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Effects of the Novel Cholecystokinin Analogue Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on Feeding and Cortisol Release in Pigs

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EBENEZER, I. S., R. F. PARROTT AND J. A. GOODE. *Effects of the novel cholecystokinin analogue Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on feeding and cortisol release in pigs.* PHARMACOL BIOCHEM BEHAV **54**(1) 255–259, 1996. – Suc-Try-N-(Me)-Nle-Asp-Phe-NH_2 is a succinylated tetrapeptide derived from the *C*-terminal sequence of cholecystokinin octapeptide (CCK-8), which has been shown to have high agonist affinity for CCK_B receptors. To test the validity of the hypothesis that implicates central CCK_B receptors in the aetiology of stress-related disorders, such as anxiety and panic, we argued that activation of these receptors by a CCK_B receptor agonist should (i) suppress feeding motivation in hungry animals and (ii) increase plasma concentrations of the stress hormone cortisol. The effects of systemic and central administration of Suc-Try-N-(Me)-Nle-Asp-Phe-NH_2 were, therefore, investigated on operant food intake and cortisol secretion in pigs. Intravenous administration of Suc-Try-N-(Me)-Nle-Asp-Phe-NH_2 (0.5–5 μg/kg) did not affect operant feeding in food-deprived pigs, although the highest dose (5 μg/kg) produced a small but significant ($p < 0.05$) increase in plasma cortisol levels 5–30 min after injection. Intracerebroventricular injection of Suc-Try-N-(Me)-Nle-Asp-Phe-NH_2 (1–5 μg) had no effect on operant feeding and cortisol secretion in this species. The results obtained in this study indicate that central CCK_B receptors are unlikely to be involved in stress-related behaviours in pigs.

Suc-Try-N-(Me)-Nle-Asp-Phe-NH_2 Operant food intake Cortisol CCK Stress Anxiety Panic

CHOLECYSTOKININ (CCK) is a peptide that is widely distributed within the central nervous system (CNS) and the gastrointestinal tract (7). CCK acts at two pharmacologically distinct receptor subtypes, namely, CCK_A and CCK_B receptors (18). CCK_A receptors are found mainly in the periphery, but they are also present in discrete brain regions (5,15,18). By contrast, CCK_B receptors are located mainly in the brain, although CCK_B receptors have also been found on vagal afferents (5,15). Pharmacological studies have shown that, while the sulphated CCK octapeptide (CCK-8S) has a high affinity for both receptors, related peptides, such as CCK tetrapeptide (CCK-4) and the unsulphated CCK octapeptide (CCK-8US), show higher affinities for CCK_B receptors than for CCK_A receptors (5). The recent availability of specific agonists and antagonists for the CCK receptor subtypes have suggested possible physiological roles for CCK within the CNS and periphery. Although most of the work on CCK has focused on its functional role at CCK_A receptors (e.g., 2,5,8,11,13), relatively little is known about the actions of CCK that are mediated by CCK_B receptors. Results from a number of preclinical and clinical studies have suggested that central CCK_B receptors may be involved in the aetiology of anxiety and panic attacks (1,4,14,23). However, the evidence for the involvement of CCK_B receptors in such a role is still unclear (10,24).

We have previously argued that, if central CCK_B receptors are involved in the mediation of anxiety and panic attacks, then activation of these receptors by a CCK_B receptor agonist should (i) suppress feeding motivation in hungry animals, and (ii) increase plasma concentrations of stress hormones, such as cortisol (10). However, the results obtained from a number of studies have proved to be fairly equivocal. Thus, Della-Fera and Baile (6) reported that intracerebroventricular (ICV) administration of the CCK_B agonist pentagastrin induced panic-like behaviour and inhibited food intake in sheep. By contrast, Ebenezer et al. (10) found that ICV administration of pentagastrin did not affect feeding nor the secretion of the stress hormone cortisol in this species. Similarly, systemic or central

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injections of various of CCK_B agonists have failed to affect food intake in pigs (19), rats (25), and baboons (13). However, Asin et al. (2) and Parrott (20) have reported that high doses of the CCK_B agonist A63387 given ICV inhibit food intake in rats and pigs, respectively.

Recently, Kaufmann et al. (17) synthesised a novel tetrapeptide derived from the *C*-terminal sequence of CCK-8 (i.e., Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2, which has a high affinity for central CCK_B receptors ($K_i = 4 \times 10^{-9}$ M) compared with pancreatic CCK_A receptors (CCKA/CCKB ratio < 10,000). It is also believed to be one of the most potent CCK_B agonists available, and may, thus, prove to be a useful pharmacological tool for studying the possible biological roles that are mediated by CCK_B receptors. To the best of our knowledge, this peptidergic CCK_B agonist has not been previously tested in vivo. Thus, in the present study we investigated the effects of intravenous (IV) and ICV administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on operant food intake and cortisol secretion in the pig. It was hoped that these experiments would throw futher light on the role of CCK_B agonists in the aetiology of stress-like behaviours. We carried out this investigation in the pig because previous work from this laboratory has shown that the pig is an excellent animal to study the peripheral and central effects of CCK and its analogues on endocrine responses and operant feeding (8,9,11,12,19,20,21,22).

METHOD

Experiment 1: Effect of IV Administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on Food Intake and Cortisol Release

Six male prepubertal Large White pigs, weighing between 35–40 kg, were used in these experiments. The animals were housed separately in metabolism cages, and were trained to press the 2 operant switch panels located on the front end of the cages with their snouts to obtain food and water reinforcements on a fixed ratio of 5. A single food reinforcement weighed 10 g and a single water reinforcement measured approximately 10 ml.

Each pig was surgically prepared under halothane anaesthesia with a catheter in either the right or left external jugular vein, as described previously (11). Sterile precautions were observed thoughout the surgical procedures. The patency of the catheters were maintained by flushing them at least once a day with heparinised saline solution.

Following recovery from surgery, the animals were maintained on the following feeding schedule: At 900 h, a buzzer sounded signalling that the feeder was activated for 20 min and that the pigs could press the food panel for reinforcement during that period. At 1600 h, the buzzer sounded again, signalling that the feeder was activated for 60 min. Water was available ad lib throughout the experiment. The occurrence of feeding was monitored by means of a computer-based data logging system. When the daily response rates of the pigs for food reinforcement had stabilized, drug studies commenced.

During the experimental sessions, the animals received either vehicle solution (control) or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (0.5, 1, and 5 μg/kg) intravenously (IV) in a randomised manner. The animals were injected with vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 at 15.55 h (i.e., 5 min prior to the buzzer signalling the afternoon). The number of reinforcements obtained by each pig was monitored in 10-min bins over the 60-min feed period. Each pig received all treatments, and at least 2 days were allowed between successive drug trials.

Four of the pigs were subsequently used to investigate the effects of the peptide on cortisol secretion, using a method previously described (21). The animals were injected with vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5 μg/kg, IV) at 1430 h. Blood samples (10 ml) were taken 30, 15, and 0 min before and 5, 15, 30, and 45 and 60 min after IV injection and held on ice until the end of sampling. Following centrifugation, the plasma was stored at −300°C pending radioimmunoassay for cortisol (see ref. 22 for details). Each pig received both treatments and at least 2 days separated successive blood sampling sessions.

Experiment 2: Effect of ICV Administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on Food Intake and Cortisol Secretion

Large White male prepubertal pigs ($n = 4$, b.wt. 40–45 kg) were surgically prepared under halothane anaesthesia with a stainless steel guide tube directed towards the left or right lateral ventricle for chronic intracerebroventricular (ICV) injections, using a method previously described (21). The animals were also prepared with catheters in an external jugular vein, as described above, for withdrawal of blood samples. We assessed the success of implantation of the ICV cannula in each animal by the drinking response elicited by ICV administration of the dipsogenic neuropeptide angiotensin II (250 nmol). All the animals started to drink water within 2 min of receiving the peptide, confirming the success of ICV injection.

Operant food intake was measured in these pigs following ICV injections of either vehicle solution (control) or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (1 and 5 μg), using an experimental protocol similar to that described in Experiment 1.

The effects of ICV administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 on cortisol secretion were also assessed in these animals. The pigs were injected with vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5 μg, ICV) and blood samples were taken and analysed for plasma cortisol as described in Experiment 1.

Drugs

Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 was synthesised by the Micochemical Facility at the Babraham Institute, Cambridge. A stock solution consisting of 1 mg of the peptide dissolved in 1 ml acetic acid (0.02% v/v) was diluted in physiological saline (0.9% w/v) to make up the different injection concentrations. The vehicle solution consisted of acetic acid (0.02% v/v) diluted in physiological saline so that the concentration of acetic acid in the vehicle equalled that of the 5 μg/kg dose of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 for IV injection and 5 μg for ICV injection. For IV administration, drug and vehicle solutions were injected as a 500 μl bolus and this volume was flushed down the catheter with 1.5 ml of heparinized saline. For ICV administration, drug and vehicle solutions were injected as a bolus dose of 100 μl and flushed down the catheter attached to the ICV cannula with 200 μl of saline.

Statistics

The results obtained in the feeding experiments were analysed by analysis of variance (ANOVA) with repeated measures. As previously (21,22), treatment effects for plasma cortisol levels were examined by an analysis of variance that calculated the net change in the area under the response curves (27). Results were compared for pretreatment (−30, −15 and 0 min), early (+5, +15, +30 min) and late (+45 and +60 min) posttreatment periods.

RESULTS

Experiment 1

Figure 1 shows the mean + SEM number of reinforcements obtained by the pigs after IV administration of vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (0.5–5.0 μg/kg). Statistical analysis of the results indicated that none of the doses of the peptide affected operant food intake at any of the measurement intervals during the 60-min feeding period. That is, there were no significant effects of drug treatment, $F(3, 15) = 0.3317$) nor drug treatment × time interaction, $F(15, 75) = 0.5971$.

The effects of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg/kg) on cortisol secretion are shown in Fig. 2. The peptide significantly increased plasma cortisol levels during the 5–30-min period after IV administration; $F(1, 3) = 10.5480$, $p < 0.05$. The effect was fairly short-lasting, with the cortisol levels returning to near control values 45–60 min after injection of the CCK_B receptor antagonist.

IV administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 did not produce any overall changes in behaviour in the animals compared with the vehicle. However, two of the animals did show retching-like behaviour immediately after IV injection of the 5 μg/kg dose, but did not vomit. The pigs recovered within 1–2 min of the injection and pressed the operant panel normally for food as soon as the feeder was activated. One of these pigs given the 5 μg/kg dose also displayed a mild polydipsia at the end of the 60-min feeding period. However, this behaviour was not seen in any of the other animals.

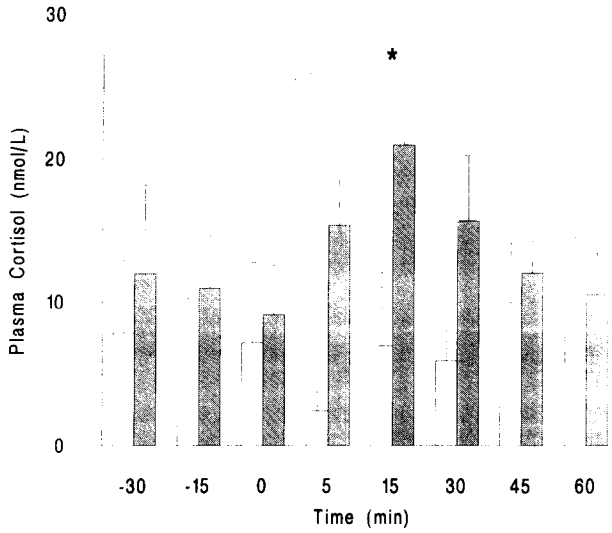

FIG. 2. Plasma concentrations of cortisol (mean + SEM) in pigs before (−30, −15 and 0 min) and after (+5, +15, +30, +45, and +60 min) after IV administration of vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg/kg). $n = 4$ pigs. *$p < 0.05$ net change in area under curve compared with control. Vehicle: □; Suc-Trp-N(-Me)-Nle-Asp-Phe-NH_2 (5.0 μg/kg): ■.

Experiment 2

Figure 3 shows the mean + SEM number of reinforcements obtained by the pigs after ICV administration of vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (1.0–5.0 μg). Statistical analysis of the results indicated that none of the doses of the CCK_B agonist affected operant food intake at any of the measurement intervals during the 60-min feeding period, that is. there were no significant effects of drug treatment, $F(2, 6) = 3.4451$; ns, nor drug treatment × time interaction, $F(10, 30) = 0.5895$; ns.

The effects of ICV administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg) on cortisol secretion are shown in

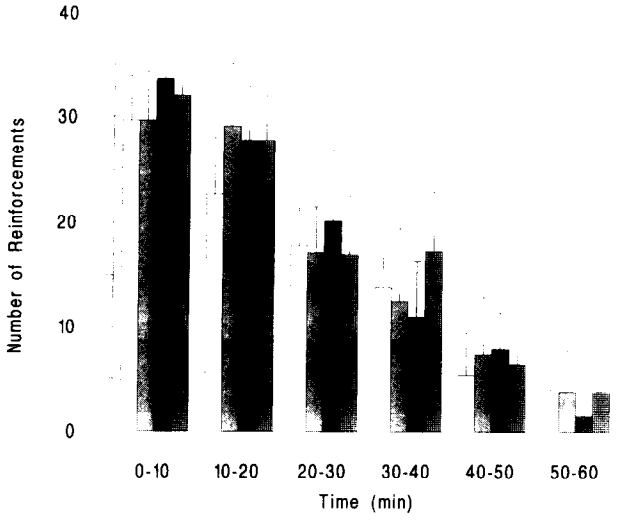

FIG. 1. Effects of IV administration of vehicle and Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (0.5–5.0 μg/kg) on the number of food reinforcements obtained by pigs in 10-min periods for 60 min. Food intake was measured 5 min after IV injection of vehicle or drug (see text for further details). $n = 6$ pigs. Vertical lines represent +SEM. Vehicle: □; Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (0.5 μg/kg): ▨; Suc-Trp-N-(Me)-Nle-Asp-Phe-NH_2 (1.0 μg/kg): ■; Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg/kg): ▩.

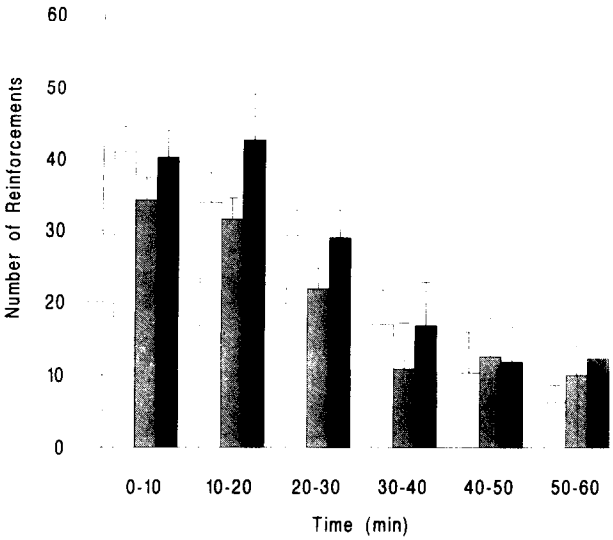

FIG. 3. Effects of ICV administration of vehicle and Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (1.0–5.0 μg) on the number of food reinforcements obtained by pigs in 10-min periods for 60 min. Food intake was measured 5 min after ICV injection of vehicle or drug (see text for further details). $n = 6$ pigs. Vertical lines represent +SEM. Vehicle: □; Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (1.0 μg): ▨; Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg): ■.

Fig. 4. The peptide did not significantly affect cortisol release compared with control values during the 60-min period following administration. Interestingly, there were slight increases in cortisol levels 45–60 min after both vehicle and drug treatment. The reason for this is not known, but it could be due to the acetic acid vehicle solution in which the drug was dissolved, or a late response to the ICV injection and blood sampling procedures.

ICV injection of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (1–5 μg) produced no overt behavioural changes in the animals.

DISCUSSION

We have previously demonstrated that doses of CCK-8S in the range 0.7–1.3 μg/kg, IV and 1–1.3 μg ICV significantly increase plasma levels of the stress hormone cortisol and inhibit operant feeding in young pigs (12,21). We, therefore, considered that the range of IV and ICV doses of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 used in this study was adequate to show any effects of the peptide on operant feeding and cortisol secretion in this species.

The results obtained in Experiment 1 show that IV administration of the CCK_B agonists Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (0.5–5 μg/kg) does not affect operant feeding in food-deprived pigs. These results are consistent with a previous study in which it was found that systemic administration of the CCK_B agonist pentagastrin did not inhibit operant food intake in this species (19). Moreover, these results are also in agreement with the observations that CCK_A receptor antagonists (8,11), but not CCK_B receptor antagonists (3), inhibit the hypophagic effects of peripheral exogenous CCK-8S on food intake in pigs. However, Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5 μg/kg, IV) did cause a short-lasting but significant elevation in plasma cortisol levels during the 30 min after injection. These results are at variance with those obtained by Itoh et al. (16), who have shown that systemic administration of pentagastrin did not increase plasma levels of the stress hormone corticosterone in rats. The increase in the stress hormone levels in this experiment were most probably due to a peripheral effect of the peptide because ICV administration of the CCK analogue does not affect plasma cortisol levels (see Fig. 4). Because CCK_B receptors are pharmacologically very similar to peripheral gastrin receptors (7), it is possible that the high plasma levels of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 activated these receptors in the gastrointestinal tract of the pig, causing excess acid secretion and the resulting mild stress response. It is, perhaps, pertinent to observe in this context that the animals had not consumed food for almost 5 h prior to the IV injection of the drug.

The results obtained in the 2nd experiment shows that ICV administration of Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 does not affect operant food intake or secretion of cortisol in pigs. The results on food intake are consistent with previous observations in this species, in which it was demonstrated that ICV administration of pentagastrin did not affect feeding behaviour (19). Furthermore, they are also in agreement with the results of a study in sheep, in which we found that ICV pentagastrin did not inhibit feeding nor increase the release of the stress hormone cortisol (10). By contrast, it has previously been demonstrated that CCK-8S (1.3 μg, ICV) causes a marked suppression of food intake in pigs and also increases plasma cortisol levels approximately 3-fold from control values (21). These results, therefore, suggest that the inhibitory effect of centrally administered CCK on food intake and the accompanying elevation in plasma levels of the stress hormone cortisol are not mediated by CCK_B receptors. However, it is noteworthy that Parrott (20) has observed that although ICV administration of another CCK_B agonist, A63387, in the dose range (1–5 μg) did not inhibit operant food intake in pigs, a very high dose (i.e., 20 μg ICV), did cause a small reduction in feeding. Similarly, Asin et al. (2) have reported that a high dose of A63387 given ICV significantly reduced food intake in rats. The reason for the hypophagic response produced by high doses of A63387 in pigs and rats is not known, but it could be due to nonspecific actions at CCK_A receptors. Interestingly, however, a very high dose of A63387 did not reduce food intake in baboons (13).

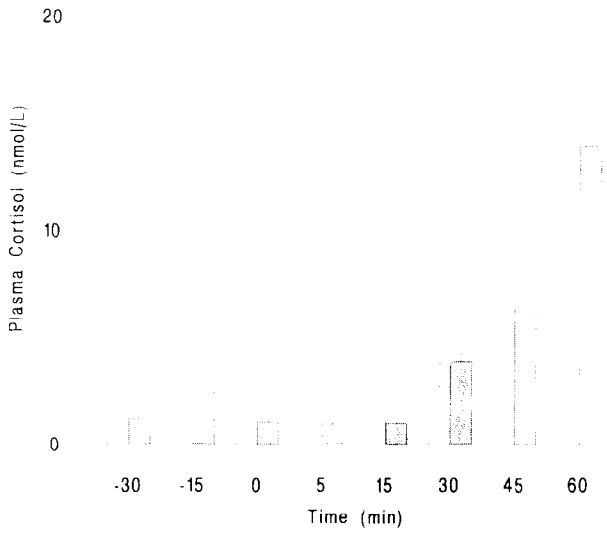

FIG. 4. Plasma concentrations of cortisol (mean + SEM) in pigs before (−30, −15 and 0 min) and after (+5, +15, +30, +45, and +60 min) after ICV administration of vehicle or Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg). n = 4 pigs. Vehicle: □; Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 (5.0 μg): ■. There were no significant effects of drug treatment on cortisol secretion.

In summary, the results of this study have shown that neither systemic nor central administration of the novel CCK_B agonist Suc-Trp-N(Me)-Nle-Asp-Phe-NH_2 has any acute effects on food intake in food-deprived pigs and that ICV injection of a high dose of the peptide did not increase secretion of the stress hormone cortisol in this species. These results thus indicate that central CCK_B receptors are unlikely to be involved in stress-related behaviours associated with anxiety or panic in pigs.

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