysis system. Results are expressed as of signal over ACE/background, to account for potential background variation (Baram & Lerner, 1991; Yi *et al.*, 1993; Yi & Baram, 1994).

CRH is found in hippocampal interneurons and may be released by stress

No direct CRH-containing neuronal pathways between the amygdala or hypothalamus and the hippocampus have been documented. Therefore, we tested the hypothesis that CRH producing

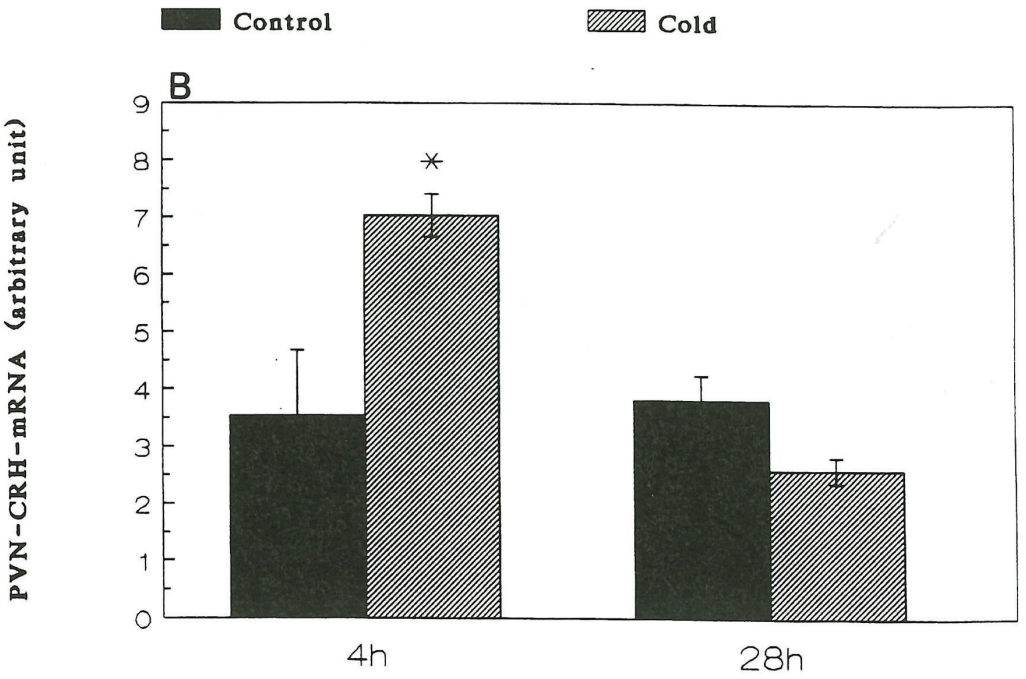

Fig. 4. Effect of cold-separation stress on CRH-mRNA in the paraventricular nucleus of the nine-day-old rat. Pups were subjected to age-appropriate maximal tolerated cold-stress (see text). CRH-mRNA was determined using in situ hybridization histochemistry. Values denote mean ± SEM and were derived as detailed in the Methods section. Asterisk denotes significantly different from control ($p < 0.05$). Modified from Yi & Baram (1994) and printed with permission.

neurons may be present in the hippocampal formation. Immunocytochemistry revealed a population of interneurons which contain CRH (Fig. 5). The CRH-immunoreactive cells possess the morphological features of interneurons and their distribution is similar to that of GABA neurons (Ribak *et al.*, 1978). As described above, synthetic CRH causes excitation of CA3 hippocampal neurons. However, it is not clear whether seizure-inducing stressors, such as hyperthermia, result in a release of CRH from interneurons in the hippocampus. Studies are ongoing to investigate this issue.

Discussion

This chapter addresses the paradox that, despite enhanced excitability of the brain during development, the majority of seizures observed are not spontaneous but must be triggered by fever, anoxia, trauma and similar stressors. We focus on potential mechanisms by which proconvulsant stimuli lead to abnormal excitation in discrete brain regions and result in seizures. The specificity of some of these 'reactive' seizures to restricted developmental periods is also discussed. We propose a scenario in which mechanisms normally involved in the brain's response to threatening or injurious stimuli mediate these seizures. We hypothesize that, in the developing brain, adverse signals such as fever or trauma may result in seizures by up-regulating the release of the excitatory neuropeptide, CRH. This CRH activates specific receptors, leading to enhanced neuronal excitability in a number of limbic circuits (for example, increased firing of CA3 pyramidal neurons).

The strengths of the CRH hypothesis are that it offers a plausible mechanism for the important observation about seizure induction in the immature human and rat. Furthermore, the hypothesis offers discrete predictions which are amenable to experimental testing. For example, it predicts that CRH is a potent convulsant during development and that the peptide's effects decrease with age. The hypothesis further predicts that proconvulsant stressors increase the levels of CRH at sites which are relevant to the origin of reactive seizures and where CRH-receptor expressing neurons can respond by enhanced excitability.

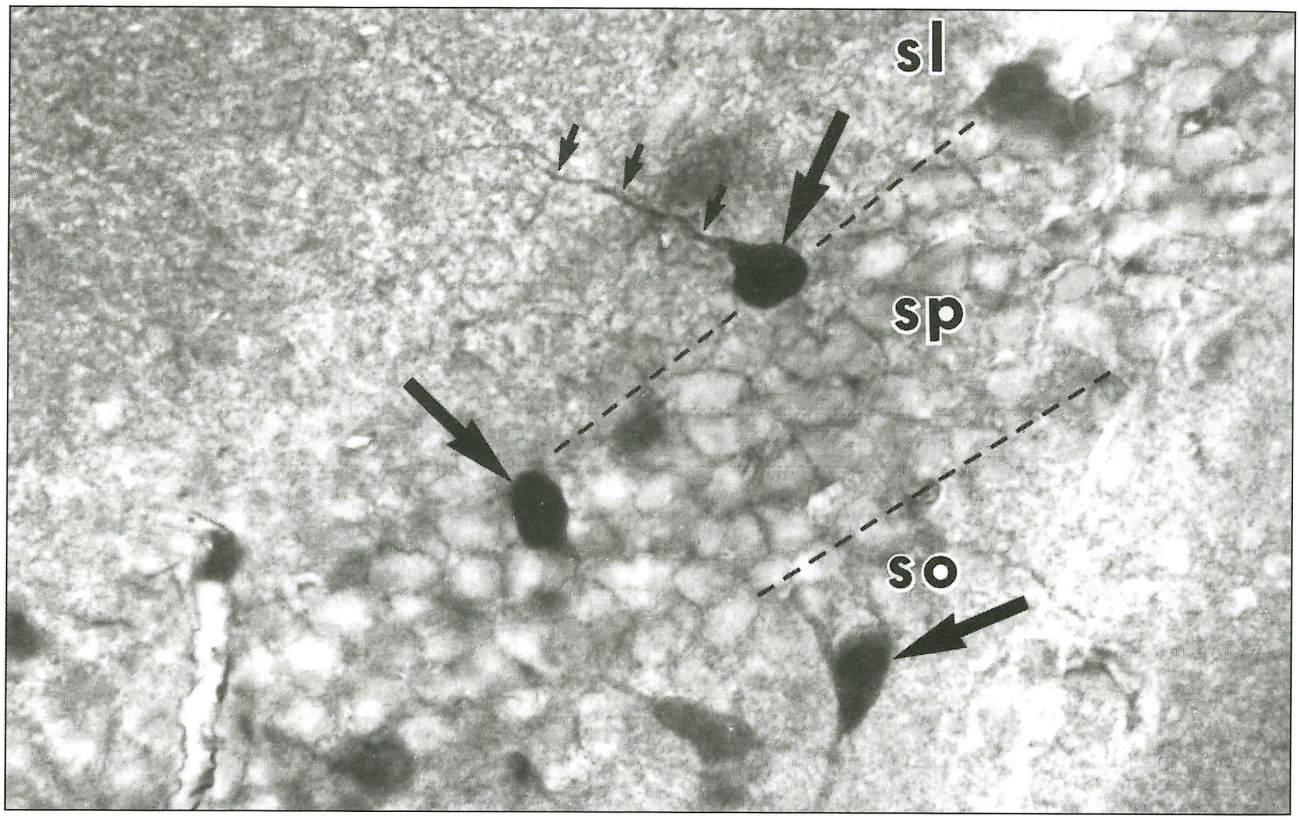

Fig. 5. A photomicrograph of the CA3 hippocampal region showing CRH-immunoreactive non-pyramidal neurons (large arrows). One of the cells, located at the border (dashed line) of strata pyramidale (sp) and lucidum (sl) extends a CRH-immunolabelled dendrite into sl (small arrows). The dendrite of the indicated CRH-labelled neuron in the stratum oriens (so) is located in the same layer, a feature of interneurons. (modified from Yan et al., 1998).

In support of the CRH hypothesis, the experimental findings described demonstrate that the neuropeptide excites neurons in the amygdala and hippocampus both *in vivo* and *in vitro,* leading to prolonged seizures in the immature brain. Studies of hippocampal neurons suggest that CRH enhances the firing of CA3 pyramidal cells (Hollrigel *et al.,* 1998). These effects of the peptide are mediated via activation of specific receptors and are maximal during the second postnatal week in the rat. This is considered to be at least partially due to the high abundance of these CRH receptors in the hippocampus and amygdala during this developmental period.

Clearly, the fundamental role of CRH in the central nervous system (CNS) is not seizure generation. A large body of literature has demonstrated that CRH is a neuropeptide with both neuroendocrine and neuro-transmitter properties (Vale *et al.,* 1981; Young, 1992). The peptide is the primary mediator of the neuroendocrine stress response (Lightman & Harbuz, 1993). The endocrine effects of CRH originate from clusters of peptidergic cells in the hypothalamic paraventricular nucleus (Herman & Cullinan, 1997). CRH is also a neuromodulator in a number of limbic and autonomic brain circuits (Fox & Gruol, 1993; Curtis *et al.,* 1995). CRH-producing neurons are widely but specifically distributed in the brain (Sawchenko *et al.,* 1993), including the central nucleus of the amygdala, which is considered a major source for non-endocrine CRH-mediated neurotransmission (Herman & Cullinan, 1997). Target neurons for the actions of this peptide, expressing specific receptors, are found in many brain regions including the hippocampus (Chalmers *et al.,* 1995; Avishai-Eliner *et al.,* 1996). Physiological effects of CRH on hippocampal neurons include facilitating memory retention and increasing protein-phosphorylation (Lee *et al.,* 1992; Behan *et al.,* 1995). Abnormalities of CRH-mediated neurotransmission may contribute to a number of adult neurological disorders such as depression (Nemeroff, 1992) or Alzheimer disease (Behan *et al.,* 1995).

In general, CRH is an excitatory neurotransmitter (Young, 1992; Curtis *et al.,* 1995). CRH-induced excitation has been demonstrated in the mature rat amygdala (Ehlers *et al.,* 1983; Rainnie *et al.,* 1992; Weiss *et al.,* 1993) and hippocampus (Marrosu *et al.,* 1988; Smith & Dudek, 1994). In the adult rat *in vivo*, the administration of CRH into the cerebral ventricles (i.c.v.) results in epileptiform discharges in the amygdala and the hippocampus, and in limbic seizures with a latency of 3–7 h (Ehlers *et al.,* 1983). There is indirect evidence suggesting that CRH may also be involved in excitotoxic neuronal death in the mature brain: increased levels of CRH have recently been reported in brain regions undergoing injury after kainic-acid-induced status epilepticus (Piekut *et al.,* 1996). Furthermore, the administration of CRH receptor blockers has been reported to decrease ischaemic neuronal injury (Lyons *et al.,* 1991; Maecker *et al.,* 1997).

The data presented in this chapter suggest that CRH may play a unique neuromodulatory role in the developing brain: both the proconvulsant potency of CRH (see above) and the peptide's excitotoxic actions are enhanced in the immature brain (Baram & Ribak, 1995; Ribak & Baram, 1996). These potent effects of CRH are probably due to the large number of limbic CRF_1 receptors which are available to be activated by synthetic CRH during *in vivo* and *in vitro* experiments. A likely cause for the marked reduction in the proconvulsant effects of CRH in the mature brain derives from the demonstration that the abundance of the receptors mediating the peptide's effects diminishes rapidly during the third postnatal week in the rat (Avishai-Eliner *et al.,* 1996).

It should be noted that the studies described above involve rats during the first and second postnatal weeks. Several issues regarding the species and the developmental age of this animal model require discussion. The infant rat was chosen because of the significant body of knowledge which confirms a developmental susceptibility to seizures in this species. Much of the information about brain excitability during development in general is derived from neuroanatomical, electrophysiological and molecular studies of the rat. The period of 'peak excitability' is generally considered to occur during the second postnatal week (Jensen *et al.,* 1991; Swann *et al.,* 1993; Kubova & Moshé, 1994). In addition, the rat and human CRH molecules are identical, and the CRH gene shows a 91 per cent homology in the coding region between these two species, suggesting a remarkable conservation of the function of this peptide across these two species. Furthermore, known regulatory mechanisms of CRH gene expression in the human CNS are considered very similar to those in the rat. The age of the rat which is comparable to infancy and early childhood in the human has not been addressed satisfactorily. Indirect species correlations, comparing corpus callosum development in the cat to both human and rat development constitute a rather imprecise approach (Berbel & Innocenti, 1988). Older evidence based on the rates of brain growth and myelination suggests that the 5 –7-day-old rat may be 'equivalent' to the human newborn (Dobbing & Sands, 1973, 1979). Rat brain development during the period of 10–15 postnatal days thus best corresponds to the stage of brain development at which human infants are most susceptible to 'reactive' seizures such as febrile seizures (Hjeresen & Diaz, 1988; Baram *et al.,* 1997b).

If developmental, 'reactive' seizures involve CRH, then circumstances leading to them, such as fever, should result in elevated levels of the endogenous CRH in strategically located neurons in the hippocampus and amygdala. As illustrated above, a number of stresses, including those which are known to elicit age-specific seizures, result in augmented CRH production and release in specific CNS regions, including the amygdala and hypothalamus. The effect of hyperthermia on secretion of CRH from the newly described CRH-immunoreactive neurons in the hippocampus is currently under investigation.

Increased neuronal excitability by CRH is obviously only one of several potential mechanisms underlying the enhanced propensity of the immature human and rat to generate 'evoked' seizures. The CRH hypothesis does not propose a role for this peptide in mediating seizures arising in brain regions which are low in – or devoid of – CRH and its receptors. An additional weakness of the proposed CRH hypothesis is the need to account for the differential effects of stressors on seizures: certain stresses such as hyperthermia (fever), trauma or hypoglycemia, which increase CRH levels, also lead

to seizures. In contrast, other stressors such as hypothermia, which also increase CRH production (Yi & Baram, 1994) are not proconvulsant.

The induction of seizures is determined in both the mature and developing brain by a complex balance of excitation and inhibition (Johnston, 1996). The components of the excitatory and inhibitory influences differ in immature brain as compared with the adult (Ben-Ari *et al.,* 1994; Holmes, 1997). The immature brain is considered more excitable, but this is manifested by enhanced sensitivity to seizure induction by a variety of manipulations, as opposed to increased prevalence of spontaneous seizures. A number of neuroanatomical and neurochemical characteristics of the immature developmental state probably combine to mediate this fact, and no one single mechanism may be singled out. Neuropeptides are emerging as important modulators of neuronal excitability in several limbic and cortical circuits (Schwarzer *et al.,* 1996). These compounds, such as somatostatin, cholecystokinin, NPY and CRH, co-exist and are co-secreted with classical neurotransmitters at presynaptic terminals (Schwarzer *et al.,* 1996). CRH is positioned to be a neuromodulator affecting neuronal excitability, which is regulated by environmental input. This neuropeptide is thus a likely contributor to the mechanisms by which signals such as fever or anoxia enhance excitation and lead to seizures in the developing brain. A better understanding of these mechanisms is of paramount importance to the design of effective anticonvulsants which are appropriate for reactive seizures in the developing brain.

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