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ACTH Does Not Control Neonatal Seizures Induced by Administration of Exogenous Corticotropin-Releasing Hormone

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Summary: ACTH has been used extensively for treatment of massive infantile spasms (MIS) and other intractable seizures. The mechanisms by which ACTH exerts anticonvulsant effects are unknown. ACTH is a neuropeptide with both endocrine and neuromodulatory properties; its efficacy against MIS could derive from intrinsic anticonvulsant properties or from hormonal effects, either directly or through glucocorticoids. We tested ACTH activity against exogenous corticotropin-releasing hormone (CRH)-induced seizures in the infant rat model. CRH was administered into the cerebral ventricles of 85 infant rats aged 5–13 days. ACTH was used either 20–60 min before CRH administration or "chronically" (pretreatment with four doses of ACTH every 6 h, before CRH administration). In a separate group of rat pups, we measured plasma corticosterone to ascertain ACTH availability. Administration of CRH, an age-specific endogenous convulsant, resulted in a prolonged series of seizures after 2- to 55-min latency. There was no difference in latency between controls (9.5 ± 1.2 min) and ACTH-treated rats (12.4 ± 2.8 min for combined acute and chronic groups). CRH-induced seizure duration (88.2 ± 9 vs. 74.7 ± 9.4 min) and severity of seizures was also unchanged by ACTH treatment. ACTH reached the circulation and caused significant increase in plasma glucocorticoids. ACTH does not block the convulsant action of exogenous CRH in infant rats. An alternative mechanism for the anticonvulsant effect of ACTH may be suppression of synthesis and secretion of an endogenous convulsant, i.e., CRH. **Key Words:** Epilepsy—Infantile spasms—Corticotropin-releasing hormone—Infants—Epilepsy models—Anticonvulsant effects—Rats.

Since the 1958 report of Sorel and Dusaucy-Bauloye (1958) regarding the efficacy of ACTH for treatment of massive infantile spasms (MIS), the peptide has been used extensively for treatment of this entity, as well as for other intractable seizures. The time course of ACTH effects, the "all-or-none" response of MIS, and the lasting effect on the seizures once ACTH therapy is discontinued, single out ACTH from conventional antiepileptic drugs (AEDs) (Hrachovy et al., 1983; Aicardi, 1986; Hrachovy and Frost, 1989; Snead, 1989; Snead and Simonato, 1991).

We hypothesized that the anticonvulsant effects of ACTH derived from its hormonal action, i.e., a negative-feedback effect on corticotropin-releasing hormone (CRH) synthesis or activity (Baram, 1993). We previously showed that CRH is a potent and rapid convulsant in infant rats (Baram and Shultz, 1991; Baram et al., 1992). The present study was designed to investigate the effect of ACTH, given acutely or "chronically," on seizures induced by CRH in neonatal and infant rats.

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METHODS

Animals and surgical procedure

Infant rats (n = 117) aged 5–13 days were products of timed-pregnancy Sprague-Dawley–derived rats, obtained from Zivic-Miller (Zelionple, PA, U.S.A.). Pregnant mothers and pups were housed under a 12-h light/dark cycle and fed ad libitum. Delivery times were monitored and were accurate to within 12 h; day of birth was considered day 0. Litters were mixed so that study groups contained pups from several litters and were culled to 12 pups. Infant rats were subjected to operation 24 h before CRH administration and returned to their mothers. CRH was always administered between 9:00 and 10:30 a.m. to minimize diurnal variations in seizure

susceptibility (Oliverio et al., 1985) and in endogenous CRH and GC levels (Watts et al., 1989).

CRH was administered intracerebroventricularly (i.c.v.) through a permanent stainless-steel cannula chronically implanted in the lateral ventricle (Baram and Schultz, 1991). The stereotaxic coordinates, using bregma as a landmark were P 0.6, L 1.8, and V 3.0 in infant rats aged 5–7 days; P 0.6, L 2, and V 3.3 in pups aged 9–11 days, and P 0.7, L 2.0, and V 3.3 in rats aged 12–13 days.

Experimental paradigm

On the morning of the experiment, infant rats were transferred to heated Plexiglas cages. After a 30-min habituation period, CRH 112–150 $\times 10^{-12}$ mol or saline was administered i.c.v. in 1 μl by microinfusion pump. ACTH (ACTHARGEL, Rhône-Poulenc Rorer, Collegeville, PA, U.S.A.) 4 IU/kg was given intraperitoneally (i.p., 2 μl/g weight). In the acute studies, ACTH or vehicle was injected 20, 30, or 60 min before CRH infusion. In the chronic experiments, ACTH (4 IU/kg per dose) was given every 6 h for four doses, with the last dose given 30 min before CRH infusion.

Experimental groups consisted of CRH alone (n = 43), CRH preceded by ACTH (n = 42), ACTH alone (n = 20), and saline/no drug (n = 12). Litters of infant rats were studied at the age of 5, 6, 7, 9, 10, 11, or 13 days. Each animal was subjected to ACTH or CRH only once. Several groups of animals were videotaped, and seizures were scored without knowledge of the treatment. In a separate group, pups were implanted with bipolar electrodes aimed at the amygdala (Baram et al., 1992,1993) to verify the epileptiform nature of CRH-induced phenomena (Baram and Hirsch, 1992) and the effect of ACTH. Other groups (n = 4 per age) of rats aged 6 or 12 days were injected with ACTH or saline and killed 30 min later for plasma corticosterone (CORT) analysis in comparison to noninjected pups. CORT was measured by radioimmunoassay (RIA) (Baram and Schultz, 1990).

Scoring and statistical analysis

We determined time to onset and duration and severity of CRH-induced seizures. Pups were scored at 5-min intervals for a minimum of 4 h and until abnormal behavior subsided. CRH-induced seizures were scored as described previously (Baram et al., 1992). Automatisms (jaw myoclonus and licking) progressed to focal leg clonus and focal tonic extension. The most severe manifestations consisted of "swimming" seizures that lasted >6 h. In pups aged ≥10 days, CRH resulted in jaw myoclonus followed by "wet-dog shakes," running, and swimming seizures.

Groups were analyzed for correlation of treatment with latency, duration, and severity of seizures. Because no differences were evident between acute and chronic ACTH groups, they were combined as already described. Significance of differences among groups, for both seizures and plasma CORT, were analyzed by Student's *t* test (STATIT statistics program). The power for differences between groups was determined with PRIMER biostatistics program, third edition.

RESULTS

CRH administration resulted in a prolonged series of seizures after a latency of 2–55 min. Rats administered vehicle or ACTH alone had no seizures. There was no difference in time to seizure onset (latency) between rats given CRH alone (9.5 ± 1.2 min) and those pretreated with ACTH (12.4 ± 2.8 min for combined acute and chronic groups) (Table 1). CRH-induced seizure duration (88.2 ± 9 vs. 74.7 ± 9.4 min) and severity of seizures were also unchanged by ACTH treatment (Table 2).

Sample size was adequate: The power for a difference of 5/6 standard deviation (SD) in latency between the entire group of ACTH-treated versus the nontreated group was 0.948. For the groups aged 5–7 days power (β) for a 1-SD difference in latency between groups was 0.84 (Table 1).

ACTH administered into the peritoneal space reached the circulation in amounts sufficient to cause a highly significant increase in plasma CORT (Table 3). This increase was evident 30 min after injection, at the time CRH was infused. Plasma CORT levels for the combined 6- and 12-day-old rat groups (n = 8 per group) were 2.47 ± 0.24 μg/dl for controls, 3.78 ± 0.54 μg/dl for saline injected, and 11.83 ± 0.83 μg/dl for rats administered ACTH (SEM ± SE).

DISCUSSION

ACTH has been used as an anticonvulsant for intractable human epilepsy since the early 1950s

TABLE 1. *Latency of CRH-induced seizures*

Age (day)	CRH alone (n)[a]	CRH and ACTH (n)[a]	p-Value
5–7	11.22 ± 1.8 (18)	13.74 ± 3.4 (19)	0.264
9–13	8.45 ± 1.6 (20)	11.25 ± 4.5 (19)	0.28

CRH, corticotropin-releasing hormone.

The power (β) for a difference between groups of 1 SD is 0.84 for the group aged 5–7 days. For the entire group, the power for detection of a difference of 6 min (5/6 SD) is 0.948.

Values are mean ± SE.

[a] Precise latency was not available for 7 pups aged 5–7 days and for 1 pup aged 9–13 days.

TABLE 2. *Duration of CRH-induced seizures: Five-minute epochs*

Age (day)	CRH alone (n)[a]	ACTH and CRH (n)[a]	p-Value
5–7	19.55 ± 2.8 (22)	15.32 ± 2.9 (22)	0.14
9–13	15.95 ± 1.6 (20)	15.32 ± 2.6 (19)	0.42

Abbreviation as in Table 1.
Values are mean ± SE.
[a] Precise duration was not available for 1 rat aged 9 days.

(Snead, 1989,1992). It has been used primarily for MIS (Sorel and Dusaucy-Bauloye, 1958), for which it is the preferred drug (Aicardi, 1986; Snead, 1990). ACTH has proven useful for a variety of other intractable childhood seizures (Snead, 1992).

The mechanisms by which ACTH exerts anticonvulsant effects have been a focus of intensive investigation (Woodbury, 1952; Urban et al., 1974; Holmes and Weber, 1985; Croiset and de Wied, 1992; Pranzatelli, 1994). ACTH is a neuropeptide with both endocrine and neuromodulatory properties. The direct effects of ACTH on the CNS have been well documented and consist of both behavioral alterations (de Wied and Ferrari, 1986) and electrophysiologic (Urban et al., 1974) and direct neuropharmacologic (Pranzatelli, 1994) activities. Anticonvulsant properties have been demonstrated in immature rodents through use of the kindling paradigm (Holmes and Weber, 1985). These intrinsic neuromodulatory actions of ACTH exist in a fragment ($ACTH_{4-10}$) devoid of hormonal effects (Urban, 1986). This portion of ACTH, however, is ineffective for treatment of MIS (Pentella et al., 1982; Willig and Lagenstein, 1982).

The lack of efficacy of ACTH fragments devoid of corticotrophic function and the efficacy of glucocorticoids (GC) for MIS suggest that the anticonvulsant potency of ACTH for infants with MIS may derive from its hormonal properties (Riikonen, 1983). GC itself may have intrinsic anticonvulsant properties by virtue of direct effects on neuronal membrane excitability (McEwen et al., 1991; Joels and de Kloet, 1992). ACTH may thus be superior to prednisone in conventional dose-regimens used for MIS by causing sustained higher plasma GC levels (Snead et al., 1989). GC, unlike ACTH, readily penetrate the blood–brain barrier. GC may also act through specific receptors to modualte expression of several genes in CNS, including those of neurotransmitter or second-messenger systems (McEwen et al., 1991).

An alternative hormonal mechanism of ACTH anticonvulsant action is through negative feedback suppression of an intrinsic convulsant, CRH (Ehlers et al., 1983; Marrosu et al., 1988; Rosen et al., 1994), as part of the hypothalamo-pituitary adrenal axis (Vale et al., 1981; Jingami et al., 1985). CRH exerts excitant properties on a wide variety of neurons in several species. In vitro studies of hippocampal slice preparation (Aldenhoff et al., 1983), cerebellar Purkinje neurons (Fox and Gruol, 1993), and in vivo electrographic investigations (Ehlers et al., 1983; Valentino et al., 1983; Siggins, 1990) amply document that CRH increases neuronal excitability. In adult rats, CRH administered i.c.v. results in epileptiform discharges in amygdala and hippocampus (Marrosu et al., 1988), and 3–7 h later, in overt, "limbic" seizures (Ehlers et al., 1983).

We have recently showed CRH to be a far more rapid and potent convulsant when administered to infant rats (Baram and Schultz, 1991; Baram et al., 1992). Seizures occur with a latency of as little as 2 min and with CRH doses as low as 7.5×10^{-12} mol (as compared with $1{,}500 \times 10^{-12}$ mol in adults). We speculated that if ACTH were a direct anticonvulsant, it should block CRH-induced seizures whereas a lack of efficacy would indicate an indirect hormonal mode of action, though not necessarily downregulation of CRH synthesis or secretion.

ACTH was not effective against exogenous CRH-induced seizures in the infant rat model in either an acute or subacute experimental paradigm. Neither latency nor length or severity of CRH-induced status epilepticus was altered. EEG correlates of behavioral seizures (Baram et al., 1992) also were unchanged (data not shown). ACTH doses (4 IU/kg per dose) were equivalent or higher than those shown to be effective in human infants (150 IU/m^2/day, 3.5 IU/kg per dose for a typical 7-kg, 0.35-m^2 infant). Furthermore, ACTH reached the circulation and resulted in significant increase in plasma CORT (Table 3).

Seizures induced by CRH administration in infant rats may not provide an optimal model for study of ACTH anticonvulsant action in human MIS (Baram, 1993). As far as can be extrapolated from comparative studies, the full-term human resembles a 7–10-day-old rat with regard to brain growth,

TABLE 3. *Plasma corticosterone after ACTH or saline injection*

Age (day)	Non-injected	Saline	ACTH
6 days	2.66 ± 0.22	4.87 ± 0.6	13.65 ± 0.7[a]
12 days	2.29 ± 0.42	2.68 ± 0.43	10.01 ± 0.6[a]
Combined:	2.47 ± 0.24	3.78 ± 0.54	11.83 ± 0.8[a]

Four rats per group. Values are Mean ± SEM, in μg/dl. Corticosterone measured in trunk plasma 30 min after injection. Elevation in saline injected pups represents response to stress of injection. [a]$p < 0.05$.

DNA content, and myelinization. A 13-day-old infant rat is beyond the exponential phase of brain growth and may thus be comparable to a 5–10-month-old human (Dobbing and Sands, 1973). CRH-exposed pups in these experiments may therefore be termed "neonatal" (age <10 days) and "infant" (age 10–13 days). In rats of both ages, these experiments suggest that the mechanism of ACTH anticonvulsant efficacy does not involve direct suppression of CRH-induced enhanced neuronal excitability.

CRH administered i.c.v. at the doses used does not result in activation of the pituitary and adrenal (Baram and Schultz, 1991). Status epilepticus induced by the peptide is stressful, however, and increases plasma CORT, presumably through ACTH (Baram and Schultz, 1991). This plasma-ACTH increase probably is insignificant, since the ACTH doses used are $>10^4$-fold higher than maximal, stress-induced ACTH plasma levels in neonatal rats (Walker et al., 1991; Yi et al., 1994).

ACTH does not block the convulsant action of exogenous CRH in infant rats. Alternative mechanisms, such as "hormonally mediated," feedback downregulation of endogenous convulsants, may account for the antiepileptic efficacy of ACTH in infant brain.

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