Corticotropic-releasing Factor Decreases Vasogenic Brain Edema

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ABSTRACT

We report the first series of studies comparing the anti-edematous effects of human corticotropin-releasing factor (hCRF) and dexamethasone in an experimental model of vasogenic peritumoral brain edema. Both hCRF and dexamethasone effectively decreased blood-brain barrier (BBB) permeability of intracerebral RG2 gliomas in rats as observed by contrast-enhanced T1-weighted magnetic resonance imaging. A decrease in the water content of tumor and peritumoral brain tissue was observed with proton-density magnetic resonance imaging and confirmed by direct wet/dry tissue measurements. The calculated ED50 for hCRF was 59 μg/kg s.c. twice a day, and that for dexamethasone was 0.61 mg/kg i.m. twice a day; the hCRF:dexamethasone dose-potency ratio was 120:1 on a molar basis. The anti-edematous action of hCRF is not mediated by the release of adrenal corticosteroids. A direct action of hCRF on the tumor microvasculature results in restoration of BBB integrity and up-regulation of BBB-specific protein expression. The average survival time with chronic treatment was prolonged significantly in the hCRF-treated group (35 days) compared with the dexamethasone-treated group (28 days; P < 0.05) and the saline-treated control group (22 days; P < 0.0001). hCRF, as an alternative to corticosteroid therapy, may provide substantial benefits with respect to reducing the major side effects encountered with long-term, high-dose corticosteroid treatment.

INTRODUCTION

Corticosteroids have been used in the treatment of brain tumors and associated peritumoral brain edema for >30 years since the report of Gallicich et al. (1). Although treatment of brain edema with corticosteroids can result in dramatic clinical improvement, their use is often associated with detrimental side effects, especially at high doses and long-term administration (2–8). Recently, hCRF and other peptides of the corticotropin-superfamily have been shown to inhibit vascular leakage of plasma constituents in response to injury and to the injection of vasoactive compounds (9–14). hCRF is a naturally occurring, 41-residue neuropeptide with a molecular weight of 4,700. This neurohormone is produced in the hypothalamus and is mainly responsible for stimulating the HPA axis (15). hCRF has been shown to be active in other physiological functions, including stress reactions (16), and there is some evidence that suggests a neurotransmitter function for this neurohormone (17–19). As an anti-edematous agent, hCRF prevents the vascular leakage induced by a variety of inflammatory mediators that selectively act on postcapillary venules and veins in skin (9) and also inhibits leakage from lung alveolar capillaries (10–11). Preliminary studies in humans showed that hCRF inhibits the flare response to intradermal histamine (12). When hCRF was injected s.c. in rats at a dose of 30 μg/kg before celiotomy, it prevented vascular leakage from muscle capillaries (13). These latter observations suggested that hCRF acts widely throughout the microcirculation to preserve endothelial cell integrity. The possible mechanisms by which hCRF acts to inhibit vascular leakage have been discussed in the literature (11, 14), but the precise mechanisms are not yet established.

We report a series of animal studies that evaluate the anti-edematous potential of hCRF in a well-established animal model of vasogenic peritumoral brain edema. hCRF-treated animals were compared with similar groups of dexamethasone- and saline-treated animals. Treatment response was monitored by MRI and by direct measurements of tumor and peritumoral brain water content. A dose-response profile of hCRF and dexamethasone was also obtained. Similar experiments were performed in adrenalectomized animals to assess the influence of the HPA axis on treatment response. Also, we measured plasma corticosterone levels in corresponding groups of adrenalectomized and nonadrenalectomized rats before and after 3 days of treatment with hCRF and dexamethasone. Immunohistochemical studies were performed to assess morphological changes in tumor microvessels induced by hCRF and dexamethasone. Finally, we assessed the effects of chronic hCRF and dexamethasone treatments on survival of rats bearing i.c. tumors.

MATERIALS AND METHODS

hCRF was chemically synthesized and purified by HPLC and supplied in lyophilized form by Neurobiological Technologies, Inc. (Richmond, CA). One mg of lyophilized hCRF was dissolved in 1 ml of 0.1 N HCl and further in saline to a total volume of 10 ml; the pH was adjusted to 7.4 with NaOH. Dexamethasone sodium phosphate solution (4 mg/ml) was obtained from American Reagent Laboratories, Inc. (Shirley, NY).

i.c. Tumor Model. The experimental protocol was approved by the Institutional Animal Care and Use Committee, i.e. tumors were produced by i.c. inoculation of the RG2 cell line, which was derived from an ethyl nitrosourea-induced rat glioma as described previously (20–24). Fischer 344 rats weighing 200–250 g and adrenalectomized Fischer 344 rats weighing 150–200 g were purchased from Taconic (Germantown, NY). The conditions for maintaining adrenalectomized rats included 20 μg/ml of dexamethasone in 0.9% saline for drinking, regular chow, and a stress-free environment with a temperature at 28°C. Dexamethasone supplementation was discontinued 2 days before i.c. tumor inoculation.

The animals were anesthetized with a gas mixture containing 5% isoflurane and oxygen and maintained with a mixture of 1.5% isoflurane, 70% nitrous oxide, and 30% oxygen. A 2% lidocaine gel was applied to the ears, and Ihe scalp was incised, and a 2-mm burr hole was made 3 mm to the right of the sagittal suture and 1 mm posterior to the coronal suture; a 26-gauge short-bevel needle was inserted to a depth of 7 mm under stereotactic control and aseptic conditions. A sterile 10-μl suspension containing 1 × 105 viable tumor cells was slowly injected into the caudate nucleus. The animals were returned to their cages, observed, and weighed daily for signs and symptoms of intracranial tumor growth.

MRI Studies. The intensity of BBB breakdown and the extent of peritumoral edema in the i.c. RG2 tumors was assessed by MRI, which was performed on a General Electric CSI system with a 4.7-Tesla, 33-cm bore.


magnet equipped with shielded gradients. A standard imaging coil was used. The i.c. tumor-bearing rats were anesthetized with the mixture of 1.5% isoflurane, 70% nitrous oxide, and 30% oxygen. Once adequate sedation was obtained, the rats were placed in a special holder to stabilize the head. The rats were then placed in the coil with the brain centered in the midpoint of the solenoid resonator. The anesthetic gas mixture was supplied constantly to the animal via the tubing attached hermetically to the holder system throughout the imaging session. Proton-density and $T_1$-weighted contrast-enhanced images were obtained. The contrast-enhanced images were obtained between 4 and 6 min after i.v. injection of Gd-DTPA (0.2 ml/kg). Coronal slices (3-mm thick plus 1-mm gap between slices) using a 256 $\times$ 256 matrix, 60-mm field of view, and standard echo sequences ($TR = 300$ ms, $TE = 10$ ms and 2 excitations per phase-encoding step) were obtained through the area of the i.c. tumor. Tumor and edematous peritumoral brain tissues were detected as the areas of hyperintense signal on proton-density images ($TR = 3000$ ms, $TE = 10$ ms and 2 excitations per phase-encoding step); areas of BBB disruption were detected as the contrast-enhancing regions on $T_1$-weighted images.

**Measurements of Corticosterone Levels in Blood Plasma.** The animals were anesthetized with a gas mixture containing 5% isoflurane and oxygen and maintained with a 1.5% isoflurane, 70% nitrous oxide, and 30% oxygen mixture. Venous blood was drawn and immediately centrifuged to obtain plasma, which was quickly frozen and kept at $-80^\circ$C until further use. Plasma corticosterone concentrations were measured in duplicate by RIA (ICN Biomedicals, Costa Mesa, CA). Briefly, plasma samples were diluted 25–200-fold, mixed with $^{125}$I-labeled corticosterone, and incubated at room temperature for 2 h with a specific antisera against rat corticosterone. Cross-reactivity of corticosterone antibody with deoxycorticosterone, progesterone, or estrone was 0.34, 0.10, 0.02, and <0.01%, respectively. After incubation, the corticosterone bound to antibody was precipitated by a mixture of polyethylene glycol and goat antirabbit gamma globulins. The amount of $^{125}$I radioactivity in the precipitates of duplicate samples was measured (COBRA QC, Model 5010; Packard), and plasma corticosterone concentrations were obtained from a comparison of measured radioactivity with a simultaneously run standard curve.

**Tissue Water Content.** Tumor, peritumoral edematous brain, and other structures of the brain were sampled, dried at 90°C in a constant temperature oven, and reweighed daily until a constant dry weight was achieved (usually 3 to 4 days). Water content of the tissue was calculated as:

$$\% \text{ tissue water} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100$$

**Immunohistochemistry.** Animals in deep anesthesia were decapitated with a guillotine, and the whole brains were extracted, frozen, embedded in M-1 cryomatrix (Lipshaw, Pittsburgh, PA), and kept at $-80^\circ$C until further use. Frozen 20-μm-thick sections were obtained for double-label immunohistochemical staining of microvessels and BBB-specific endothelial protein. Sections were reacted with rabbit antihuman polyclonal antibody for Von Willebrand/ factor VIII (DAKO, Glostrup, Denmark) and stained using Vectastain avidin-biotin complex-alkaline phosphatase and Vector Blue alkaline phosphate substrate kits (Vector Laboratories, Inc., Burlingame, CA). Then sections were reacted with SM171 mouse antirat monoclonal antibody, which recognizes EBA on the rat brain microvessels (Stenbern Monoclonals, Inc., Baltimore, MD) and stained using avidin-biotin complex kit (Vector Laboratories, Inc.) and $3,3'$-diaminobenzidine tetrahydrochloride as a chromogen (Sigma Chemical Co., St. Louis, MO). Finally, sections were counterstained with methyl green nuclear stain (Vector Laboratories, Inc.).

**Survival Study.** The animals were observed and weighed daily after i.c. inoculation of RG2 glioma cells. When animals developed symptoms of ataxia, severe paraes, seizures, peripneumonic cristiatisions, or posturing, or were in an agonal stage, they were interpreted as “near death” and were euthanized with an i.v. injection of 1.0 ml of Euthanasia-5 solution (Henry Schein, Inc., Port Washington, NY). Life expectancy of the near-death animal was estimated to be <1 day. The day of euthanasia was treated as the day of death in the survival analysis.

**Statistics.** Simple descriptive statistics of group data was performed using univariate analysis. Furthermore, group data were compared using ANOVA analysis and paired and unpaired Student’s $t$ tests; a value of $P < 0.05$ was considered significant. The survival distribution was determined from the Kaplan-Meier survival curves. All statistical analyses were performed using StatView 4.02 software (Abakus Concepts, Inc., Berkeley, CA).

**RESULTS**

**BBB Permeability and Tissue Water Content.** To compare the effects of hCRF and dexamethasone on the permeability of the BBB and on water content of tumor and peritumoral brain tissue, rats bearing i.c. RG2 tumors were divided into three groups of 18 rats. Each group received treatment with either hCRF (100 μg/kg s.c., b.i.d.), dexamethasone (1 mg/kg i.m., b.i.d.), or saline (0.1 ml i.m., b.i.d.). Therapy was initiated on day 7 after tumor inoculation and was continued for 3 days. Two sequential MRI studies were performed on each animal: the first, before treatment, on day 7 after tumor inoculation; and the second, after 3 days of treatment, on day 10 after tumor inoculation. $T_1$-weighted Gd-DTPA contrast-enhanced MRIs were used to monitor qualitative changes in BBB permeability. Proton-density MRIs were used to monitor qualitative changes in tissue water content. Tumor, peritumoral brain tissue, and other brain structures were assayed directly for tissue water content after 3 days of treatment.

Treatment with hCRF resulted in a reduction in the intensity of contrast enhancement of i.c. RG2 tumors on $T_1$-weighted MRI (Fig. 1, A and B). A reduction in contrast enhancement reflects a decrease in the permeability of tumor capillaries. The effect of hCRF on the BBB was similar to that obtained with dexamethasone treatment (Fig. 1, C and D). In contrast, there was an increase in the intensity of contrast enhancement of saline-treated control animals (Fig. 1, E and F), which suggests an increase in the permeability of tumor blood vessels during the 3-day interval.

Both hCRF and dexamethasone treatments decreased the proton-density signal of i.c. RG2 tumor and peritumoral brain (Fig. 2, A and B). A decrease in tissue proton density reflects a decrease in tissue water content. In contrast, there was an increase in proton density of tumor and peritumoral brain in saline-treated control animals (Fig. 2, E and F), which suggests an increase of tumor and peritumoral brain edema.

Direct measurements of tissue water content in tumor and peritumoral brain using wet/dry weight measurements confirmed the antiedematous effects of hCRF and dexamethasone observed with MRI. The tissue water content of the tumor, peritumoral brain (ipsilateral hemisphere), and contralateral hemisphere was significantly lower in the animals treated with hCRF and dexamethasone as compared with controls (Fig. 3).

**Dose Response.** A dose-response curve for hCRF and dexamethasone treatment of peritumoral brain edema was obtained in rats bearing i.c. RG2 glioma. The animals were divided into three treatment groups (30 rats/group) as described in the previous experiments. Doses of 10, 30, 60, 100, and 300 μg/kg of hCRF and 0.1, 0.3, 0.6, 1, and 3 mg/kg of dexamethasone were used (6 rats/dose). Therapy with different doses of hCRF and dexamethasone was initiated on day 7 after tumor inoculation and was continued for 3 days. Treatment response was assessed in terms of tumor and peritumoral brain water content, which was measured by the wet/dry weight method. Both hCRF and dexamethasone showed a dose-dependent, anti-edematous effect on tumor and peritumoral brain edema (Fig. 4). The percentage of tissue water content in tumor and peritumoral brain demonstrates an inverse-S relationship with increasing dose for both drugs. The calculated $ED_{50}$ for hCRF was 59 μg/kg b.i.d. (s.c.), and that for dexamethasone was 0.61 mg/kg b.i.d. (i.m.). The hCRF/dexamethasone dose potency ratio was ~120:1 on a molar basis.

**Adrenalectomized Animals.** To assess the noncorticosteroid-mediated effects of hCRF on vascular permeability and peritumoral brain

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Fig. 1. Effects of hCRF and dexamethasone on vascular permeability (BBB) of i.c. RG2 gliomas. 

T1-weighted Gd-DTPA contrast-enhanced MRIs of rat heads in the coronal plane are presented. Pre-
and posttreatment images of the same animal are arranged vertically in pairs. Treatment groups in-
cluded: hCRF (A and B); dexamethasone (C and D); and saline-treated control (E and F). The con-
trast-enhancing area localizes the i.e. tumor in the right hemisphere and reflects the extent and the
intensity of BBB disruption before treatment (A, C, and E). There is a decrease in contrast enhance-
ment of the i.e. tumor because of an apparent decrease in the permeability of tumor vessels in
both hCRF- (B) and dexamethasone- (D) treated animals. Conversely, in the saline-treated control
animals, there is a further increase in BBB perme-
ability, visualized as an increase in the size and intensity of the contrast-enhancing area of the tu-
mor (F).

Fig. 2. Effects of hCRF and dexamethasone on the water content of i.c. RG2 gliomas and peritu-
moral brain tissue. Proton-density MRIs of the rat heads in the same coronal planes, obtained during
the same imaging session as those in Fig. 1. Pre-
and posttreatment images of the same animal are arranged vertically in pairs. Treatment groups in-
cluded: hCRF (A and B); dexamethasone (C and D); and saline-treated control (E and F). The area
of the increased proton density localizes to the i.e. tumor and peritumoral edematous brain in the right
hemisphere and reflects increased local tissue water
content, which is present in all tumors before treat-
ment (A, C, and E). There is a significant decrease
in proton density of the i.e. tumor because of ap-
parent decrease of local tissue water content in both
hCRF- (B) and dexamethasone- (D) treated ani-
mals. Conversely, in saline-treated control animals,
there is a further increase in local tissue water
content, visualized as an increase in size and signal
intensity in area of the tumor and peritumoral
edematous brain (F).

Fig. 3. Tissue water content after therapy in rats with the i.c. RG2 tumors and the percentage of
water content of the i.e. tumor, peritumoral brain
(ipsilateral hemisphere), and contralateral hemi-
sphere. Values are means; bars, SD. *, statistically
significant difference (P < 0.05).
edema, adrenalectomized Fischer 344 rats bearing RG2 i.c. tumors were studied. The possibility of hCRF induction of corticosteroid secretion by the adrenal glands was eliminated in these animals. The study protocol involved three treatment groups identical to those described above for nonadrenalectomized animals. Given the fragility of adrenalectomized animals under stress, proton-density MRI was not performed, because it requires a longer duration of gas anesthesia (35 min) compared with the T1-weighted MRI (5–6 min). A parallel study was also performed in nonadrenalectomized animals bearing i.c. RG2 gliomas to serve as an internal control.

hCRF treatment significantly decreased the permeability of i.c. RG2 glioma capillaries in adrenalectomized rats, as observed on T1-weighted Gd-DTPA-enhanced MRI (Fig. 5, A and B). The change in tumor vascular permeability induced by hCRF was comparable with that observed with dexamethasone (Fig. 5, C and D). In contrast, there was progressive disruption of the BBB during the 3-day period of saline treatment in control animals (Fig. 5, E and F). The water content of i.c. RG2 glioma and peritumoral brain tissue in hCRF and dexamethasone-treated adrenalectomized animals was significantly lower compared with similar saline-treated control animals (Fig. 6A). The magnitude of the anti-edematous effect was similar for hCRF and dexamethasone treatment in adrenalectomized animals. Furthermore, the responses obtained in adrenalectomized animals were essentially identical to that obtained in the parallel study involving nonadrenalectomized animals (Fig. 6B).

**Plasma Corticosterone Levels.** Three sets of studies were performed. In the first two sets, plasma corticosterone was measured after 3 days of treatment in both adrenalectomized and nonadrenalectomized animals used in MRI studies. Blood was collected in the afternoon (4:00–7:00 p.m.). In all treatment groups of adrenalectomized animals, corticosterone was below a detectable level (<1 ng/ml). In nonadrenalectomized animals, plasma corticosterone level was lower in hCRF-treated animals (193 ± 92 ng/ml) than in the saline-treated control animals (424 ± 165 ng/ml; P < 0.01), which was above the normal range (25, 26). Dexamethasone-treated animals had substantially lower corticosterone levels (3.9 ± 1.7 ng/ml) than did hCRF-treated and control animals (Fig. 7A).

In the third set, plasma corticosterone was measured before and after therapy in rats bearing i.c. tumors. Adrenalectomized animals were not studied in this set. Animals with i.c. tumors received hCRF, dexamethasone, or saline in the same doses as in previous experiments (six rats/group). Three groups of naive (nontumored) rats (also six rats/group) were studied in parallel. Two groups of naive rats received hCRF 100 μg/kg b.i.d., s.c. and i.p., respectively; the third group received saline s.c. and served as the control. During the 4 days before treatment, animals were conditioned to reduce the stress associated with their handling. One day before the initiation of treatment, in the afternoon (4:00–7:00 p.m.), animals were anesthetized as described above, and the venous blood was drawn. Treatment was initiated the next day in the afternoon at the same time. The last (sixth) injection was administered in the morning (9:00–10:00 a.m.), and bloods were drawn in the afternoon (4:00–7:00 p.m.) with the rats under anesthesia as described above. Administration of hCRF did not increase pretreatment (baseline) plasma corticosterone levels. There was a relative decrease in plasma corticosterone levels in all groups of rats receiving hCRF (Fig. 7B), which was statistically significant (P < 0.0001) when pre- and posttreatment data on corticosterone levels in all groups of rats receiving hCRF in the third set were pooled and analyzed together by paired Student’s t test. The most dramatic fall in plasma corticosterone levels was observed in dexamethasone-treated animals. It is interesting to note that in tumored animals receiving saline, plasma corticosterone levels increased as compared with the pretreatment levels and were similar to those observed in saline-treated control animals in the first two sets. There was essentially no change in plasma corticosterone levels of the nontumored control animals receiving saline.

**Immunohistochemistry.** The effects of hCRF and dexamethasone on the expression of EBA by microvessels of the i.c. tumors were assessed in animals from the second set of plasma corticosterone measurement studies described above. In the control group, there was no EBA staining in the center or in the periphery of the i.c. tumors (Fig. 8C). In contrast, hCRF treatment (100 μg/kg s.c., b.i.d., for 3 days) induced EBA expression by microvessels in both the center and the invasive border of the tumor (Fig. 8A). Dexamethasone treatment (0.1 mg/kg i.m., b.i.d., for 3 days) resulted in a similar but less intense induction of EBA expression by the tumor microvessels as compared with hCRF treatment (Fig. 8B).

**Survival Study.** The effects of hCRF and dexamethasone on survival were studied in animals inoculated i.c. with RG2 glioma cells as described above. Three groups of i.c. tumor-bearing rats (15 rats/group) received 100 μg/kg hCRF b.i.d. (s.c.), 1 mg/kg dexamethasone...
b.i.d. (i.m.), and 0.1 ml saline b.i.d. (i.m.), respectively. Treatment was initiated the day after tumor inoculation and continued as long as the animal survived. The average survival time determined from Kaplan-Meier plots was significantly prolonged for the hCRF-treated group (35 days) compared with dexamethasone-treated (28 days; \( P < 0.05 \)) and the saline-treated control group (22 days; \( P < 0.0001 \)) (Fig. 9A). A significant weight loss of 50% of the initial weight was observed in dexamethasone-treated animals, whereas hCRF-treated animals exhibited only a 10–15% weight loss (Fig. 9B).

**DISCUSSION**

Peritumoral brain edema is a major complication of intracranial neoplasms. This edema is primarily vasogenic in origin, and high-dose glucocorticoid administration has been the treatment of choice in its management. Although corticosteroid treatment of brain edema can result in dramatic clinical improvement, long-term use is often associated with detrimental side effects, particularly at the high doses required for efficacy (2–8, 27, 28). The severe myopathies and muscle wasting (29–32), osteoporosis (33) and avascular osteonecrosis (34–36), gastrointestinal bleeding (37, 38), and psychological effects ranging from personality changes (39, 40) to frank psychoses (41, 42) that are observed during corticosteroid therapy can be more debilitating to patients than the neurological deficits directly related to the tumor. Therefore, it is important to continue to search for anti-edematous agents with similar or even greater potency and with less toxicity, particularly that associated with chronic high-dose corticosteroid administration.

hCRF and other peptides of the corticoleerin superfamily have been shown to inhibit transendothelial leakage of plasma-derived fluid and tissue swelling in response to injury or to the injection of vasoactive compounds (14–19). In this study, we show that hCRF is as effective as high-dose dexamethasone in the treatment of peritumoral brain edema in the i.c. RG2 rat glioma, which is a well-established brain tumor model. Microscopic features, blood flow, and permeability characteristics of the i.c. RG2 glioma have been described elsewhere (20–24). Previous studies have shown that treatment with i.m. dexamethasone (1.5 mg/kg b.i.d. for 2.5 days) consistently reduced or eliminated the filtration of plasma-derived fluid across tumor capillaries.
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Fig. 7. Effects of hCRF and dexamethasone on plasma corticosterone levels. Corticosterone concentration in plasma of rats treated with hCRF s.c. or i.p., dexamethasone i.m., and saline i.m. (control) for 3 days. A, plasma corticosterone levels in animals with i.e. RG2 tumors measured after treatment only. Values are means; bars, SDs of data pooled from the first two study sets. B, plasma corticosterone levels in animals with i.e. RG2 tumors and in nontumored animals before and after treatment. Values are means; bars, SD.

The pretreatment levels of plasma corticosterone in our studies (third set) were above the normal maximal level, which we think is a reflection of inadequate conditioning and stress during the first anesthesia for blood sampling. In addition, we collected blood samples in the afternoon (4:00–7:00 p.m.), when plasma corticosterone levels are known to be high (~300 ng/ml), as shown in studies on circadian rhythms in rats (25, 43). The absence of a further increase, and a tendency toward a decrease in plasma corticosterone levels in rats receiving s.c. or i.p. hCRF, can be explained in at least two ways. One explanation is that the repeated injections of CRF cause a “blunting effect” on adrenocorticotropic hormone release and decrease peak levels of plasma corticosterone in response to subsequent stimulation of the HPA axis by CRF (44–46). This blunting effect results from the corticoid-mediated feedback inhibition of endogenous CRF and adrenocorticotropic hormone release (45–47). Thus, multiple injections of supraphysiological doses of hCRF could attenuate the circadian peak of corticosterone in plasma, which was measured 8 h after the last hCRF injection. Another, but less likely, explanation is that the animals became more conditioned during the course of treatment and were less stressed during the second anesthesia for blood sampling. As expected, dexamethasone treatment resulted in suppression of the HPA axis and a highly significant drop of plasma corticosterone to almost nondetectable levels.

The anti-edematous activity of hCRF appears to be mediated by direct vascular action and may involve at least two different domains of the hCRF peptide. One domain corresponds to the first nine residues at the amino terminus of hCRF and is required for CRF receptor activation (48). Two types of CRF receptors have been described. The CRF1 receptor is expressed mainly in the brain and pituitary gland (49, 50) and in pituitary CRF2, which is also expressed in the heart and muscle (51, 52). Activation of CRF receptors results in the increase in cytosolic Ca\(^{2+}\) concentrations (49, 53) and in proopiomelanocortin production in pituicytes (54). Characterization of CRF receptors on the endothelial cells has not yet been reported. However, binding of iodinated hCRF to endothelial cells occurs in close proximity to the sites of vascular leakage (13, 55). Also, hCRF binding to the endothelial cells and the major anti-edematous effect of hCRF can be blocked by a CRF receptor antagonist, α-helical CRF (9–41, 56). The CRF receptor-mediated increase in endothelial Ca\(^{2+}\) levels may also explain a vasodilatory effect of hCRF described by others (15, 57, 58).

In the current study, we have shown that hCRF treatment induced expression of EBA by the brain tumor microvessels. EBA was absent in the vasculature of saline-treated control brain tumors. The antibody that we used for immunohistochemical staining of EBA reacts with...
of the i.e. RG2 gliomas. Double-label immunohistochemistry for factor VIII (light concentration improves the endothelial and BBB function (61, 62).

endothelial cAMP and Ca++ cause the up-regulation of expression of the luminal plasma membrane of central nervous system nonfenes-
sels from a control animul treated with saline.

animal treated with dexamethasone. C. no EBA expression is seen in the tumor microves
treated with hCRF. fl. patchy expression of EBA by the tumor microvessels from the staining. X 100. A, strong expression of EBA by the tumor microvessels from the animal treated with dexamethasone. The prolongation of survival with chronic dexa

Fig. 8. Effects of hCRF and dexamethasone on the expression of EBA by microvessels of the i.c. RG2 gliomas. Double-label immunohistochemistry for factor VIII (light blue-gray) and EBA (brown) of i.c. RG2 gliomas. Sections are counterstained with ethyl green nuclear stain (light green). Arrows, localization of the characteristic immuno-staining. ×100. A, strong expression of EBA by the tumor microvessels from the animal treated with hCRF. B, patchy expression of EBA by the tumor microvessels from the animal treated with dexamethasone. C, no EBA expression is seen in the tumor microvessels from a control animal treated with saline.

the luminal plasma membrane of central nervous system nonfenes-
trated endothelium and was shown to detect alterations in the BBB in lesions in Lewis rats with experimental allergic encephalitis (59, 60).

We suggest that the activation of CRF receptors and increase in endothelial cAMP and Ca^{2+} cause the up-regulation of expression of BBB-specific structural proteins such as EBA, which may explain, at least in part, the morphological basis of anti-edematous effect of hCRF. It has been shown that elevation of the intracellular cAMP concentration improves the endothelial and BBB function (61, 62).

Other studies have shown that the direct, nonsteroid-mediated anti-
edematous and anti-inflammatory effects of hCRF are exerted by another domain located near the carboxy terminus of hCRF (63). Sequences (indicated by a single-letter amino acid code) RKLME near the carboxy terminus of hCRF (35–39) and RKLLD of ovine CRF (35–39) were found to be similar to the highly conserved sequence RKLLE of the coil regions of several intermediate filaments (64–66). Intermediate filaments such as keratins, desmins, vimentins, neurofilaments, and others play an important role in the maintenance of cell morphology and in the regulation of vascular permeability (67–69). Studies using hCRF carboxy terminus fragment peptides similar to the IATYRKLLE portion of these cytoskeletal proteins suggested that these peptides may affect vascular permeability by modulating the activity of intermediate filaments and change cell-cell and cell-matrix adhesions (63). These cell-cell and cell-matrix adhesions are especially important for maintaining the integrity of a normal BBB.

The anti-edematous mechanism of dexamethasone is different from that of hCRF. Dexamethasone treatment had only a small effect on the EBA expression by the tumor vasculature, but the anti-edematous effect was somewhat stronger than that of hCRF. The effect of dexamethasone on peritumoral brain edema is known to be mediated through steroid receptors and results in a decrease in the permeability of tumor capillaries and in a lower rate of plasma-derived edema fluid moving into the brain (23, 24, 70). A recent study on the effects of dexamethasone on transcapillary transport in experimental RG2 brain tumors in rats and in avian sarcoma virus-induced canine brain tumors did not support the hypothesis that dexamethasone reduces brain tumor capillary permeability (71, 72). The authors have suggested that dexamethasone has an effect on the bulk flow away from the tumor margin. Other evidence suggests that dexamethasone treatment causes a fluid shift from extra- to intracellular compartments because of effects on the cell membrane K^+-Na^+ pump (73) and that this fluid shift decreases the extracellular space and increases the resistance to the intraparenchymal movement (spread) of edema fluid through the brain (24).

Chronic administration of hCRF and dexamethasone revealed other important differences. Survival of animals with i.c. RG2 gliomas was significantly longer with hCRF treatment than in those animals treated with dexamethasone. The prolongation of survival with chronic dexamethasone treatment was accompanied by severe weight loss and muscle wasting, and this was not observed in hCRF-treated animals. This is consistent with myopathic/atrophie effects of dexamethasone on skeletal musculature that arise from the ability of corticosteroids to inhibit protein synthesis by reducing muscle ribosomal capacities and degrading contractile proteins predominantly in fast-twitch type I muscle fibers (74, 75). In a separate study using histochemical staining for ATPase (76), we also have found that chronic dexamethasone administration causes a dramatic loss of type I fast-twitch fibers and a decrease in the weight of the individual muscles, but there are no atrophic changes in the musculature of animals receiving chronic hCRF treatment. The reasons for the initial 10% weight loss in hCRF-treated animals remain to be explained.

In conclusion, our findings demonstrate that hCRF has substantial anti-edematous effects that are comparable to those obtained with dexamethasone in a well-established animal model of vasogenic peritumoral brain edema. hCRF, as an alternative to corticosteroid therapy, may provide substantial benefits with respect to reducing the major side effects encountered with long-term, high-dose corticoste-
roid treatment.

\* J. G. Tjuvajev, unpublished observations.
Fig. 9. The effects of hCRF and dexamethasone on survival and weight of the animals with i.c. RG2 gliomas. A. Kaplan-Meier plot of cumulative survival in animals treated with hCRF (○), dexamethasone (●), and saline (○). B. Percentage of initial body weight change over time after initiation of treatment. Groups are the same as in A. Values are means; bars. SDs of all the animals in corresponding treatment groups that have survived at each day.

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