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Behavioral and Hormonal Effects of Centrally Injected "Anxiogenic" Neuropeptides in Growing Pigs

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PARROTT, R. F., S. V. VELLUCCI AND J. A. GOODE. *Behavioral and hormonal effects of centrally administered "anxiogenic" neuropeptides in growing pigs.* PHARMACOL BIOCHEM BEHAV **65**(1) 123–129, 2000.—Records of behavior (alertness, posture, oro-nasal responses, activity level, and vocalization pattern) were made in prepubertal pigs (n = 6) during a 60-min period following central injections of equimolar (21 nmol) doses of porcine CRH (pCRH), urocortin (UCN), octadecaneuropeptide (ODN), or saline vehicle (SAL). Blood samples were also collected at 15-min intervals before, during, and after the test, and used to determine plasma cortisol, prolactin, and growth hormone concentrations. The pigs became excited and highly active after pCRH, and to a lesser extent following UCN administration, but were subdued when given ODN or SAL. None of the peptides significantly affected prolactin or growth hormone release, but both UCN, and especially pCRH, increased cortisol concentrations. The emotional responses induced by pCRH and UCN are consistent with observations in rodents, which indicate that centrally administered CRH-like peptides have anxiogenic effects. In contrast, ODN, which inhibits benzodiazepine binding at the $GABA_A$ receptor and is anxiogenic in rodents, lowered plasma cortisol and had no overt behavioral effects. Hence, at the dose administered, there was no evidence to indicate that ODN acted as an anxiogen in this species. © 1999 Elsevier Science Inc.

Pigs CRH Urocortin Anxiety Peptide Central Injection Behavior Cortisol

CORTICOTROPHIN-releasing hormone (CRH) acts within the brain to coordinate the response to stress (12), and may mediate certain psychopathological states (1). Experimentally, central administration of CRH induces physiological and neuroendocrine responses, increased arousal, and behavior consistent with its putative role as an anxiogen (12). However, although extensively studied in rodents, less is known about the actions of this neuropeptide in other species. Nevertheless, understanding of neural mechanisms involved in stress and anxiety is important in farmed species where the constraints imposed by current husbandry practice can compromise welfare. In this context, the domestic pig, which is usually intensively reared, represents a species for which more basic knowledge is needed.

Porcine CRH (pCRH) was isolated in 1985 (35), and subsequently shown to exist in two forms differing by a single amino acid [isoleucine or asparagine at position 40; (21)]. However, the first studies with pigs, carried out in this laboratory before pCRH was available, used the ovine (oCRH) and rat/human (rCRH) peptides; intracerebroventricular (ICV) injection of oCRH-inhibited operant feeding (30) and oCRH was more effective than rCRH in activating the hypothalamo–pituitary–adrenocortical (HPA) axis (31). Other workers subsequently reported marked effects of centrally administered pCRH on behavior, the HPA axis, and immune function in swine (23,37); these studies used the most abundant form of the peptide [Asn^{40}; (21)]. Recent work in this laboratory has included the sequencing of the pCRH gene (26) and quantification of its expression in the hypothalamus (44).

The CRH receptor exists as two subtypes (R1 and R2), the second of which occurs as α, β, and γ splice variants (3,40). Subtype R1 is present mainly in the brain and pituitary, R2α occurs primarily in CNS neurons, R2β is more prevalent in the periphery, and R2γ only found in the human brain (3,40).

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Specific ligands for these subtypes, therefore, may provide new information on the actions of CRH in different brain regions. A new development in this field was the identification of urocortin (UCN), a CRH-like peptide, in rat (43) and human brain (11). Binding studies indicate that UCN seems to have a higher affinity for CRH receptors than related peptides (rCRH, oCRH, urotensin, sauvagine), although the subtype involved differs between species [human, R1; rat, R2α; mouse, R2β: (40)].

Central injections of UCN (0.01–10 μg; 0.002–2 nmol) produced dose-related decreases in feeding in rats (39,41) and the highest dose also reduced water intake (41). However, compared to rCRH, UCN had very little anxiogenic activity in the elevated plus-maze (41). Nevertheless, mice given ICV injections of UCN (0.3–0.9 μg; 0.06–0.2 nmol) displayed increased anxiety when tested in the light/dark box and open field (28). The latter effect was reversed by diazepam, and these workers also found UCN to have similar effects to rCRH in rats tested in the elevated plus-maze (28). With regard to the effects of UCN on the HPA axis, studies in rodents have reported significant stimulatory effects using in vitro systems and following intravenous (IV) administration (2,29,42). However, although ICV injections have been found to reduce plasma vasopressin concentrations (24), effects on the release of stress-responsive hormones have not been described.

Benzodiazepines (BZs) normally reduce the anxiogenic effects of CRH (12). The fact that BZs potentiate GABA-dependent inhibition points to an interaction between CRH- and GABA-mediated neuronal systems which, conceivably, may involve an endogenous inhibitory ligand for the BZ/$GABA_A$ receptor (10). Because BZ inverse agonists act in this way (25), it has been postulated that endogenous CRH might release a similar type of neuromodulator (10). Moreover, such a compound has already been described: this is the octadecaneuropeptide fragment (ODN) of a large naturally occurring peptide, diazepam binding inhibitor. The latter, DBI, was originally isolated from the rat brain (20) and has since been studied in great detail (5,15). It is present in various brain regions and shows considerable homology between species, including the pig (5).

In rats given ICV injections of DBI, the anticonflict action of diazepam was reduced and a proconflict effect similar to that of a benzodiazepine inverse agonist was demonstrated (20). Subsequently, it was found that ODN retained this proconflict effect of DBI at ICV doses between 3 and 10 μg ([1.5–5 nmol; (16,17)], and that its anxiogenic action could be prevented by the BZ antagonist flumazenil (38). Moreover, recently, others have investigated the effects of centrally administered ODN on CNS gene expression in rats: there was a reduction in CRH mRNA in the hypothalamus (18,19) that could be reversed by flumazenil (18), and an increase in prolactin message in the pituitary (45).

From the above, it is apparent that central administration of UCN and ODN may provide new insights into neural mechanisms of stress and anxiety. Because neither peptide has been studied in this way in an ungulate species, the aim of the present experiment was to compare the effects of equimolar ICV doses of pCRH, UCN, and ODN on behavior and cortisol, prolactin, and growth hormone release in growing pigs.

METHOD

Six Large White breed prepubertal boars were supplied at an initial weight of approximately 25 kg. They were housed in individual metabolism cages where they learned to make fixed-ratio (FR) operant responses for food (FR.5) and water (FR.2) by pressing switch panels with their snouts. Food could be obtained during a 2-h period (0900–1100 h) each day, the start of which was signalled by a buzzer, whereas water was continuously available. The animals were regularly handled and soon settled down to a pattern of activity in the morning and rest during the afternoon.

Each pig was prepared under closed-circuit halothane anesthesia, using sterile precautions, with a catheter in the jugular vein and an ICV (lateral ventricle) cannula (200-μl dead space), both of which were protected by an elasticated bandage wound around the neck. After recovery from surgery, and following standard practice in the laboratory (32), cannula placements were verified by confirming the ability of an ICV injection of angiotensin II (420 μl g in 200 μl saline vehicle, SAL, followed by 300 μl SAL) to induce operant drinking. All of the pigs responded after a short delay; the number of reinforcements (mean ± SEM) was 31.2 ± 5.6. Venous catheters were flushed daily with sterile heparinized SAL.

The treatments were ICV injections of SAL, pCRH, ODN (anxiety peptide), and UCN given in a 400-μl volume and followed by 300 μl SAL. The peptides were obtained from different sources (pCRH, American Peptide Co., Sunnyvale, CA; ODN and human UCN, Bachem UK Ltd., Saffron Walden, Essex), made up in sterile SAL and stored as frozen aliquots until required. Equimolar doses (21 nmol) were administered but, because of differing molecular weights, the amounts varied (pCRH/UCN, 100 μg; ODN, 40 μg); these doses were scaled up from a preliminary study that used smaller quantities of UCN and ODN. All experimental procedures were carried out in accordance with the UK Animals Scientific Procedures Act 1986 (Project Licence No 80/1269).

Each pig was tested four times (i.e., once per treatment), but individual animals received the treatments in a different sequential order. Two pigs given different treatments were tested each day, and 2 nontreatment days separated consecutive tests for individual animals. Because the pigs were very well adapted to human contact, it was possible to collect blood samples for hormone analysis with minimum disturbance during each test. Behavior was observed for 60 min (1430 to 1530 h), starting immediately after the ICV injection. However, blood samples were collected before (−15.0 min) the injection and both during (15,30,45, and 60 min) and after (75 min) the recording period. Blood tubes were stored on ice and subsequently centrifuged. The resultant plasma was divided into aliquots and stored at −30°C pending radioimmunoassay for cortisol, prolactin, and growth hormone, as previously described (9,33).

The 60-min test period was divided into 5-min time bins. In each of these, an assessment was made of the animal's arousal state (drowsy, alert, or agitated), posture (standing, sitting, or lying), oro-nasal behavior (drinking, chewing, nose rubbing), behavioral intensity (high or low activity), and pattern of vocalization (infrequent or regular grunting). These behavioral categories were not mutually exclusive, i.e., it was possible for different activities (e.g., standing and lying) to be recorded in the same time bin. However, no attempt was made to count the number of events/bin or to measure event durations. The behavioral record, therefore, provided a simple measure of the occurrence, or not, of a particular behavior or state in each 5-min bin, giving a maximum possible score of 12 over the 60-min period. A record was also made in each test of other behaviors (see the Results Section) which occurred, often at low frequencies.

Behavioral scores for all six pigs under the same treatment

condition were used to derive medians and interquartile ranges. A nonparametric analysis of variance for related samples (Friedman test) was carried out to examine whether there was an overall difference between the treatments. If this was the case, separate control vs. treatment comparisons were made using a nonparametric test for paired samples (Sign test, two tailed). The effects of the treatments on cortisol, prolactin, and growth hormone concentrations were examined by comparing averaged values before (−15, 0 min) and (after 30–75 min) the ICV injection, using the paired *t*-test (two tailed).

RESULTS

Behavioral scores (medians and interquartile ranges) obtained under the four treatment conditions are indicated in Fig. 1 (A–D). Drowsiness and agitation, indices relating to state of arousal, are presented in Fig. 1A. There were significant ($p < 0.01$) differences between treatments for both behavioural states and, not surprisingly, the scores were inversely related. Drowsiness (eyes closed) was apparent on occasions in all animals when treated with SAL or ODN, but was never observed after they received pCRH or UCN. Compared to the SAL condition, pigs given pCRH or UCN were significantly ($p < 0.03$) less drowsy. Conversely, only one animal (the same pig) displayed agitation following SAL or ODN administration; in both cases this occurred in the fourth 5-min period. All pigs displayed agitation after pCRH and four of six did so following UCN injection. The median latency for this response was approximately 13 min for pCRH and 18 min for UCN (NS). Compared to the SAL condition, animals given pCRH or UCN were significantly ($p < 0.03$) more agitated.

The results illustrated in Fig. 1B are scores relating to changes in posture. There were no significant differences between treatments with respect to sitting but there were for standing ($p < 0.01$) and lying ($p < 0.02$). All pigs stood on occasions in each treatment condition but, compared to SAL, the incidence of standing was higher ($p < 0.03$) after pCRH or

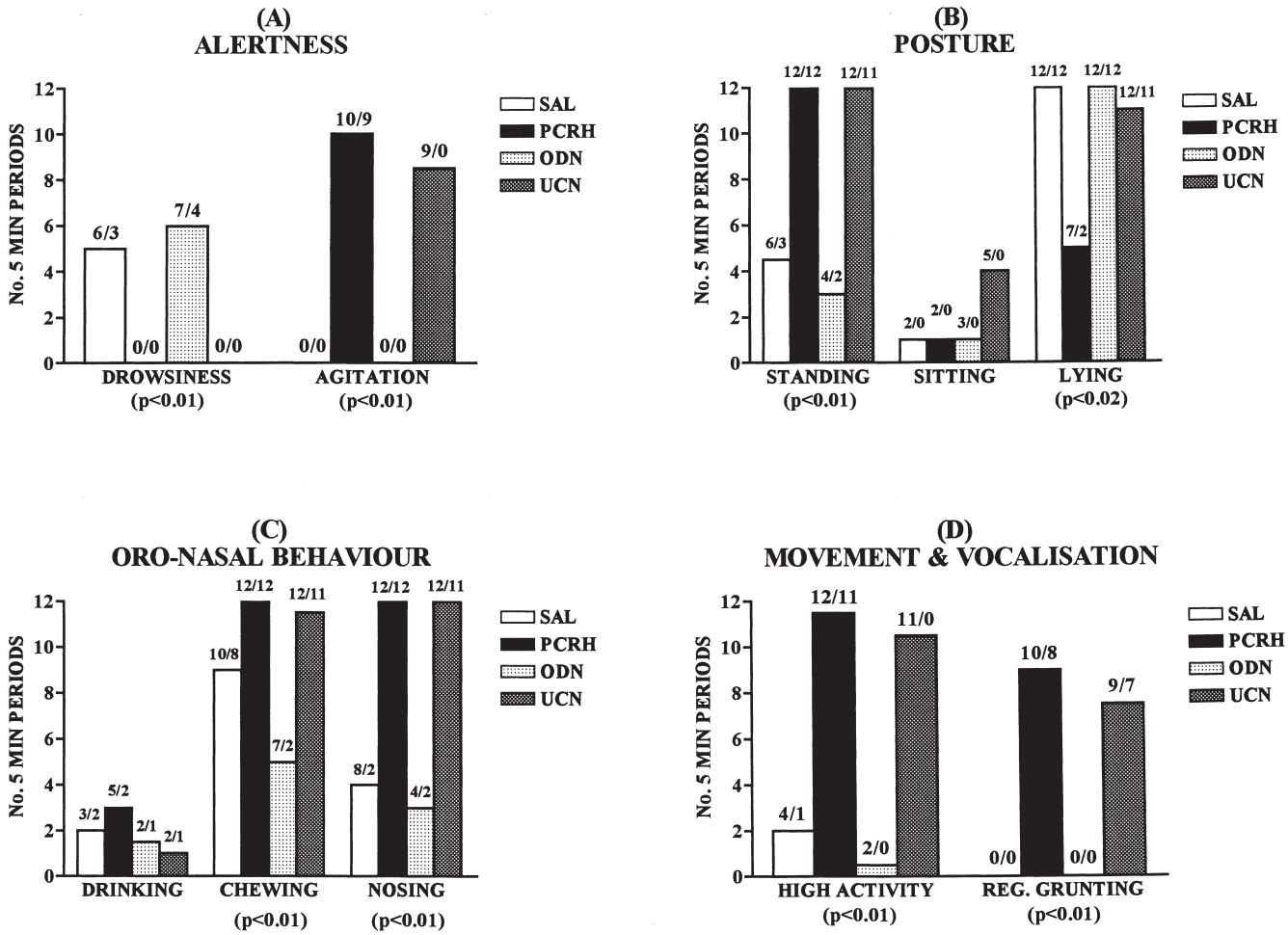

FIG. 1. Behavioral responses (alertness, posture, oro-nasal behavior, movement, and vocalization) of pigs ($n = 6$) to ICV injections of SAL, pCRH, ODN, and UCN in 60-min tests. A behavioral score, i.e., the number of 5-min periods (max = 12) in which a particular activity or state was recorded, was derived for each animal under the four treatment conditions. These scores were then used to obtain the median number of responses per treatment, shown as histograms, and their respective upper and lower interquartile ranges, given above each column. Between-treatment comparisons for a particular behavioral index were made using the Friedman test, and significant differences are indicated in parentheses. Details of further statistical analysis are given in the text.

UCN. Lying was observed in all animals and treatment conditions but was less frequent ($p < 0.03$) after pCRH than SAL administration.

Eating was not recorded in this experiment as no food was available during the tests; however, oral and nasal activities were scored (Fig. 1C). These included, drinking, chewing, either in vacuum or of the cage bars or bowls, and nosing of the cage floor, sides, and bowls. Although nearly all the animals drank on occasions, no treatment differences were found. However, the incidence of chewing differed ($p < 0.01$) between treatments. Chewing occurred in the majority of tests, but appeared to be less frequent after SAL or ODN administration; treatment with pCRH resulted in more chewing activity ($p < 0.03$) than SAL. Nosing was exhibited by the animals on every test occasion, but the amount of activity differed ($p < 0.01$) between treatments. More nosing occurred ($p < 0.03$) following pCRH or UCN administration than after SAL.

An overall assessment of the level of activity and the pattern of vocalisation was made at the end of each 5-min period; the scores derived from these data are shown in Fig. 1D. Two treatments, pCRH and UCN, produced a consistently high level of activity characterized by repeated changes in position and posture, together with frequent oral behavior that persisted throughout most of the 5-min period. All the pigs showed high activity on some occasions after SAL administration by only three of six did so following ODN. Activity levels differed between treatments ($p < 0.01$) and, compared to SAL, pCRH, and UCN resulted in higher ($p < 0.03$) behavioral scores. Vocalization often occurred during the tests but the pattern differed between treatments ($p < 0.01$). In particular, grunting induced by pCRH and UCN tended to increase in volume with time. All animals receiving these treatments exhibited this response and, in consequence, the frequency of regular grunting was higher ($p < 0.03$) after pCRH and UCN injection than following SAL.

In addition to the above, the number of animals in each treatment group showing eliminative, oral/olfactory/vocal, or aspects of motor behavior was quantified (Table 1). Because these are only nominal data, no statistical analysis was carried out; however, some trends are apparent. In the case of eliminative activity, urination did not distinguish between treatments, whereas defecation was noted in all animals treated with pCRH. The oral/olfactory/vocal category includes behaviors that may represent different behavioral states. Thus, yawning, which relates to drowsiness, was seen in three of six pigs treated with SAL but only in one of six after pCRH. Sneezing was recorded in only a few animals in each treatment condition, whereas only pCRH and UCN induced sniffing. Similarly, gagging, often seen after chewing, and playing with bowls or water was a characteristic of pigs treated with pCRH or UCN. Barking, which was a development of regular grunting, occurred especially when the animal was approached, for example, for blood sampling. This behavior was induced in all pigs by pCRH, and in five of six by UCN, and often continued for some time after the 60-min test period. With regard to motor activity, rubbing and shaking occurred under most treatment conditions. However, turning around in the cage seemed to be more frequent in animals treated with pCRH and UCN. Also, scampering, i.e., excited jumping from one position to another tended to occur most often in pigs given pCRH.

TABLE 1

TOTAL NUMBER OF PIGS ($n = 6$) SHOWING ELIMINATIVE AND ASPECTS OF ORAL, OLFACTORY, VOCAL, AND MOTOR BEHAVIOR IN 60-MN TESTS FOLLOWING ICV INJECTION OF SAL, pCRH, ODN, OR UCN

	SAL	pCRH	ODN	UCN
Eliminative				
Urination	3	2	3	1
Defecation	2	6	4	3
Oral/olfactory/vocal				
Yawning	3	1	2	2
Sneezing	2	1	1	1
Sniffing	0	3	0	4
Gagging	0	3	0	4
Bowl play	0	5	0	6
Barking	1	6	0	5
Motor				
Rubbing	0	2	1	3
Shaking	1	2	2	2
Turning	2	4	1	5
Scampering	2	4	1	2

The effects of the various treatments on plasma cortisol concentrations are illustrated in Fig. 2. These data additionally include a sample taken 15 mm before the ICV injection and another taken 15 min after the end of the observation period. These results show that hormone concentrations were similar before the ICV injection and different afterwards. The paired *t*-test (two-tailed) was used to compare plasma cortisol before (−15, 0 min) and after (15–75 min) the various treatments. Concentrations (nmol/1; mean ± SEM) did not differ in the SAL condition (before 21.48 ± 9.95; after, 21.99 ± 5.37), whereas ODN produced a small decrease (before, 29.81 ± 4.37; after, 21.84 ± 5.41; $p < 0.05$). By contrast, pCRH injection markedly increased hormone concentrations (before, 28.66 ± 6.33; after, 116.81 ± 11.08; $p < 0.001$). A similar, but smaller, change was produced by UCN treatment (before, 26.99 ± 10.76; after, 79.36 ± 16.64; $p < 0.01$) and comparison between the two treatments indicated that the stimulatory action of pCRH was greater ($p < 0.01$) than that of UCN.

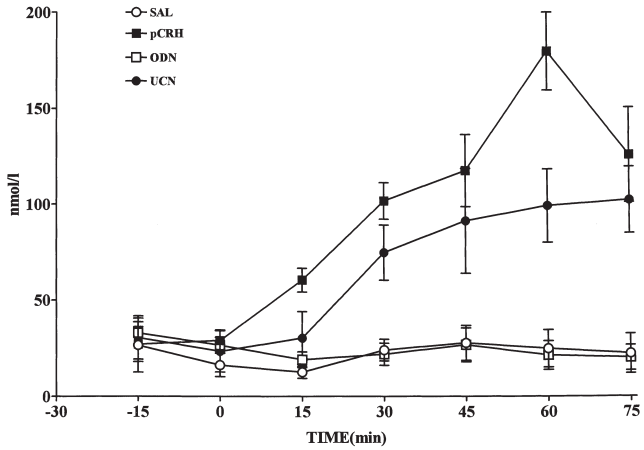

FIG. 2. Plasma cortisol concentrations (nmol/l; mean ± SEM) in blood samples obtained from pigs under the experimental conditions described for Fig. 1. The ICV injections were given at time zero. Within-treatment comparisons (−15 and 0 min preinjection versus 15–75 min, postinjection) indicated significant increases in response to pCRH ($p < 0.001$) and UCN ($p < 0.01$), a significant decrease after ODN injection ($p < 0.05$), but no change following SAL administration.

Changes in plasma prolactin are illustrated in Fig. 3. Comparing the data in the same way as for cortisol, it was found that plasma prolactin (pmol/l; mean ± SEM) increased slightly following SAL injection (before, 46.28 ± 7.04; after, 56.76 ± 11.00; $p < 0.05$) but no changes were detected after pCRH (before, 44.00 ± 8.80; after 43.68 ± 10.12) or UCN (before, 54.12 ± 16.72; after, 56.33 ± 14.52). There appeared to be a small increase in prolactin following ODN administration (before, 43.12 ± 14.08; after, 62.92 ± 17.16), but, because only three of six animals showed a rise in hormone concentration, this change was not statistically significant.

Effects of the treatments on growth hormone release are indicated in Fig. 4. In the SAL condition, hormone concentrations were rather high before the injection and significantly ($p < 0.05$) lower afterwards (before, 157.5 ± 59.85; after, 18.45 ± 13.5); a similar trend was apparent for ODN (before, 78.30 ± 56.65; after, 27.45 ± 21.6). By contrast, both pCRH (before, 46.8 ± 19.8; after, 125.55 ± 58.95) and UCN (before, 10.8 ± 9.0; after, 55.35 ± 30.15) tended to produce a change in the opposite direction.

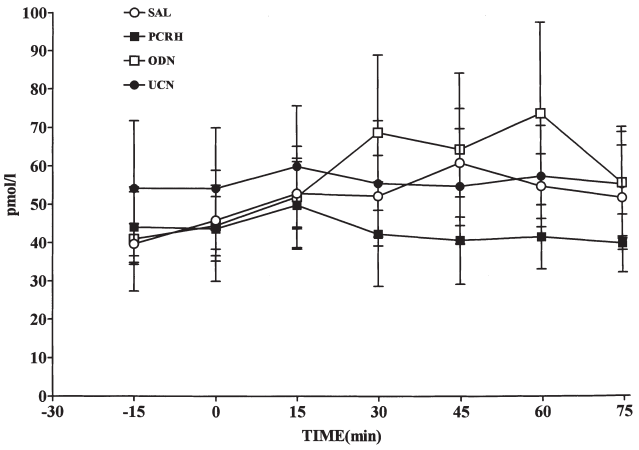

FIG. 3. Plasma prolactin concentrations (pmol/l; mean ± SEM) in blood samples obtained from pigs under the experimental conditions described for Fig. 1. Within-treatment comparisons (−15 and 0 min preinjection vs. 15–75 min postinjection) indicated a significant increase after SAL ($p < 0.05$), but no change in response to the other treatments.

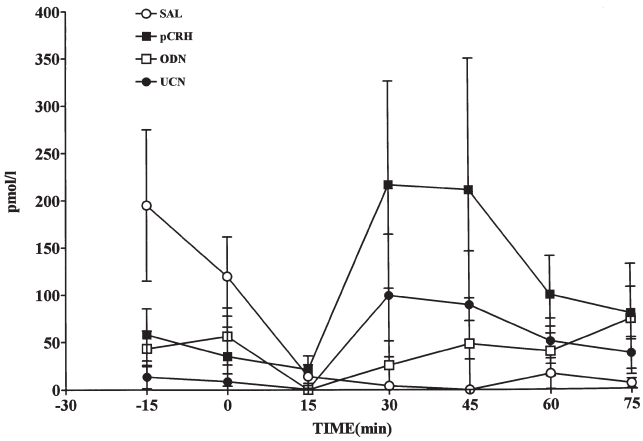

FIG. 4. Plasma growth hormone concentrations (pmol/l; mean ± SEM) in blood samples from pigs under the experimental conditions described for Fig. 1. Within-treatment comparisons (−15 and 0 min preinjection vs. 15–75 min postinjection) indicated a significant decrease after SAL ($p < 0.05$), but no change in response to the other treatments.

DISCUSSION

Central administration of pCRH or UCN induced cortisol release and produced marked behavioral activation. The animals became aroused and excited: they made frequent changes in posture and orientation, engaged in vigorous oronasal activity, and were highly vocal. Hence, the emotional state induced by these neuropeptides has much in common with anxiety (12). However, as this is normally assessed in animals using defined experimental paradigms, it may be inappropriate to use the term "anxiety" to describe the responses recorded in the present study. It also has to be borne in mind that because an observer was present for each test, animal/human interactions may have affected the behavioral scores. There were, however, no aversive effects due to the ICV injection procedure, as indicated by the unchanging plasma cortisol concentrations after SAL administration.

Previous reports have shown that ICV injection of pCRH in pigs increases plasma ACTH and motor activity [15–50 μg; 3–32 nmol; (23)] and stimulates cortisol release while inducing hyperactivity and vocalization [50 μg; 10 nmol; (37)]. The present results are, therefore, entirely consistent with these findings. In this context, however, it is of note that oCRH produces identical responses in pigs at lower ICV doses [20 μg; 4 nmol; (31)]. This may be related to the fact that the ED_{50} for in vitro ACTH release using the most abundant form of pCRH (Asn^{40}) is 8.7 ng/ml, whereas values for oCRH and the IIe^{40} variant of pCRH are 3.5 and 3.2, respectively (21). Thus, it is possible that the IIe^{40} form, which is not commercial available, may have greater behavioral activity.

In the rat CNS, the CRH receptor subtypes R1 and R2 have different distributions (3) and UCN has high affinity for the R2 receptor (43). This peptide also occurs in human brain (11), but has not yet been described in the porcine CNS. The problem with the interpretation of the action of UCN is that CRH receptor subtypes for which it has the greatest affinity vary between species (40). Moreover, the R1 receptor is relatively unselective, being capable of binding several CRH-like peptides (40). With regard to the anxiogenic effects of UCN in rats, one study (41) found UCN to be less effective than rCRH, while another (28) reported a similar potency. Moreover, antisense data suggest that these actions are mediated by the R1 receptor (22). In the present experiment, ICV injections of UCN produced the same type of behavior as pCRH but at a slightly lower intensity. In particular, the incidence of lying differed between the two treatments: compared to SAL, this was significantly reduced by pCRH but not UCN.

The ability of centrally administered UCN to activate the HPA axis, albeit less effectively than pCRH, was demonstrated for the first time in this study. Previous experiments in rats showed that peripherally administered UCN was an effective stimulus to ACTH release. This response involves the R1 receptor (2) but because UCN antiserum does not alter the increase in ACTH induced by adrenalectomy (29) or electric shock (42), a physiological action of UCN was considered improbable. Interestingly though, IV UCN is more potent (ACTH, 1.5×) than rCRH in rats (29,42) whereas UCN given ICV was less potent (cortisol, 0.6×) than pCRH in pigs.

Stress in the pig, as in humans, not only activates the HPA axis but also stimulates the release of prolactin and growth hormone (14). In this study, there was no effect of pCRH or UCN on plasma prolactin, although there was a suggestion of increased growth hormone release. This contrasts with the findings of a previous experiment in another ungulate, the sheep, where it was shown that oCRH given ICV at a behaviorally effective dose affected neither hormone (33). However, the sheep is a species in which growth hormone is not stress responsive (6), whereas, studies in humans suggest that its release is enhanced in neurotic individuals (27). Therefore, the possibility of a relationship between the pulsatile pattern of growth hormone secretion (9) and "anxiety" states in swine seems to be worthy of further investigation.

Central injections of ODN have anxiogenic effects in rats that can be antagonized by ligands for the BZ site on the $GABA_A$ receptor (16,17,20,38). However, on a molar basis, the doses of ODN required to produce these responses (1.6–5 nmol) are considerably greater than the effective anxiogenic doses of CRH [0.1–0.2 nmol; (41)]. Therefore, it is possible that the dose of ODN used in this study (21 nmol), which was the same as that of pCRH, was too low to produce an effect. Behavioral responses have, however, been reported in pigs given much smaller ICV doses of pCRH [15 μg; 3.2 nmol; (23)] so some activational effect of ODN might have been expected. In contrast, ODN appeared to exhibit BZ-like effects in that it tended to produce more drowsiness, less standing, and oro-nasal behavior, and lower overall activity than SAL. Also, ODN was the only treatment to reduce plasma cortisol concentrations, although, as with SAL, plasma prolactin increased in some animals. In this connection, it is of interest that low ICV doses of ODN (0.5 nmol) in rats increase hypophysial prolactin mRNA (45) and reduce CRH message in the paraventricular hypothalamic nucleus via a BZ-dependent mechanism (18,19). Taken together, these findings are difficult to reconcile with an anxiogenic mode of action, as reported in rats (16,17,20,38).

Concentrations of DBI in the CSF of panic disorder patients correlate with those of CRH (36), supporting the speculation that CRH might release an ODN-like BZ ligand (10). However, there was no increase in DBI in the CSF of the high anxiety group (36). Moreover, a recent review (4) has suggested that DBI and ODN are biochemical artefacts rather than endogenous neuropeptides. With respect to the pig, the actions of BZ agonists, and especially antagonists, have been little studied. It is known that conditioned suppression of operant responding for food can be reduced by diazepam (7) and less effectively by chlordiazepoxide (8) or lorazepam (34). Intravenous administration of the BZ inverse agonist ethyl-beta-carboline-3-carboxylate (βCCE) also inhibits operant food but not water intake (13), although, at these doses (60 μg/kg), it does not produce agitation, vocalization, or an increase in plasma cortisol (Vellucci and Parrott, unpublished). Similarly, the motor effects of the putative anxiogen pentylenetetrazol [(PTZ;(4)] can be reversed in pigs by diazepam but, again, no overt emotional responses were observed (Vellucci and Ebenezer, unpublished). At present, therefore, none of these proposed anxiogenic agents (ODN, βCCE, or PTZ) has been found to induce the same type of behavioral response in pigs as centrally administered pCRH or UCN.

In conclusion, concern for the psychological well-being of intensively housed pigs requires greater understanding of CNS mechanisms relating to stress and anxiety. The results of this and previous studies in pigs suggests that interactions between pCRH and the $BZ/GABA_A$ receptor may be highly relevant to this problem. However, this subject clearly needs to be examined in greater detail.

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