CORTICOTROPIN RELEASING FACTOR AND GASTRIC EROSIONS

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Several neuropeptides have been shown to either promote or inhibit the development of gastric erosions in rats; recent reviews have been presented by Taché[1] and Hernandez[2].

One of these peptides, corticotropin releasing factor (CRF) inhibits pentagastrin stimulated gastric acid secretion after either central[3] or peripheral[4] administration. However, the results reported concerning the effects of CRF on gastric erosions have not been consistent.

When administered peripherally, CRF (10 μg, subcutaneously) was shown to potentiate ulcer formation during cold restraint in one study[5]. We have previously reported a protective effect of 8-10 μg of CRF (intraperitoneal injection) during water restraint (1-2 h)[6,7]. The latter study revealed that while this protective effect was evident in rats aged 100 days, it was not in 200-day old rats. In fact, our data indicate that CRF exerts ulcerogenic properties in older (220 days) rats[7], as opposed to the effect seen in younger rats. Interestingly, this study also revealed a trend for ulcerogenic properties in unrestrained, 20 h fasted younger (100 days) animals. It may be that CRF exerts different gastric effects in rats depending on the condition (stress/non-stress) under which the rats are tested.

As for the effects seen after central administration, Nakane *et al.*[8] reported an ulcerogenic effect in 24 h food-deprived rats but not in restrained rats after intracisternal administration of 5 μg CRF. No significant potentiation of cold supine restraint-induced gastric erosions was observed. Conversely, others[9] have reported no increase in gastric ulcers in 24 h fasted, non-restrained rats 4 h after ICV administration of 10 μg CRF. Moreover, Krahn *et al.*[10] showed an inhibitory effect of intracerebroventricular (ICV) administration of 5 μg CRF during cold restraint. This effect was reversed by the CRF antagonist α-helical CRF (9-41) (ICV). The antagonist itself was inactive. Intraventromedial hypothalamic administration of 2 μg CRF (per side) inhibited cold restraint-induced mucosal damage in another study[11].

While the mechanisms behind the effects of CRF on gastric acid secretion have been carefully investigated, especially by Taché and coworkers[3,4,12], this seems not to be the case regarding the effect on restraint induced gastric erosions.

The inhibitory effect of ICV-administered CRF on gastric acid secretion is blocked by vagotomy and adrenalectomy but not by hypophysectomy or naloxone treatment. This effect therefore, seems to be a CNS phenomenon independent of

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hypophysiotropic actions but mediated through vagal and adrenal mechanisms[3]. However, the importance of gastric acid secretion for the development of gastric erosions have been called into question. Though the presence of some acid seems to be a prerequisite for erosions to develop, experimental studies have revealed restraint stress in rats to induce a decreased gastric acid secretion in rats[13,14]. The mechanisms by which centrally administered CRF affects stress-induced gastric erosions may therefore be different from those that cause the inhibition of gastric acid secretion. Previous experiments from our laboratory employing peripherally administered CRF have, on the other hand, given strong indications that the ulceroprotective effect of CRF is also independent of its hypophysiotropic actions[6,7].

In the experiment to be presented here, we undertook to elucidate the central mechanisms behind the effect of CRF on restraint stress-induced gastric erosions. Specifically, we wanted to investigate a possible interaction with the locus coeruleus (LC) noradrenergic system. Both the CNS noradrenergic system in general and the LC system in particular have been implicated in the development of restraint induced gastric erosions[15]. Anatomical studies have revealed coexistence of CRF immunoreactivity with noradrenaline (NA) within the LC[16] and alterations in CRF-like immunoreactivity in the LC after exposure to stress[17]. Moreover, electrophysiological studies have shown administration of CRF ICV or directly to LC neurons to increase discharge rates of these neurons and have given evidence for a neurotransmitter role for CRF in the LC during hemodynamic stress[18,19,20].

Experimental rats were in this study pretreated with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride (DSP-4), a highly selective and long-lasting noradrenergic neurotoxin. This drug affects mainly nerve terminal projections originating in the LC, while noradrenergic pericarya in this nucleus appear unaffected[21]. There is also a transient toxic effect on sympathetic neurons outside the brain with an almost normal appearance within one week after drug administration[15,21,22]. The transient nature of this peripheral effect has been shown in several organs; iris, stomach, salivary glands and the heart.

ICV administered neuropeptides can "leak" into the peripheral vascular system[23]. To control for such an occurrence, CRF was also administered intraperitoneally (IP) to DSP-4 treated animals.

PROCEDURES

35 male Sprague-Dawley rats (Möllegaard, Denmark) were treated IP with a single dose (0.4 ml) of DSP-4 (50 mg/kg in 0.9% NaCl) (Astra Läkemedel, Sweden). Control animals (N=12) were injected with an equivalent quantity of physiological saline. The average weight of the rats was 300 grams at the start of the experiment. Animals were single housed and maintained in light-(12:12 h) and temperature-regulated animal quarters. They had free access to food and water. Twenty experimental and all control animals were later implanted with chronic cannulas in the left lateral ventricle of the brain. Four DSP-4 animals died or were sacrificed due to physical illness with serious weight loss (three from the implanted group).

All groups were during the following weeks, tested for plasma catecholamine and corticosterone response to either ICV or IP administration of CRF. An open field test was also performed. The results from these experiments (to be presented elsewhere) were in accordance with previous reports. However, no interaction

between the effects of CRF and pretreatment with DSP-4 was observed on any parameter.

Two weeks after the open field test, all animals were deprived of food but not water for 24 h prior to immobilization stress procedures. The immobilization procedure was water restraint for 120 min at 19.3 $\pm$ 0.2°C. Human CRF (Bissendorf, West Germany) 8 μg (1.68 nmol) dissolved in 8 μl 0.9% NaCl or only 8 μl saline was administered ICV 15 min before onset of the restraint period. Intraperitoneally treated rats were injected with equivalent doses in a volume of 0.4 ml at the same time point. The animals were sacrificed immediately after the restraint session. The stomachs were removed, cut along the lesser curvature, and cumulative ulcer length was scored. At the same time, brains from six DSP-4 treated and six control animals were rapidly taken out of the skull, the neocortex removed and frozen (liquid nitrogen). They were later analyzed for NA and dopamine content employing high-performance liquid chromatography (HPLC) methods.

RESULTS

HPLC analyses of cerebral cortex NA content revealed a 60% reduction after DSP-4 treatment (Mann-Whitney U Test: $Z=2.88$, $p<0.004$). Means, ng/g wet weight tissue (SEM); DSP-4: 132.53 (2.17), Control: 310.16 (13.96). No difference between groups was observed on dopamine content ($F<1.0$).

The cumulative ulcer length scores were statistically treated on square root transformed data due to heterogeneity of variance. In the ICV treated groups, a significant effect of CRF was shown ($F(1,25)=12.9$, $p<0.002$) and of DSP-4 ($F(1.25)=6.9$, $p<0.02$) (Two-way ANOVA). Subsequent Newman-Keuls tests revealed a significant decrease in water restraint-induced gastric erosions after CRF administration ($p<0.05$) in Control animals, while not in DSP-4 treated animals. In fact, the DSP-4 treated rats exhibited a significantly higher ulcer score after ICV injection of CRF than Control animals (Newman-Keuls test, $p<0.05$) (Fig. 1)

Intraperitoneal administration of CRF to DSP-4 animals, however, did cause a significant decrease in ulcer score (Newman-Keuls test, $p<0.05$) (Fig. 1).

DISCUSSION

The gastric ulcer scores from the present experiment give support to those previous reports that have shown a protective effect of centrally administered CRF under restraint[10,11,24]. This protective effect of ICV administered CRF was not evident in DSP-4 treated rats. Although not completely abolished after DSP-4 treatment, the ulceroprotective effect of ICV CRF administration seems dependent on an intact LC noradrenergic system. The still-present reduction of gastric pathology after peripheral administration of CRF to DSP-4 animals indicates that this interaction occurs centrally.

Our data support the proposal that CRF may have neurotransmitter/ neuromodulator functions in the central nervous system [16,20]. Furthermore, the functional relationship between CRF and the LC noradrenergic system during acute and chronic stress as proposed by Chappell *et al.*[17] seems to be of major importance regarding the effect of CRF on stress-induced gastric pathology.

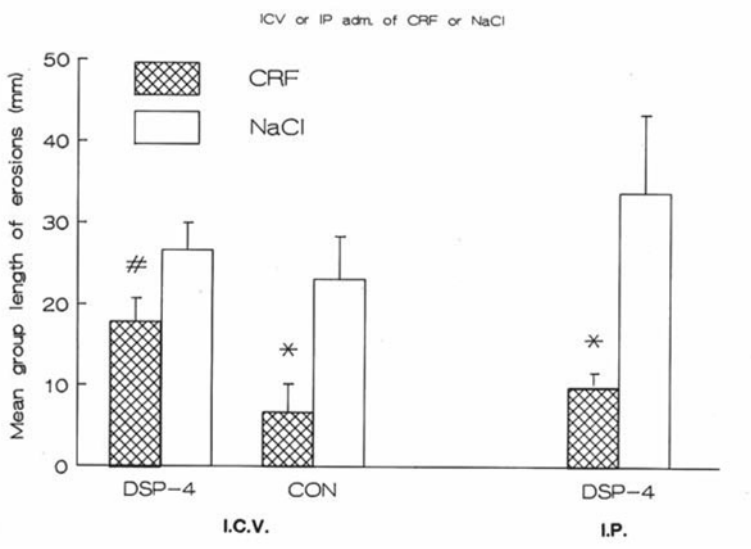

Figure 1. Mean group post restraint cumulative length of gastric erosions (mm ± SEM) after ICV or IP administration of CRF or NaCl to DSP-4 treated (DSP-4) and Control (CON) rats. *, $p<0.05$ compared to Saline group. #, $p<0.05$ compared to DSP-4 group. Reprinted with permission from Physiology and Behavior, H.K. Bakke, A. Bogsnes and R. Murison, Studies on the interaction between ICV effects of CRF and CNS noradrenaline depletion, Vol. 47, No 6 1990, Pergamon Press PLC.

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