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Journal
Brain Research, 61(1)

ISSN
1385-299X

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Publication Date
1991-07-01

DOI
10.1016/0165-3806(91)90118-3

Published in final edited form as:
Brain Res Dev Brain Res. 1991 July 16; 61(1): 97–101.

Corticotropin-releasing hormone is a rapid and potent convulsant in the infant rat

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Abstract

Corticotropin-releasing hormone (CRH) administered into the cerebral ventricles of rats during the first postnatal week caused a specific and stereotyped behavior sequence: rhythmic chewing and licking (jaw myoclonus) were followed by 'litnbic'-type seizures. The onset of the seizures was much more rapid (2–45 min vs 3–7 h) than in adult rats, and the convulsant doses were much lower (50×10^{-12} mol per gram brain weight vs 750×10^{-12} mol per gram brain weight in adults). CRH potency in inducing seizures varied inversely with age. CRH-induced seizures occurred prior to any changes in serum corticosterone, and were eliminated by the administration of a CRH antagonist, as well as of phenytoin. Electrocorticographic correlates of CRH-induced behaviors in the infant rat were inconsistent, suggesting a subcortical origin of CRH-induced paroxysmal events in the immature brain.

Keywords

Corticotropin-releasing hormone; Corticotropin-releasing factor; Seizure; Brain development; Rat; Infant

INTRODUCTION

Corticotropin-releasing hormone (CRH) is a hypothalamic hormone which acts on the pituitary gland to stimulate ACTH secretion in response to stressful stimuli[34–35]. Once its structure was elucidated, it became clear that, like other hypothalamic hormones, it is distributed widely throughout the brain[33]. CRH receptors have been identified in the brain[10], and their number has been reported to be maximal on the fifth postnatal day[10,19]. A neurotransmitter or neuromodulatory role for extrahypothalamic CRH has been suggested[8,20,21,34].

CRH has been shown to excite neurons both in vivo and in vitro[29,36]. The peptide increases both spontaneous and evoked spike-discharge from locus-ceruleus neurons in vivo and produces neuronal depolarization in CA1 and CA3 hippocampal pyramidal cells in the slice preparation in vitro. CRH administered into the cerebral ventricles (i.c.v.) to adult rats causes epileptiform discharges in the amygdala after a 1- to 3-h delay, which then spread to the dorsal hippocampus[12]. These discharges progress, over 3–7 h to behavioral and electrographic seizures. The doses needed for frank seizure generation in adult rats are 1.5–3.75×10^{-9} mol (0.75–1.88×10^{-9} mol/gram brain weight)[11,12].

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This study was designed to test the hypothesis that the neonatal rat is more sensitive to the convulsant effects of CRH than the mature animal. We set out to delineate the behavioral and electrocorticographic effects of the peptide, their specificity and their response to anticonvulsant compounds. We utilized infant rats, starting on the 5th postnatal day.

MATERIALS AND METHODS

Animals

Timed-pregnancy rats were obtained from Zivic-Miller, (Zelionple, PA), and were housed under a 12-h light/dark cycle, and fed ad libitum. They were handled daily to prevent cannibalism[23]. Delivery times were monitored and were accurate to within 12 h. The day of birth was considered day zero. The pups were kept with the mothers, and litters were culled to 12 pups. Pups were mixed, such that study groups contained pups from several litters. Pups were subjected to surgery 24 h prior to recording, and returned to their mothers. All recordings were done at 08.30–11.30 h, to minimize diurnal variations in seizure susceptibility[27] and in endogenous glucocorticosteroid levels. CRH was injected via a stereotaxically placed cannula in all pups, except in those utilized for hormonal measurements alone (when the peptide was injected ‘free-hand’ as described below). Separate groups of animals were used for dose–effect and time-to-on set experiments.

Surgery and recording

Cortical stainless steel electrodes were implanted under halothane anesthesia, using an infant-rat stereotaxic apparatus, as previously described[6,31]. Two frontal and two posteroparietal electrodes were used. The technique allows for electrocorticogram (ECoG) recording from freely moving animals, via long, flexible wires. Recordings were carried out in heated, shielded Plexiglas chambers, using a Grass 78E Polygraph. Pups were continuously observed throughout the recording.

A stainless steel cannula was chronically implanted into the lateral ventricle. The stereotaxic coordinates, using Bregma as a landmark were: (P 0.6, L 1.8, V 3.0) in 5-day-old infant rats; (P 0.6, L 2, V 3.3) in 10-day-old pups, and (P 0.7. L 2.2, V 3.5) in 16-day-old animals.

After a 30-min habituation period, CRH or saline were administered i.c.v. in 1–2 μl, using a micro-infusion pump. Doses used were 7.5–600 × 10^{-l2} mol, or 22.5–1800^{-12} mol/gram brain weight. Phenytoin, 20 mg/kg (1 μl/g), was injected into the peritoneal cavity 45 min prior to CRH, a time determined optimal for its anticonvulsant effect[37,38]. Phenytoin alone at the same dose was administered to control animals, and others (2 per age) were not injected. Alpha-helical(9–41) CRH (Peninsula, CA), a competitive CRH antagonist[28], was administered i.c.v. in 2 μl buffered saline (pH = 7.2), 30 min prior to CRH injection. Doses used were 5 and 10 μg. Epileptic phenomena were defined as those events not seen prior to CRH administration, recognized as epileptic in infant[2,9,26] and adult rats[7,18,24], and abolished by a non-sedating (behaviorally, compared to uninjected controls) dose of phenytoin.

Hormonal effects of CRH and CRH-induced seizures

Five-day-old pups were divided into 6 experimental groups: 3 groups received CRH (150 × 10^{-12} mol) in a dye solution injected i.c.v. using a ‘freehand’ technique. Briefly, a Hamilton 25-μl syringe was fitted with a needle-cover allowing 3 mm of tip protrusion. The needle was inserted using firm pressure 1 mm posterior and lateral to bregma — which is easily visible on the soft and penetratable skull at this age. In our hands, as verified by the presence of dye in the third ventricle and cisterns, this ‘freehand’ method achieved 60–70% i.c.v. injection and is applicable for injection of large numbers of neonatal rats. Three other groups

were injected with dye solution alone. Rats were sacrificed 15, 30 and 45 min after CRH administration, and trunk blood collected. Plasma was frozen at −80 °C and processed for corticosterone radioimmunoassay[4]. For each pup, i.c.v. injection was ascertained, and presence or absence of CRH-induced seizures was noted. Only pups manifesting epileptic phenomena at 15 or 30 min were included in the experimental groups.

RESULTS

Behavioral and electrographic effects of CRH

In the 5-day-old rat, CRH induced a dose-dependent spectrum of stereotyped behaviors. At the lowest effective dose, 7.5×10^{-12} mol, pups displayed jaw myoclonus ('chewing'). A larger dose (22.5×10^{-12} mol) resulted in jaw myoclonus (Fig. 1), followed by tonic extension of one or two extremities, leg clonus and focal tonic extension. Administration of the largest dose tested — 300×10^{-12} mol — resulted in 'chewing' and 'licking' in less than 2 min, which progressed rapidly to focal tonic extension and ?!swimming' seizures that lasted for over 6 h.

On the 10th postnatal day, CRH resulted in jaw myoclonus followed by 'wet-dog shakes', running and 'swimming' seizures. By the 16th postnatal day, while jaw-myoclonus was observed, even the maximal dose tested (300×10^{-12} mol) did not elicit motor seizures. Fig. 2 shows the dose-dependent spectrum of CRH-induced behaviors in 5-day-old pups, as compared those observed in 10- and 16-day-old animals. As evident from the figure, larger peptide doses were needed to elicit motor seizures in older rats: a dose causing status epilepticus in a 5-day-old pup resulted only in jaw myoclonus in the 16-day-old pup. Table I shows the time to onset of jaw myoclonus as a function of CRH dose and of animal age. By the 18th postnatal day, the 4 animals tested displayed no jaw myoclonus or other epileptic phenomena during an observation period of 9 h.

Electrocorticographic (ECoG) correlates of CRH-induced behaviors were inconsistent. Most commonly observed were semi-rhythmic sharp waves and increased beta frequency. Fig. 3 compares the ECoG (as well as cardiac (EKG) and muscle movement (EMG) monitors) before and after i.c.v. CRH in the 5-day-old rat pup. All ECoG changes were abolished by pre-administration of a CRH antagonist (Fig. 3C, and see below).

Effect of anticonvulsants and CRH antagonist on CRH-induced behaviors

In the 5-day-old pup, administration of 5 μg of alpha-helical(9–41) CRH 40 min prior to the administration of a convulsant dose of CRH, eliminated all tonic events, and attenuated dramatically the jaw myoclonus (Table II). A larger dose (10 μg) effectively blocked also the onset of 'chewing' induced by 22.5×10^{-12} mol CRH. The antagonist alone had no behavioral or ECoG effects. Phenytoin (20 mg/kg) administered i.p. to 5-day-old infant rats resulted in no overt behavioral changes. This dose of anticonvulsant, however, eliminated entirely all the effects of a maximal (300×10^{-12} mol) CRH dose given i.c.v. 40 min later (Table II).

CRH-induced behaviors are not a result of stress response

The effects of i.c.v. CRH in convulsant doses on plasma corticosterone was assessed in 5-day-old infant rats. By 15 min after administration, 5/6 animals with verified i.c.v. cannula placement displayed jaw myoclonus; by 30 min, all (4) animals with i.c.v. placement manifested jaw myoclonus associated, in two, with tonic extension. As is evident from Fig. 4, corticosterone levels did not change during the first 15 min following i.c.v. CRH administration, at a time when the pups were displaying jaw myoclonus, forepaw clonus and

some tonic flexion. Corticosterone levels were significantly higher at the 30-min time point, possibly related to the stress induced by the convulsive phenomena themselves.

DISCUSSION

CRH is an hypothalamic hormone which has convulsant properties in adult rats[11,12,25], and generally activates neurons, both in mammals and in lower species[3,29,36]. The role of endogenous CRH in seizure generation or propagation is unknown. There is some evidence suggesting a possible role for endogenous CRH in seizure susceptibility or generation in the immature brain: an age-specific human seizure disorder, massive infantile spasms (MIS), is refractory to common anticonvulsants, but responds to ACTH or glucocorticoids[1,17,30]. ACTH increases plasma glucocorticoids, which have been shown to decrease CRH gene expression, synthesis and release from the hypothalamus in both humans and rodents[22,32,39]. Thus, the possibility exists that excessive CRH synthesis or activity may be a causal factor in this type of epilepsy. MIS has been considered to be subcortical in origin[1,30], but does not respond to phenytoin[1,17].

CRH presumably activates neurons through specific receptors, which are non-randomly distributed throughout the brain[10]. Insel et al. have demonstrated, in the rat brain, a maximal number of CRH receptors on the fifth postnatal day[19]. Moreover, studies of the ontogeny of CRH gene expression suggest that, subsequent to a perinatal hiatus, the peptide's synthesis increases dramatically, starting at the end of the first postnatal week[5,14]. Thus, at the end of the first postnatal week in the rat, both CRH synthesis and receptor number are maximal, possibly contributing to enhanced convulsive potential of the peptide.

CRH-induced behaviors in the neonatal rat are specific— they are completely abolished by a 67-fold excess of a competitive antagonist. CRH-induced phenomena are probably epileptic, since they are abolished by phenytoin, an anticonvulsant, in a dose that does not cause sedation or elimination of normal behaviors. Moreover, the sequence of CRH-induced behaviors is rather similar to that described following kainic acid administration in rats of these same ages[2,9], during kindling[13], and over a longer time course, in CRH-treated adult rats[11,12].

The lack of consistent electrocortical correlates of the dramatic behaviors induced by CRH during the first postnatal weeks is similar to results reported for kainic acid[9], even during status epilepticus[16]. In both cases, a limbic origin of the seizures is likely. The jaw myoclonus — 'chewing' and 'licking' — are consistent with limbic, specifically amygdala involvement[13,18], and have been reported with kainic acid administration on the first postnatal week[16]. The amygdala has been shown to be the first to develop spikes after CRH administration to the adult rat[11,12], although the hippocampus has been implicated by other investigators25. The time course (less than 2 min vs 1–3 h), however, suggests that the underlying activation mechanisms are fundamentally different in the neonatal and adult rat. In the adult rat, CRH-induced amygdala spikes have been likened to kindling[11]. This mechanism cannot be in effect in infant rats, both because of the rapid onset of CRH-induced seizures, and because of the difficulty in achieving kindling in such immature animals[15,26].

In conclusion, our data suggest that CRH is a potent convulsant in the infant rat, and that perturbation of CRH synthesis or activity may have some importance in seizure generation in the immature brain.

Acknowledgments

Supported by NINDS Grant NS10307 (T.Z.B.). We thank O. Carter Snead III for continued encouragement, and S.R. Snodgrass for helpful comments and review of this manuscript.

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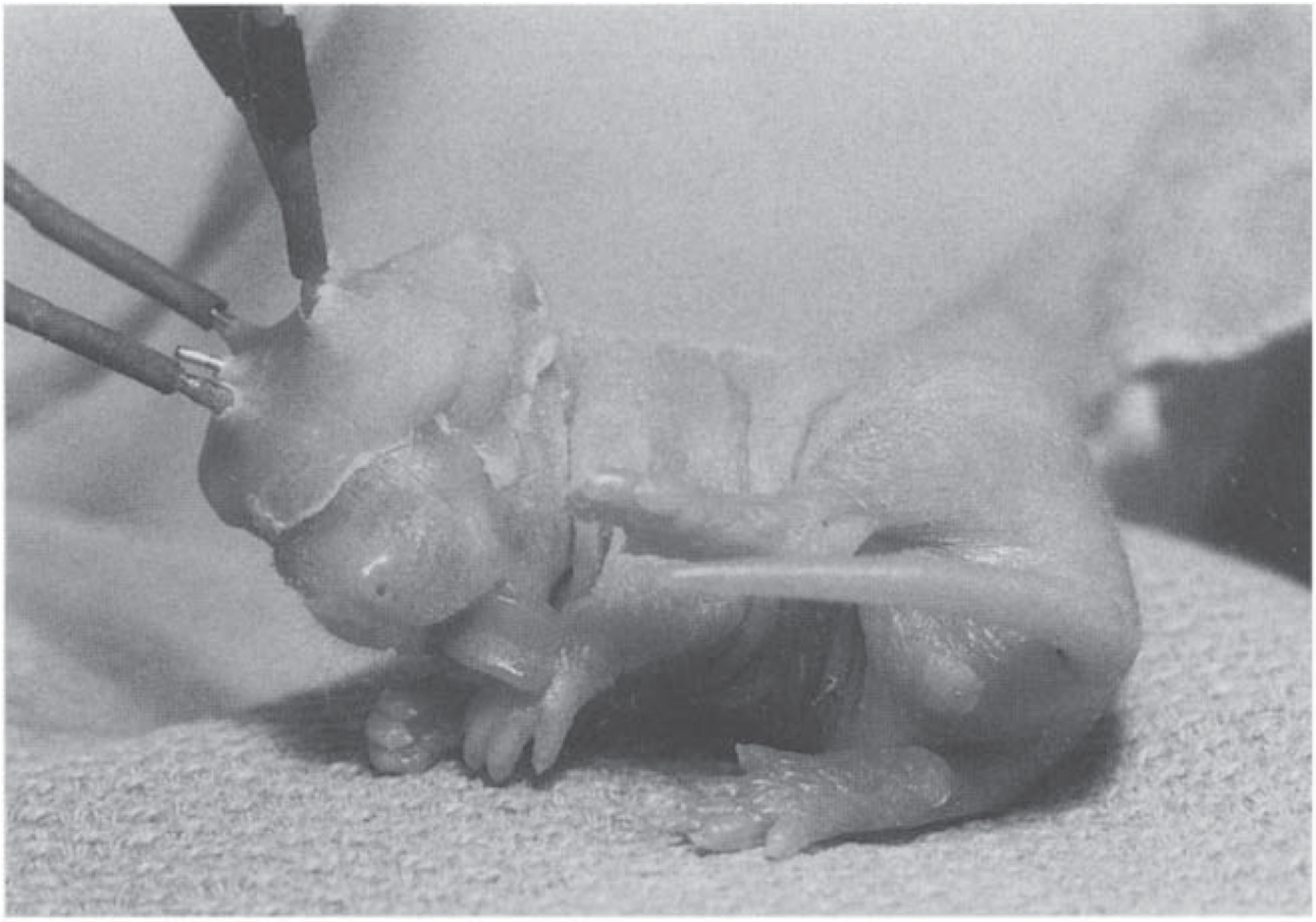

Fig. 1.
Photograph of a 5-day-old male rat pup displaying jaw and tongue myoclonus, following CRH (150×10^{-12} mol) administration into the cerebral ventricles.

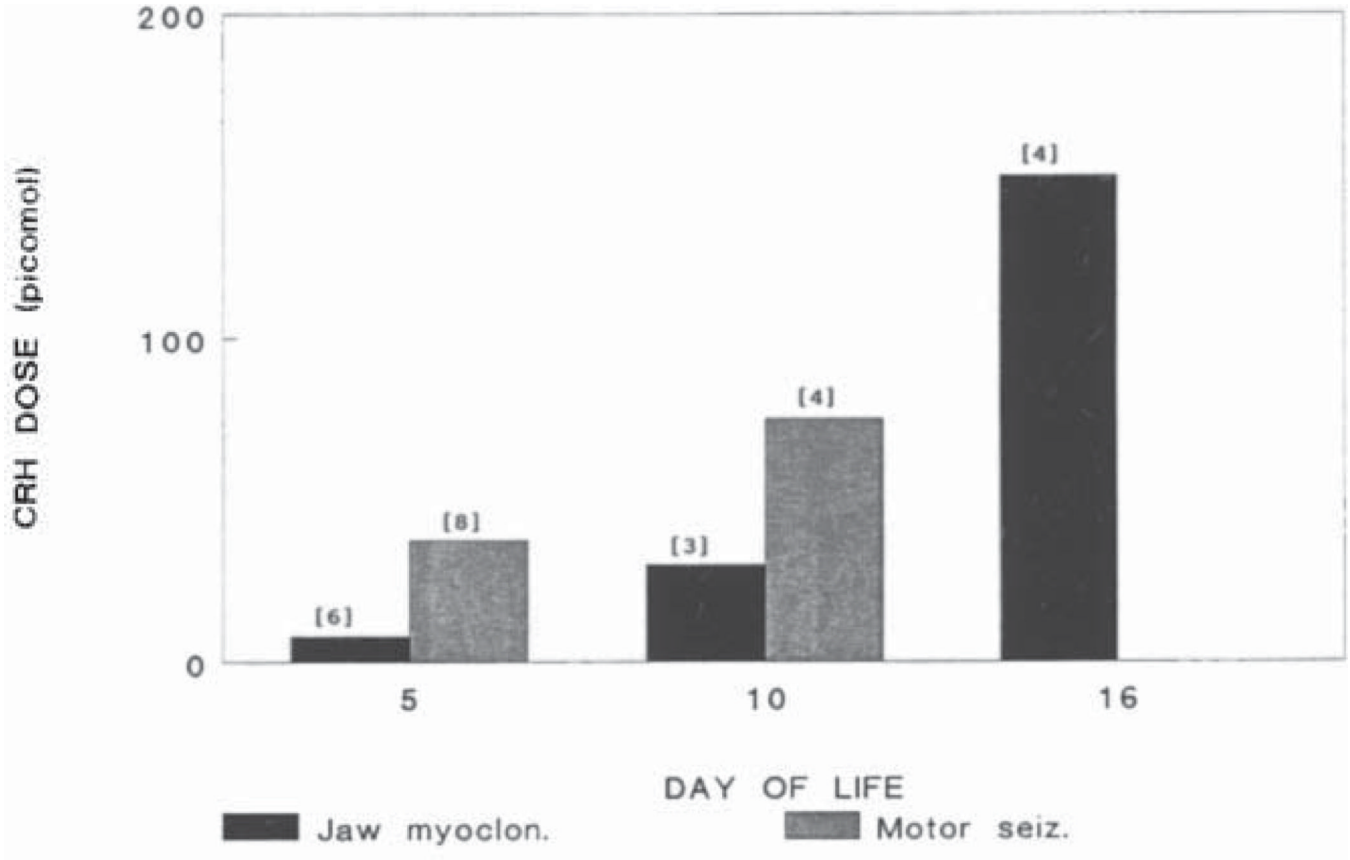

Fig. 2.
Age dependence of seizure-inducing doses of CRH. Details of motor phenomena are given in the text. Numbers in brackets denote number of animals per group.

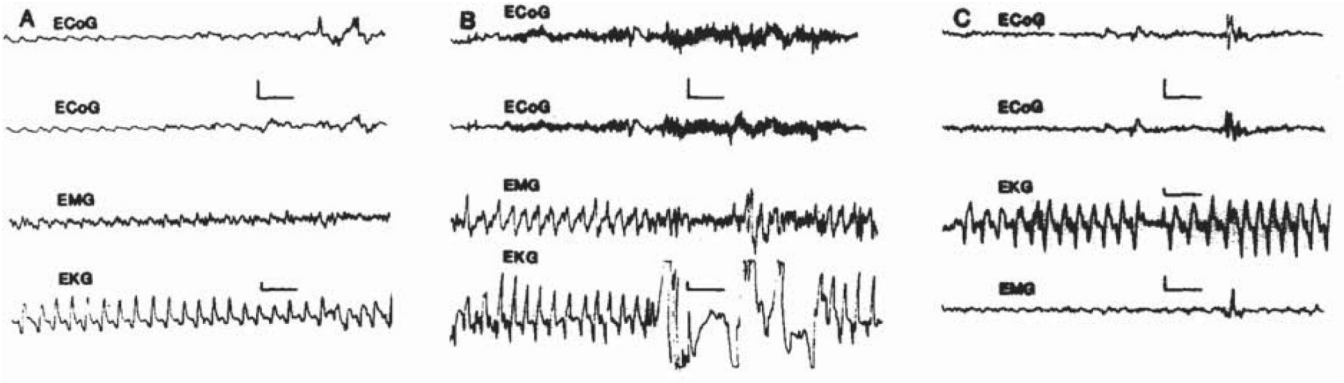

Fig. 3.

Electrocorticograms of 5-day-old rat pups, before (A) and 20 minutes after (B) intracerebroventricular (i.c.v.) administration of 22.5×10^{-12} mol CRH. C: 20 min after CRH administration, in a rat given alpha-helical CRH (10 mcg, i.c.v.) 40 min prior to the peptide. ECoG, electrocorticogram; EKG, electrocardiogram; EMG, recording from an electrode placed over angle of jaw, anterior to the ear. Horizontal bars = 1 s. Vertical bars = 50 μV.

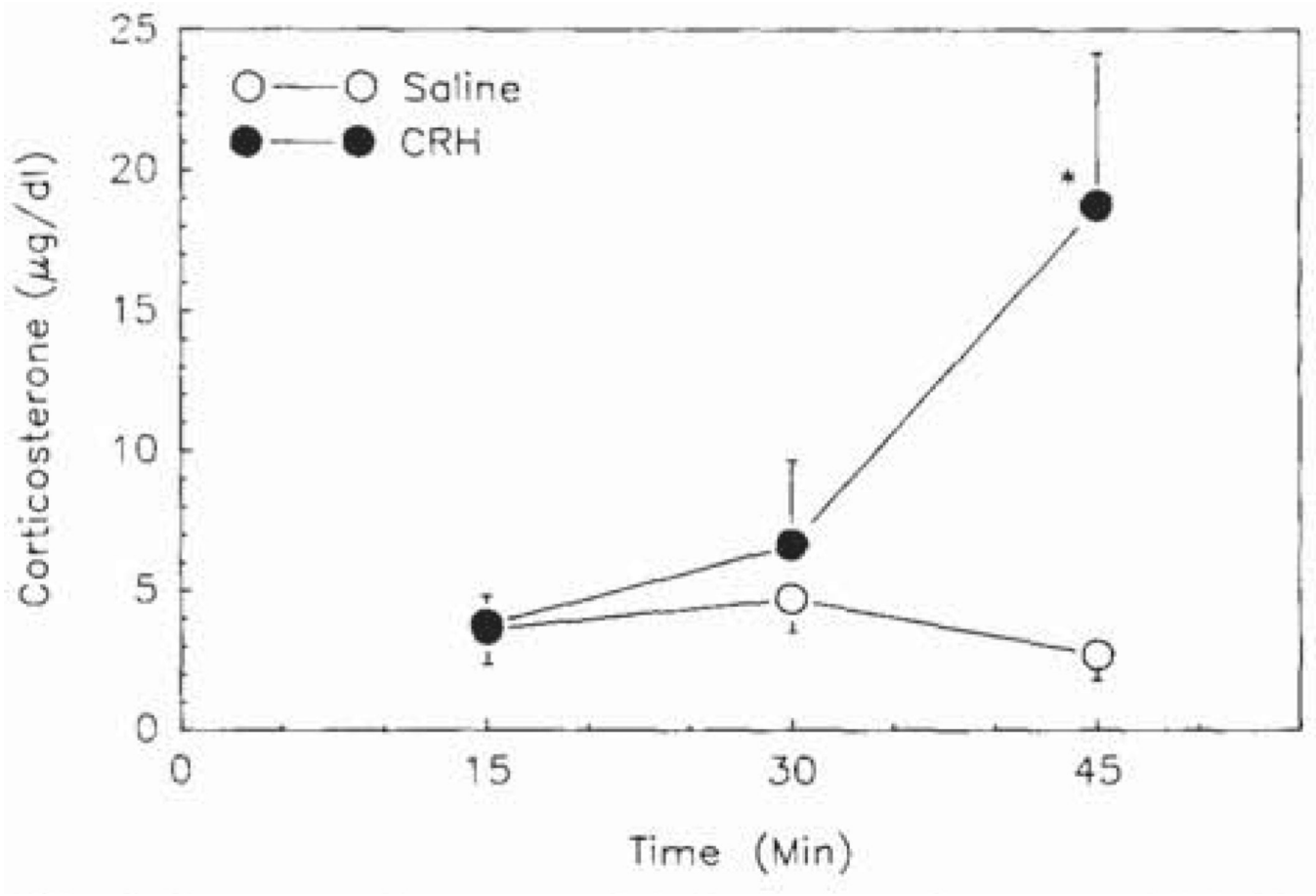

Fig. 4.

Plasma corticosterone in infant rats subjected to CRH (0.15 nmol, filled circles) or saline (open circles) injection into the cerebral ventricles (i.c.v.). At the 30 min time-point, CRH-injected rats have begun having motor phenomena, which were well established by 45 min. Bars denote standard errors; *$P < 0.05$.

TABLE I

Time to onset — and duration — of CRH-induced jaw myoctonus as a function of CRH dose andanimal age

Range of duration reflects intra-group variability. S.E., standard error; min, minutes; obs, observation; n.r., not recorded.

Age (day)	Responders/ total	CRH dose (10^{-12} mol)	Time to onset (mean ± S.E.)	Duration (min)	Obs. time (h)
6	4/4	150	9.5 ± 2	25–32	3
8	2/8	15	18,18	n.r.	>3
8	3/8	37.5	12.7 ±0.6	n.r.	>3
8	2/2	300	2,5	120–180	>4
10	2/2	37.5	40,45	<15	4
11	5/5	150	4 ± 0.35	60–180	>4
14	4/4	300	n.r.	60–180	>4
18	0/2	300	-	-	9
18	0/2	600	-	-	9

TABLE II

Effects of phenytoin and of alpha-helical CRH on CRH-induced seizures in 5-day-old rats

n, number of animals; pmol = 10^{-12} mol.

Drug/ dose	n	CRH/ pmol	Jaw myoclonus	Motor seizures
−	8	22.5	+ + +	+ + +
Antagonist 5 μg	2	22.5	+	−
Antagonist 10 μg	3	22.5	−	−
Phenytoin 20 mg/kg	2	−	−	−
−	2	300	+ + +	+ + +
Phenytoin 20 mg/kg	4	300	−	−