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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.

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 endo-
genous 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 coor-
dinates, 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 × 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., 0.2 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 pheno-
mena (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 my-
oclonus followed by "wet-dog shakes," running,
and swimming seizures.

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

RESULTS

CRH administration resulted in a prolonged se-
ries of seizures after a latency of 2–55 min. Rats
administered vehicle or ACTH alone had no sei-
zures. 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 dif-
ference 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>
<thead>
<tr>
<th>Age (day)</th>
<th>CRH alone (n)</th>
<th>CRH and ACTH (n)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>11.22 ± 1.8</td>
<td>13.74 ± 3.4</td>
<td>0.264</td>
</tr>
<tr>
<td>9-13</td>
<td>8.45 ± 1.6</td>
<td>11.25 ± 4.5</td>
<td>0.28</td>
</tr>
</tbody>
</table>

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.

* Precise latency was not available for 7 pups aged 5–7 days
  and for 1 pup aged 9–13 days.
Anticonvulsant action is through negative feedback that has been well documented and consist of both behav-
ioral 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 paradig-
ms (Holmes and Weber, 1985). These intrinsic neuromodulatory actions of ACTH exist in a frag-
ment (ACTH$_{1-10}$) devoid of hormonal effects (Urban, 1986). This portion of ACTH, however, is ine-
effective for treatment of MIS (Pentella et al., 1982;
Willig and Lagenstein, 1982).

The lack of efficacy of ACTH fragments devoid of
corticotropic function and the efficacy of gluco-
corticoids (GC) for MIS suggest that the anticon-
vulsant 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; Snead, 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 in-
tractable childhood seizures (Snead, 1992).

The mechanisms by which ACTH exerts anticon-
vulsant effects have been a focus of intensive inves-
tigation (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 proper-
ties. The direct effects of ACTH on the CNS have
derived from its hormonal properties (Riikonen,
1983). GC, unlike ACTH, readily pen-
trate the blood-brain barrier. GC may also act
through specific receptors to modulate expression
of several genes in CNS, including those of neuro-
transmitter 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 hip-
pocampal 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) am-
ply document that CRH increases neuronal excit-
ability. In adult rats, CRH administered i.c.v. re-
sults 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 × 10$^{-12}$ mol in adults). We
speculated that if ACTH were a direct anticonvul-
sant, it should block CRH-induced seizures whereas a lack of efficacy would indicate an indi-
rect hormonal mode of action, though not necessar-
ily 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 be-
havioral seizures (Baram et al., 1992) also were un-
changed (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 circula-
tion and resulted in significant increase in plasma
cortisol (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>
<thead>
<tr>
<th>Age (day)</th>
<th>CRH alone (n)a</th>
<th>ACTH and CRH (n)a</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5–7</td>
<td>19.55 ± 2.8 (22)</td>
<td>15.32 ± 2.9 (22)</td>
<td>0.14</td>
</tr>
<tr>
<td>9–13</td>
<td>15.95 ± 1.6 (20)</td>
<td>15.32 ± 2.6 (19)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Abbreviation as in Table 1.
Values are mean ± SE.

* Precise duration was not available for 1 rat aged 9 days.

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CRH-induced seizures not blocked by ACTH

DNA content, and myelination. 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 >104-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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