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A Comparison of the Effects of Corticotropin Releasing Factor and Sauvagine on Food Intake

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GOSNELL, B. A., J. E. MORLEY AND A. S. LEVINE. *A comparison of the effects of corticotropin releasing factor and sauvagine on food intake.* PHARMACOL BIOCHEM BEHAV 19(5) 771–775, 1983.—Corticotropin releasing factor (CRF) and sauvagine (SVG) when administered ICV both reduced spontaneous feeding as well as feeding induced by deprivation or the administration of ethylketocyclazocine (EKC). For spontaneous- and EKC-induced feeding, SVG produced a larger and longer-lasting suppressive effect than did CRF. Both peptides produced a conditioned taste aversion when paired with a novel saccharin taste. As with the feeding effects, SVG produced a stronger aversion than CRF. These studies further establish the similarity between CRF and SVG and suggest that they may have a disruptive effect on feeding.

Corticotropin releasing factor Sauvagine Food intake

A NUMBER of neuropeptides have been shown to affect ingestive behaviors leading to speculation that some of them play a role in the normal regulation of food intake [21, 24, 30]. One of these, corticotropin releasing factor (CRF), is a 41-residue peptide which was isolated from the ovine hypothalamus [33,34]. This peptide stimulates the release of ACTH and β-endorphin and elevates plasma levels of epinephrine, norepinephrine, and glucose [3,34]. Additionally, CRF reduces nocturnal and starvation induced feeding, while increasing grooming [1,24]. The feeding and grooming effects also occur in hypophysectomized rats, which suggests that they are not secondary to the action of CRF on the pituitary [24]. More recently, CRF has been shown to reduce feeding induced by muscimol, norepinephrine, dynorphin and insulin [18].

Sauvagine (SVG) is a peptide recently isolated from the skin of the frog *Phyllomedusa sauvagei* and characterized as a 40-residue polypeptide [6, 7, 20]. In addition to its structural similarities to CRF, sauvagine also stimulates the release of ACTH and β-endorphin [4,34]. In contrast to the elevated arterial blood pressure following CRF given centrally [8], both SVG and CRF decrease mean arterial blood pressure when given intravenously [4, 6, 16, 19].

SVG has been shown to be more potent than CRF in increasing plasma catecholamine and glucose levels when given centrally [4]. In the experiments reported here, we compare the pharmacological effects of CRF and SVG on nocturnal and deprivation-induced feeding. As we have previously shown that the kappa opiate receptor agonist ethylketocyclazocine (EKC) is a potent stimulator of food intake [27], we also tested the effects of CRF and SVG on EKC-induced feeding. Additionally, the possible aversive consequences after central injections of the two peptides were tested with a taste aversion paradigm.

METHOD

Male Sprague-Dawley rats (145–355 g) were housed singly and maintained on a 12:12 light/dark cycle. Water was ad lib (except in experiment 4) and access to food is described below. Under Nembutal anesthesia stainless steel guide tubes were stereotactically implanted into the right lateral ventricles, a procedure described elsewhere [22] and used in several previous reports [18, 23, 24, 26]. The rats were allowed at least five days post-operative recovery. Peptides were dissolved in saline (pH 2.3 or 5.5) and injected (ICV) rapidly in 5 μl volumes. CRF was purchased from Bachem Corporation (Torrance, CA) and SVG was purchased both from Bachem Corporation and Peninsula Laboratories (San Carlos, CA).

Experiment 1: Deprivation Induced Feeding

In the mid-portion of the light cycle, food deprived rats (24–25 hr) were injected ICV with 0.1, 1, 5 or 10 μg of SVG, 0.1, 1, 5 or 10 μg of CRF or saline. They were immediately given pre-weighed food pellets in their home cages, and intake was measured at 0.5, 1 and 2 hours after injection. Food weights were corrected for spillage at each measurement. Rats were tested at several doses, with at least two days between doses. If a rat was given the same dose more than once, all trials at that dose were averaged and used as a single trial. A total of 56 rats were used in this experiment.

Experiment 2: Spontaneous Feeding

Rats were injected ICV with either SVG (5μg), CRF (5μg) or saline at the beginning of the dark cycle. Three non-injected rats that were tested at the same time were included in the control group. The food intake of these three did not significantly differ from the NaCl-injected rats. Food had been removed two hours prior to injection. Immediately after injection, pre-weighed food pellets were given and food intake was measured after 1, 2, 4 and 12 hours. Food weights were corrected for spillage at each measurement. A total of 32 rats were used in this experiment.

Experiment 3: Ethylketocyclazocine-induced Feeding

Feeding was induced by injecting rats SC with ethylketocyclazocine (EKC, 10 mg/kg) within two hours after the onset of the light cycle. This dose of EKC has previously been shown to be a potent stimulator of feeding [27]. One hour after EKC injections, rats were injected ICV with 5 μg of SVG, CRF or saline. The peptides were injected one hour after the EKC because the sedative effects of EKC initially prevail over the feeding effects. Food intake was measured at 1, 2, 3, 4 and 6 hours after the ICV injections. Food weights were corrected for spillage at each measurement. A total of 33 rats were used in this experiment.

Experiment 4: Taste Aversion

Rats inexperienced with the taste of saccharin were put on a schedule in which water was available for only one hour each day. Food was ad lib throughout the experiment. After several days on this schedule, most rats immediately began drinking at the beginning of the one-hour water session. On the conditioning day, the rats were given a thirty minute access to 0.15% sodium saccharin solution, after which they were immediately injected ICV with CRF (5 μg), SVG (5 μg) or NaCl in a 5 μl volume. A fourth group received an IP injection of lithium chloride (0.36% body weight of 0.65 M LiCl). Two additional groups received an IP injection of either NaCl (0.22% body weight of 0.9% NaCl) or LiCl (0.22% body weight of 0.65 M LiCl) immediately after the saccharin exposure. The rats were not allowed to consume any other liquid on the conditioning day. On the following day, they were again given a one hour water session. The next day (Extinction day), the rats were given 30 minutes access to 0.15% sodium saccharin. Saccharin intakes on the conditioning and extinction days were measured (to the nearest ml) by weighing before and after placement on the cages.

For all experiments, intake amounts at any given time period were tested with a one-way analysis of variance. When the overall F value was significant, the least significant difference procedure (LSD, two-tailed) was used to compare specific group means.

RESULTS

Experiment 1: Deprivation-Induced Feeding

Figure 1 shows the amounts consumed at the various doses of CRF and SVG. At 0.5, 1 and 2 hours, CRF significantly reduced food intake at the 5 and 10 μg doses ($p<0.05$). Two-hour intake was significantly increased by the 0.1 μg dose of CRF. The two highest doses of SVG (5 and 10 μg) also reduced intake accumulated over 1 and 2 hours. At the 5 and 10 μg doses, there were no significant differences between the intakes of the CRF and the SVG injected rats.

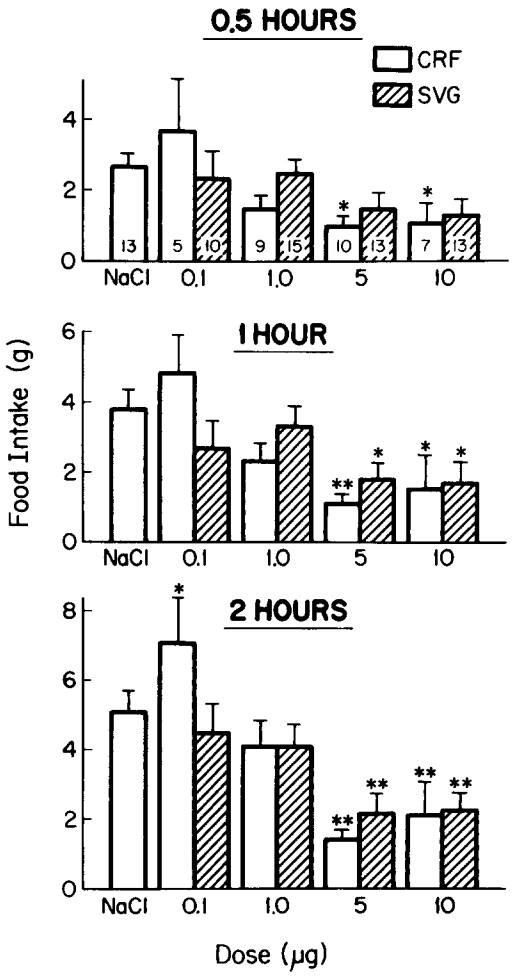

FIG. 1. Effects of several doses of CRF amd SVG on deprivation-induced feeding. An analysis of variance was performed on the cumulative intake at each time point and yielded F-values, F(8,86), of 2.48, 3.52 and 5.89 for the 0.5, 1 and 2 hour measures, respectively (all p's<0.05). Conditions marked with asterisks are significantly different from control (*$p<0.05$; **$p<0.01$).

Experiment 2: Spontaneous Feeding

Figure 2 illustrates the cumulative nocturnal food intake of SVG-, CRF- and saline-treated animals. Both peptides significantly reduced 1, 2, 4 and 12 hour cumulative intakes ($p<0.05$). Additionally, the intakes of SVG-treated rats were significantly less than those of CRF-treated rats at the 4 and 12 hour measurements.

Experiment 3: EKC-Induced Feeding

As shown in Fig. 3, CRF significantly reduced 2 and 3 hour intake, but 4 and 6 hour intakes were not significantly different from control. SVG reduced 2, 3, 4 and 6 hour intake, and its effect was also significantly greater than that seen in the CRF-treated rats at 3, 4 and 6 hours. Intake in the

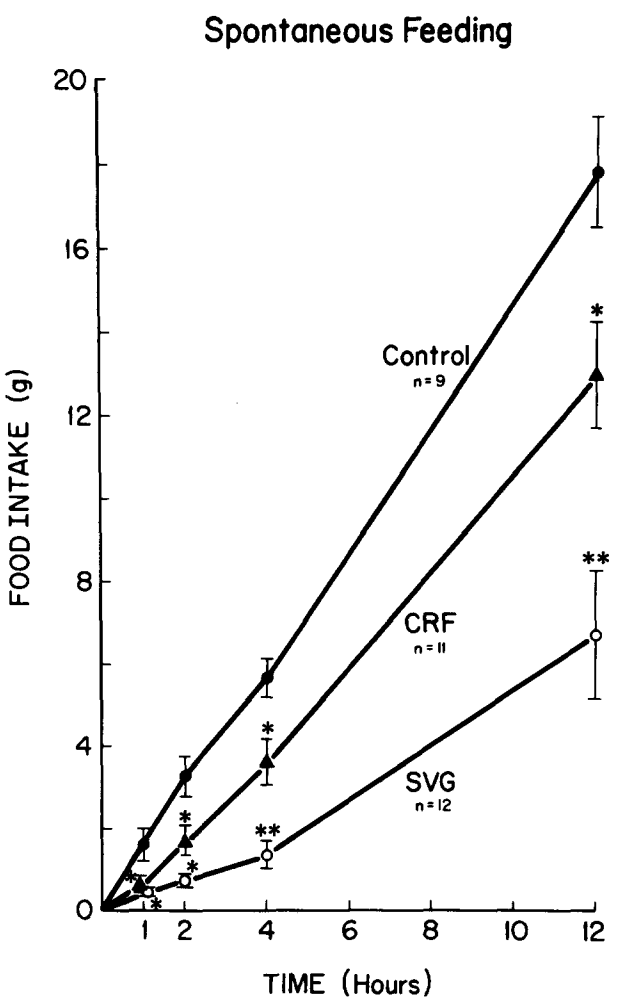

FIG. 2. Effects of CRF (5μg) and SVG (5μg) on spontaneous nocturnal feeding. An analysis of variance on the cumulative intake at each time point yielded F-values, F(2,29) of 5.93, 13.36, 20.21 and 15.34 for the 1, 2, 4, and 12 hour measures, respectively (all p's<0.05). (*) indicates a significant difference from control ($p<0.05$); (**) indicates a significant difference from the control and CRF conditions ($p<0.05$).

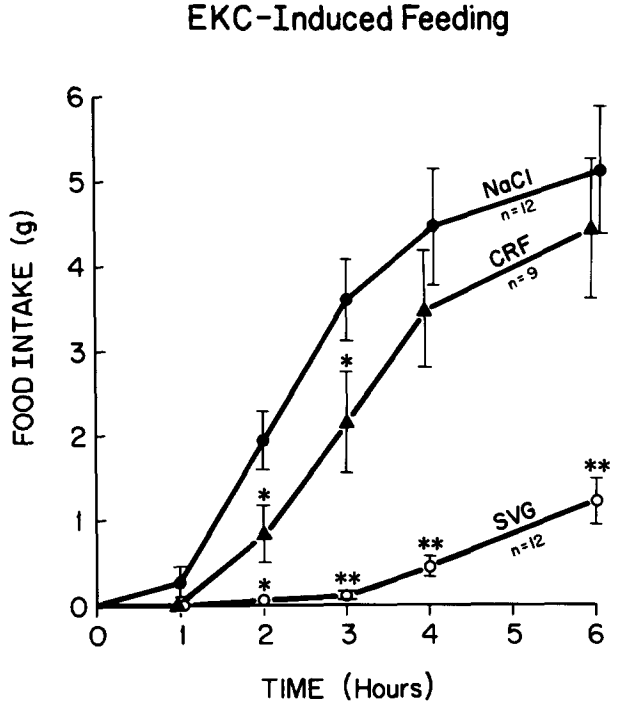

FIG. 3. Effects of CRF (5μg) and SVG (5μg) on feeding induced by ethylketocyclazocine (10 mg/kg). An analysis of variance at each time point yielded F-values, F(2,30), of 12.79, 19.71, 16.20 and 11.23 for the 2, 3, 4 and 6 hour measures, respectively (all p's<0.05). (*) indicates a significant difference from the NaCl group ($p<0.05$); (**) indicates a significant difference from the NaCl and the CRF groups ($p<0.05$).

first hour after the peptide injections was near zero in all these groups due to the continuing sedative effects of EKC; consequently, no statistical analysis was performed on the data.

Experiment 4: Taste Aversion

Figure 4 illustrates the saccharin intake of the six groups on the conditioning day (immediately prior to injections) and 2 days later during a 30 minute re-exposure (extinction). The analysis of variance indicated no significant differences in mean intakes on the conditioning day, $F(5,38)=1.65$, $p>0.05$. On the extinction day, the ANOVA indicated significant differences among the intakes of the groups, $F(5,38)=14.49$, $p<0.01$. Ths LSD procedure indicated that the SVG, CRF, and LiCl treated rats consumed significantly less saccharin than the NaCl groups ($p<0.05$). Additionally, the SVG and LiCl groups consumed less than the CRF group ($p<0.05$).

DISCUSSION

This study confirms that CRF is a potent inhibitor of food intake under a number of conditions and extends these findings to sauvagine, a structurally similar peptide. Both peptides were approximately equipotent in reducing deprivation-induced feeding. As Fig. 1 indicates, the 0.1 μg dose of CRF significantly increased 2-hour cumulative intake. In a previous study, however, this dose caused a slight (but not significant) decrease in food intake [24]. The increased intake reported here, therefore, may represent a spurious finding. The finding of a slight increase in feeding with low doses of satiety agents is one which we have often observed with a variety of feeding inhibitors (e.g., cholecystokinin and naloxone) (unpublished observations) and as such may be worthy of further investigation. In contrast to deprivation-induced feeding, SVG produced a much larger and longer-lasting suppressive effect than CRF on nocturnal feeding. That no difference in potency was found with the deprivation-induced feeding paradigm may be due to measuring intake for only two hours, since no difference in potency was seen after two hours with nocturnal feeding. These results are consistent with those of Brown *et al.* [4] who showed that SVG was more potent than CRF in elevating brain catecholamine and plasma glucose levels.

A number of studies have suggested that endogenous opioid peptides may play an integral role in the regulation of feeding [12, 21, 25, 30]. We have previously shown that

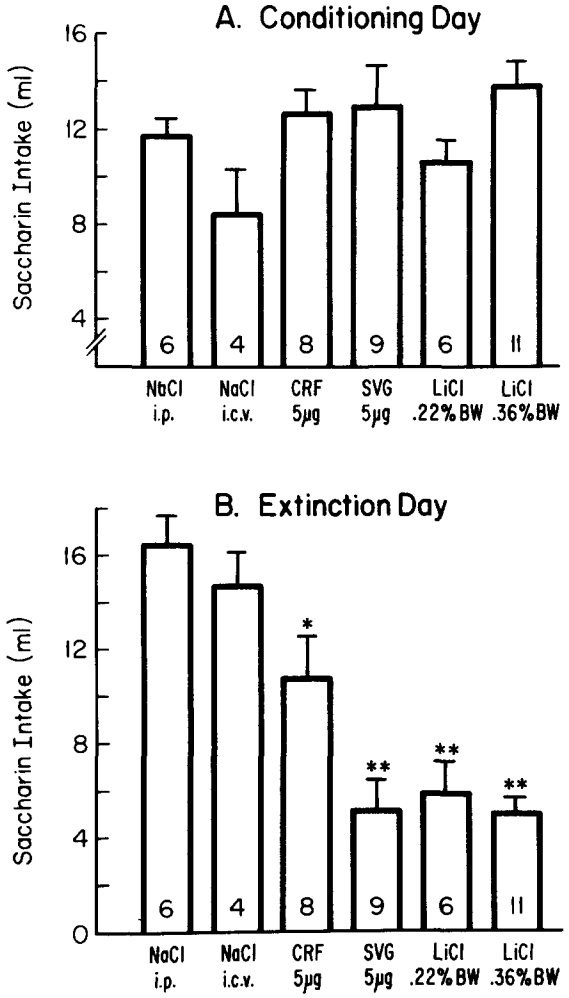

FIG. 4. A. Saccharin intake immediately before injections of NaCl, CRF, SVG or LiCl. The analysis of variance indicated no significant differences among the groups, $F(5,38)=1.65$, N.S. B. Saccharin intake two days after the injections. The analysis of variance indicated significant differences among the means, $F(5,38)=14.49, p<0.01$. (*) indicates a significant difference from the NaCl groups ($p<0.05$); (**) indicates a significant difference from the NaCl and CRF groups ($p<0.05$).

kappa opiate receptor agonists are more potent in inducing feeding than mu receptor agonists [27]. We therefore studied the effects of CRF and SVG on feeding induced by EKC. EKC is a kappa opiate agonist receptor which has been previously shown to stimulate intake [27]. As with nocturnal feeding, SVG produced a larger and longer lasting suppression of feeding than did CRF. The ability of these compounds to decrease feeding following EKC is compatible with our previous observation that CRF decreases feeding induced by centrally administered dynorphin [18], an opioid peptide that appears to be the endogenous ligand for the kappa receptor [5, 15, 35]. Dynorphin has previously been shown to be a potent inducer of feeding [23,26].

We are not aware of any report suggesting that CRF has any affinity for opiate receptors. Therefore, it seems doubtful that the inhibitory effects of CRF and SVG are directly due to opiate receptor blockade. On the other hand, it has recently been shown that CRF immunoreactivity occurs in a subpopulation of dynorphin-(1–8) immunoreactive neurons in the paraventricular hypothalamic nucleus, suggesting some functional relationship between the two peptides [29].

It should be noted that the doses of CRF and SVG that were effective in reducing intake are within the range of doses found to increase mean arterial blood pressure and plasma levels of glucose and catecholamines [3, 4, 8]. It is therefore possible that the reduction of intake could be secondary to one of these effects. Blood glucose levels, for example, have long been considered important in the regulation of feeding. It is not known, however, whether blood pressure plays any direct role in modulating intake.

When a novel saccharin taste was paired with injections of CRF and SVG, the intake of saccharin solution two days later was decreased compared to saline-injected controls. Consistent with the food intake experiments, SVG had a stronger effect than CRF on subsequent saccharin intake. It is well known that rats avoid a flavor that has been temporally paired with sickness-inducing substances [9,28]. The lack of an ability to cause an aversion to a novel taste with which a putative feeding or satiety factor is paired is often taken as supportive evidence that the substance is modulating intake by some means other than causing sickness or malaise in the animal. For example, the peripheral injections of the neuropeptides.cholecystokinin and bombesin do not cause an aversion to a novel taste [11, 13, 17], thus lending support to the suggestions that their inhibition of food intake in physiological in nature rather than a secondary effect of illness [10, 11, 13]. The present report of conditioned taste aversions formed with CRF and SVG, therefore, indicate that there may be some aversive or disruptive consequences to their injections. Additionally, the effects may indicate that the doses used have pharmacological rather than physiological effects on food intake.

An alternate explanation can be advanced for the taste aversions formed CRF and SVG. It has been shown that the strength of a conditioned taste aversion correlates well with plasma corticosterone levels [32]. Hennessy, Smotherman and Levine [14] have suggested that ACTH plays a role in the formation of an association between a taste and illness. Since both CRF and SVG stimulate the release of ACTH, it is possible that rather than causing illness themselves, the peptides enhanced the association between the saccharin taste and the stress involved in the injections. The present results cannot be completely explained by elevated ACTH levels, however, since a greater aversion was formed with SVG than with CRF. Brown *et al.* [4] have shown that CRF is more potent than SVG in releasing pituitary ACTH; one would predict, then, that CRF would cause the greater aversion.

In summary, the experiments reported here indicate that under some conditions, SVG is more potent than CRF in reducing food intake. The demonstration of a taste aversion to saccharin that has been paired with CRF and SVG, along with previous reports indicating that CRF increases grooming [1,24] suggests that these peptides may reduce intake by disrupting feeding. The recent observation that centrally administered SVG glows gastric emptying [2] suggests an alternate mechanism by which some of the effects of these compounds on feeding may be mediated.

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