

Short communication

Astressin, a novel and potent CRF antagonist, is neuroprotective in the hippocampus when administered after a seizure

Heather Maecker^a, Aparna Desai^a, Rajesh Dash^a, Jean Rivier^b, Wylie Vale^b, Robert Sapolsky^{a,*}

^a Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA

^b Clayton Foundation Laboratories for Peptide Biology, Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037, USA

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Abstract

Corticotropin-releasing factor (CRF), the principle hypothalamic regulator of the adrenocortical axis, also functions as a neurotransmitter. In this latter role, CRF causes electrophysiological activation and epileptiform activity in various brain regions. That finding, coupled with the observation that CRF mRNA is induced in endangered brain regions following necrotic insults, suggests that the peptide might contribute to necrotic neuron loss. Supporting that, a number of studies have shown that CRF antagonists decrease ischemic or excitotoxic damage to neurons. In the present report, we demonstrate the considerable neuroprotective potential of a novel and potent CRF antagonist, astressin, against kainic acid-induced excitotoxic seizures. Intracerebroventricular infusion of the peptide both 30 min before and 10 min after seizures decreased damage in some hippocampal cell fields by as much as 84%, a magnitude of protection greater than reported for other CRF antagonists against other models of necrotic neuronal injury. Administration of astressin was done against both local microinfusion (0.035 μg) or systemic infusion (10 mg/kg body weight) of the excitotoxin; furthermore, the peptide protected even if administered only 10 min following excitotoxin exposure. This fulfills a critical prerequisite for any eventual therapeutic use of CRF antagonists, namely that they need not be administered in anticipation of a neurological insult.

Keywords: Astressin; Corticotropin-releasing factor (CRF) antagonist; Seizure; Neuron loss; Hippocampus

Corticotropin-releasing factor (CRF), the first hypothalamic factor whose existence was inferred from physiological evidence, remained one of the last to be chemically characterized [25]. Following such characterization, CRF has been established as the primary releaser of ACTH in most species [26]. As has been the case for many of the hypothalamic peptides, CRF has turned out to play a variety of additional neurotransmitter or neuromodulatory roles within the CNS. Most broadly, CRF mediates sympathetic activation, thus serving a central role in integrating the adrenocortical and autonomic branches of the stress response [26]. As part of the visceral and behavioral effects of CRF within the brain, the peptide causes electrophysiological excitation, producing epileptiform activity and even neuron loss [1,2,4,6,14,16,20].

It is this marked excitatory potential of CRF that makes it relevant to necrotic neuronal injury. Seizure, hypoxia-ischemia and hypoglycemia all damage via activation of excitotoxic glutamatergic pathways. Exogenous CRF can

potentiate the excitatory potential of glutamate [4], and the chemical neuroanatomy of CRF projections suggests that such potentiation occurs physiologically [5,13]. Diminution of glutamatergic tone by hypothermia or administration of barbiturates during necrotic insults is a well-characterized route of protection (see, for example, [8,15]), suggesting that antagonism of CRF action might be protective as well. The possible efficacy of this approach is underlined by the 2.5-fold increase in CRF mRNA that occurs in ischemic brain tissue [30], suggesting activation of CRF at such times.

A number of studies with rodents now demonstrate the protective potential of CRF antagonists. Such antagonists decrease ischemic damage to the hippocampus and enhance EEG recovery [12], protect the cortex and basal ganglia against damage caused by middle cerebral artery occlusion [23,30], as well as the striatum against glutamatergic damage [23].

In the present report, we demonstrate the considerable neuroprotective potential of a novel and potent CRF antagonist, astressin, against excitotoxic seizures. Most importantly, in terms of the potential therapeutic use of such

* Corresponding author. Fax: +1 (415) 725-5356.

compounds, we have shown that astressin can be neuroprotective even if administered only after an insult, rather than in anticipation of it. Astressin [cyclo(30–33){D-Phe¹²,Nle^{21,38},Glu³⁰,Lys³³}hCRF(12–41)] has a partially constrained secondary structure and is significantly more potent at inhibiting ACTH secretion when administered in the periphery than any of the other analogs previously tested, including α -helical CRF(9–41) or [D-Phe¹²,Nle^{21,38},C α MeLeu³⁷]hCRF(12–41) [9].

Astressin was synthesized by the solid phase method, purified using HPLC and characterized both chemically and biologically as described earlier [9]. Unlike α -helical CRF(9–41) which is soluble at neutral pH, astressin will dissolve in water (> 10 mg/ml, pH around 5) with gentle warming. Kainic acid (KA; Sigma, St. Louis, MO) was dissolved in sterile saline.

Subjects were male Sprague–Dawley rats (275–325 g; Simonsen, Gilroy, CA) housed on a 12:12 light:dark cycle and fed and watered ad libitum. KA was administered either systemically or by focal injection into the dorsal hippocampus, as described previously [18,22]. For systemic administration, KA (10 mg/kg b.wt in saline) was injected intraperitoneally into rats. For focal KA injection into the dorsal hippocampus, rats were anesthetized with a ketamine/rompun cocktail, placed in a stereotaxic head holder, and the skull exposed along the midline. KA (0.035 μ g in a volume of 1 μ l) was injected unilaterally into dorsal hippocampus (stereotaxic coordinates: AP = –3.0 from lambda, ML = 2.1; DV = 4.0 mm). As expected [3], essentially all animals administered KA by either route displayed seizures within the first hour post injection. Astressin (25 μ g in 4 μ l saline) or vehicle control (4 μ l saline) were administered by injection via an indwelling guide cannula into the lateral ventricle (coordinates: AP = 4.1 from bregma, ML = 3.0; DV = 2.0) over a 2 min period at indicated times with respect to the KA administration.

Three days post KA administration rats were perfused with 4% paraformaldehyde. Coronal brain sections (30 μ m) were stained with Cresyl violet and damage was quantified by measuring the length and width of the lesion in each hippocampal cell field with a calibrated ocular grid at $\times 40$ magnification. Areas of damage within successive sections were then integrated into a measure of total volume of damage.

Statistical comparisons were made by ANOVA fol-

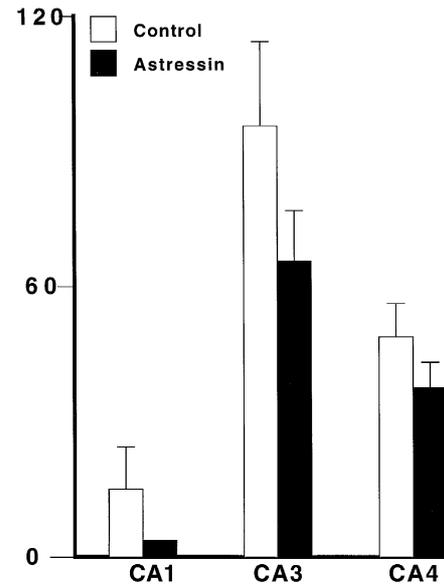


Fig. 1. Damage (in cubic microns) to CA1, CA3 and CA4 hippocampal cell fields in control rats and those treated with astressin before and after local microinfusion of kainic acid. Astressin caused an overall reduction in damage across cell fields ($P < 0.01$ by two-way ANOVA). $n = 10$ /group. In all figures, means without error bars had S.E.M.s too small to graph.

lowed by Newman–Keuls post-hoc tests, or by t -tests, as indicated. Data are expressed as means \pm S.E.M.s.

Astressin and control subjects did not differ in their rate of surviving of KA-induced seizures (98 ± 2 versus $89 \pm 8\%$, respectively; n.s. by t -test, $n = 3$ separate experiments), nor did they differ in circulating corticosterone concentrations up to 6 h after KA infusion (data not shown).

Initially, subjects were microinfused with KA into the dorsal hippocampus in order to cause local status epilepticus seizures with preferential damage to the CA3 pyramidal cell region. Astressin or vehicle were administered intracerebroventricularly (i.c.v.) both 30 min prior to and 10 min after the KA microinfusion. Astressin caused an overall reduction in damage across hippocampal cell fields, with non-significant trends towards reductions for each individual cell field were considered separately (Fig. 1 and Table 1).

We next examined epileptic damage to the hippocampus caused by systemic administration of KA, a more pathophysiologically relevant model, as it induces both

Table 1

Neuroprotection due to astressin administration

Model of excitotoxic insult and astressin administration	Percentage reduction in damage			
	CA1 (%)	CA3 (%)	CA4 (%)	Total (%)
(I) Kainic acid microinfusion, astressin before and after	–80	–33	–23	–42
(II) Systemic kainic acid, astressin before and after	–82	–74	–66	–77
(III) Systemic kainic acid, astressin after	–84	–62	–30	–70

Data represent transformation of absolute values in Fig. 1, Fig. 2 and Fig. 3 to percentage change.

seizures and convulsions, as seen in human status epilepticus. As before, astressin or vehicle was administered both before and after the KA treatment. Astressin caused a highly significant reduction of total hippocampal damage, as well as damage in all individual cell fields (Fig. 2 and Table 1).

Were CRF antagonists such as astressin to ever be of clinical use, it is essential that they be protective if delivered only after the onset of a necrotic insult (i.e., it is not possible to predict such an onset). Thus, we next tested whether astressin would protect against systemic KA when administered only 30 min after the excitotoxic insult. Again, the antagonist provided highly significant protection against seizure damage (Fig. 3 and Table 1).

Pre-insult administration of the CRF antagonist α -helical CRF(9–41) has been shown to decrease neuronal damage 40–60% induced by ischemia or excitotoxins [12,23]. In the present report, we demonstrate a superior neuroprotective potential of the CRF antagonist astressin against two newly tested models of seizure damage to the hippocampus. The ability of CRF antagonists to decrease necrotic damage, together with the induction of CRF mRNA in endangered tissue following such insults [30], suggests that the mobilization of the peptide contributes to neuronal injury. A number of mechanisms might explain this endangerment. The first concerns the neuroendocrine role of CRF as the key hypothalamic peptide controlling activation of the adrenocortical axis. Necrotic neurological insults cause massive mobilization of the axis in both humans and in experimental models of injury [7,18], and the resulting increased circulating glucocorticoid concentrations add to subsequent hippocampal, cortical and stri-

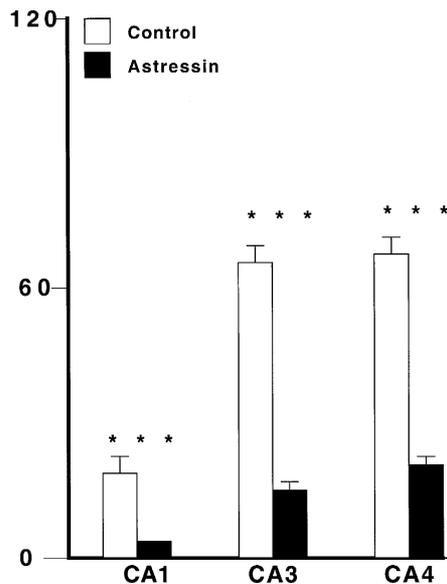


Fig. 2. Damage in control rats and those treated with astressin before and after systemic injection of kainic acid. *** indicates $P < 0.001$, Newman-Keuls post-hoc test following two-way ANOVA. $n = 16$ and 20 for control and astressin, respectively.

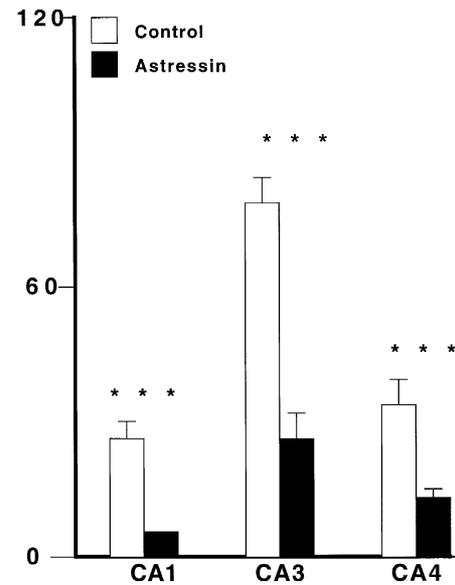


Fig. 3. Damage in control rats and those treated with astressin after systemic injection of kainic acid. *** indicates $P < 0.001$, Newman-Keuls post-hoc test following two-way ANOVA. $n = 11$ and 12 for control and astressin, respectively.

atal damage (reviewed in [17]). Thus, one might speculate that CRF enhances necrotic damage insofar as it enhances glucocorticoid secretion. However, as noted, we did not observe a difference in circulating corticosterone concentrations between astressin and control subjects post KA; in agreement with this, the ability of α -helical CRF(9–41) to decrease ischemic damage was shown to be independent of the adrenocortical axis [23].

As a second mechanism, CRF, in its neurotransmitter role, mediates sympathetic arousal during stressors, and CRF antagonists significantly blunt sympathetic tone. Elevated *pre*-ischemic catecholamine concentrations add to the resulting damage [10,19,21,29], most likely by sympathetically induced hyperthermia and hyperglycemia, two well-known enhancers of ischemic injury. Thus, CRF's deleterious actions may result from its sympathetic activation. However, CRF antagonists were reported to be neuroprotective without changing body temperature [23]. Furthermore, there is no evidence that enhancement of *pre-seizure* sympathetic tone adds to damage, yet *pre-seizure* treatment with astressin was neuroprotective. Finally, sympathetic activation during the *post*-ischemic reperfusion period is neuroprotective [11]. Therefore, it is unlikely that the deleterious effects of CRF are due to its activation of peripheral sympathetic tone.

Of additional relevance, CRF has been shown to have powerful anti-edemic properties, decreasing vascular leakage through injured endothelium [24,28]; as such, CRF can decrease the edema associated with traumatic brain injury [27]. However, the antagonism of such actions by astressin is probably not the route of neuroprotection. There is little reason to think that the necrotic neuron loss seen in

excitotoxic insults, such as utilized in the present report, is mediated by edema. Moreover, the pattern of anti-edemic actions of various CRF analogues suggests that they occur via the CRF-R2 receptor [24], whereas astressin is most likely to be exerting its protective effect through the CRF-R1 receptor [9].

The most plausible route by which CRF adds to necrotic damage is through its ability to cause electrophysiological excitation in numerous brain structures. Insofar as such excitation can lead to epileptiform activity and augment glutamatergic tone [1,2,4,6,14,16,20], this represents an obvious means by which the peptide would synergize with various excitotoxic insults.

The present report differs from prior ones, not only in its use of astressin (versus α -helical CRF(9–41)) and of a seizure model, but in its demonstration that administration of the antagonist after the insult (Fig. 3), rather than both before and after (Fig. 2), was quite protective. Whether the peptide would be protective with even greater delays in delivery remains to be tested. This finding is of heuristic value, in that it helps define the time window with which the cascade of events leading to delayed necrotic neuron death may occur. More importantly, it fulfills a critical prerequisite for any eventual therapeutic use of CRF peptide antagonists, namely that they are neuroprotective when administered after the onset of the neurologic insult.

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