Hormonal effects of apomorphine and cholecystokinin in pigs: modification of the response to cholecystokinin by a dopamine antagonist (metoclopramide) and a kappa opioid agonist (PD117302)

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Abstract. Three experiments were carried out to investigate some of the mechanisms involved in the endocrine responses of pigs to the emetic agents apomorphine and cholecystokinin. In Experiment 1, plasma levels of vasopressin and cortisol were measured in prepubertal pigs (N=5) treated with iv apomorphine (25 µg/kg) or saline vehicle. In Experiment 2, concentrations of vasopressin and cortisol were determined in pigs given iv sulphated cholecystokinin octapeptide (1.5 µg/kg), metoclopramide (300 µg/kg), metoclopramide + cholecystokinin, and an oral dose of the kappa opioid agonist PD 117302 (20 µg) alone, or followed by iv cholecystokinin. In Experiment 3, operant feeding behaviour was quantified in pigs (N=4) given cholecystokinin (1 µg/kg) or cholecystokinin preceded by oral PD 117302. Following apomorphine injection in Experiment 1, there was a rapid, transient, rise in plasma vasopressin. Cholecystokinin had a similar effect on vasopressin secretion in Experiment 2 and also induced a later rise in plasma cortisol. Pre-treatment with metoclopramide appeared to reduce both of these effects of cholecystokinin, but only the decrease in cortisol was statistically significant. However, oral administration of PD 117302 abolished the effect of cholecystokinin on vasopressin release and reduced the subsequent rise in cortisol. The inhibitory effect of cholecystokinin on feeding was unaltered by PD 117302 treatment in Experiment 3. The results obtained with apomorphine and metoclopramide, together, suggest that the neuroendocrine effects of cholecystokinin in the pig may involve an action on central dopamine receptors while the effects of PD 117302 indicate that kappa opioids may modify the hormonal responses to cholecystokinin by a peripheral action.

Nausea and emesis have a pronounced stimulatory effect on vasopressin secretion in man (1). Circulating levels of vasopressin also increase in man (2), monkeys (3), sheep (4) and pigs (5) following iv injection of cholecystokinin (CCK), a gut/brain peptide hormone that induces vomiting in some species (3,6). Moreover, the finding that apomorphine, a dopamine agonist known for its emetic properties, also induces vasopressin release in primates and carnivores (1,3) suggests that this neuroendocrine response may be mediated by dopaminergic neurons. However, a different situation may obtain in ungulate species because apomorphine does not alter vasopressin secretion in sheep when given at doses that produce behavioural effects (7). Nevertheless, the dopamine antagonist, haloperidol, does appear to reduce the vasopressin response of sheep to iv CCK (8). The pig also has unusual CNS dopamine receptors because dopamine antagonists produce the same type of behavioural responses as apomorphine (9). Therefore, it is of interest to determine whether dopaminergic neurons play a part in mediating the vasopressin response to emetic agents in pigs. This was investigated in the present study by examining the en-
endocrine response of pigs to apomorphine, and also to CCK when given in combination with the central dopamine antagonist metoclopramide.

In addition to its effect on vasopressin release, CCK has also been shown to induce cortisol and prolactin secretion in sheep (4), cortisol release in pigs (5,10) and rats (11) and prolactin secretion in rats (12). Such results suggest that exogenous CCK may act as a chemical stressor. Hence, drugs that affect the hypothalamo-pituitary-adrenocortical axis may alter the endocrine response to CCK. In this connection, it has recently been demonstrated that a kappa opioid agonist, PD 117302, will increase the nociceptive threshold of mice to noxious chemical stimuli (13) and, when given orally, reduce the cortisol response of dogs to acoustic stress (14). Thus, a second aim of this study was to determine whether oral administration of this opioid would also modify the cortisol response of pigs to exogenous CCK.

Peripherally administered CCK is also well known for its ability to reduce food intake in a variety of species and previous work has shown that such effects can also be demonstrated in the pig (15). However, because there is a close temporal link between the behavioural and vasopressin responses of pigs to CCK (5), it is possible that both effects are a consequence of the aversive properties of the peptide when given by iv bolus. Accordingly, a third objective of this investigation was to examine whether PD 117302 would modify the effect of CCK on operant feeding in pigs. An experiment of this kind might allow some conclusions to be drawn regarding the likely site of action, i.e. central or peripheral, of kappa opioids in this species.

Materials and Methods

Nine prepubertal Large White breed pigs (3 male, 2 female, Experiments 1 and 2; 3 male, 1 female, Experiment 3) weighing 24.6±2.3 kg at the start of the investigation were prepared with jugular vein catheters, under closed circuit halothane anaesthesia, using sterile precautions. The animals lived in metabolism cages throughout and were provided with food twice daily and water ad libitum.

Two experiments (1 and 2) were carried out to investigate the role of dopaminergic systems in the endocrine responses of pigs to the emetic agents. In both experi-

ments, blood samples were taken 10 min before (−10 min) and 2, 5, 10, 20 and 30 min after treatment administration by iv (2 ml) injection at 0 min (time zero). The samples were held on ice and subsequently centrifuged. The resultant plasma was divided into aliquots and stored at −30°C pending radioimmunoassay for lysine vasopressin (LVP) as previously described (16), and cortisol. Plasma concentrations of cortisol were measured by a direct radioimmunoassay using 125I-cortisol conjugate IM129 (Amersham International, UK) and cortisol antiserum (Steranti Research, UK). The sensitivity and intra-and inter-assay coefficients of variation, respectively, were 0.83 ng/l, 9.1% and 9.4% for cortisol and 0.15 ng/l, 8.3% and 12.2% for LVP.

Experiment 1 examined the effects of apomorphine sulphate (Sigma Chemical Co Ltd), given in saline at a dose of 25 µg/kg, with vehicle administration serving as a control. Experiment 2 investigated the response to CCK (sulphated octapeptide, Cambridge Research Biochemicals Ltd) reconstituted from frozen aliquots and administered in saline at a dose of 1.3 µg/kg. During the course of the experiment, the following treatments were given to individual animals at the same time of day and in the majority of cases, on different test days: CCK; metoclopramide monohydrochloride (Sigma) given in saline at a dose of 300 µg/kg at −10 min and followed by saline at time zero; metoclopramide at −10 min followed by CCK at time zero; PD 117302, generously supplied by Prof J. Hughes, Parke-Davis Research Unit, Cambridge, given at −25 min followed by CCK at time zero. This opioid was administered as a 20 mg oral dose contained in a gelatine capsule embedded in a ball of moist bread after the pigs had been trained to eat bread balls containing empty capsules.

In the behavioural study, Experiment 3, 4 catheterised pigs of similar weight to those used above were trained to press switch panels with their snouts to obtain food reinforcements (approx. 20 g) on a fixed ratio of 5 presses to 1 food delivery. The animals were deprived of food for 17 h and then a buzzer sounded to indicate that the panels were operational. Five minutes after the start of feeding, the pigs were given iv injections of CCK (1 µg/kg) or saline vehicle. The pigs were also tested with CCK after receiving a 20 mg oral dose of PD 117302 administered 20 min before the start of the test. Feeding activity was recorded for 30 min and analysed in 5-min blocks.

As previously (5), the results of Experiments 1 and 2 were analysed by using the trapezoid rule to calculate the area under the response curve for LVP and cortisol. The difference from pre-treatment values (−10 min samples) in the early (2-10 min) and late (10-30 min) post-treatment periods were then determined and any differences expressed as two-tailed probability values. Comparisons were made between saline and apomorphine in Experimental 1 and between CCK and all other treatments in Experiment 2. The results of Experiment 3 were analysed using the two-tailed paired t-test.
Results

The effects of apomorphine (Experiment 1) are illustrated in Fig. 1. Levels of LVP were consistently low after saline administration but rose abruptly within 2 min of apomorphine injection. The net change (difference from pre-treatment level) for LVP during the 2-10 min pre-treatment period was significantly different (apomorphine x saline, p<0.03) but the effect was short-lived and a difference was no longer apparent in the later, 10-30 min, post-treatment period. Cortisol concentrations also appeared to increase after apomorphine, but the net change did not differ from saline in either post-treatment period; this lack of significance is likely to be due to the variability of the control data. One pig vomited after receiving apomorphine.

Changes in LVP secretion after CCK, metoclopramide and PD 117302 treatment (Experiment 2) are shown in Fig. 2. A rapid rise in LVP was seen after CCK injection, similar to that induced by apomorphine in Experiment 1 (Fig. 1). However, plasma LVP levels did not change after metoclopramide followed by saline and were similar to those when the pigs received saline alone (Fig. 1). As a result, the net change in LVP during the 2-10 min post-treatment period differed significantly between this control treatment and CCK (p<0.04). When metoclopramide was followed by CCK, the LVP response appeared to be reduced although the net change (CCK x metoclopramide/CCK) in the 2-10 min post-treatment period did not reach statistical significance (p<0.08). In contrast, oral pre-treatment with PD 117302 abolished the effect of CCK on LVP release in the 2-10 min post-treatment period (CCK x PD 117302/CCK, p<0.04). One animal vomited after being given CCK in the absence of any pre-treatment.

The effects of CCK, metoclopramide and PD 117302 on cortisol secretion (Experiment 2) are displayed in Fig. 3. Plasma cortisol increased following CCK administration but remained unchanged in the metoclopramide/saline condition, as with saline alone (Fig. 1). In consequence, the net change differed significantly between these treatments in both the early (p<0.03) and later (p<0.01) post-injection periods. Pre-treatment with metoclopramide also reduced the effect of CCK on cortisol secretion in the 2-10 min and 10-30 min post-treatment periods (CCK x metoclopramide/CCK, p<0.001, p<0.05, respectively). Similarly, pre-treatment with PD 117302 significantly decreased the cortisol response to CCK in both post-treatment periods (CCK x PD 117302/CCK, p<0.01, p<0.05, respectively).

Fig. 4 shows the changes in operant feeding behaviour induced by iv CCK or CCK following oral pre-treatment with PD 117302 (Experiment 3). There was the expected (13) transient inhibition of feeding in the 5 min period following CCK injection (CCK x saline, p<0.02) and this effect was still apparent when the animals also received PD 117302 (PD 117302/CCK x saline, p<0.05).

Fig. 1.
Plasma concentrations (X ± SEM) of lysine vasopressin (LVP) and cortisol in pigs before (shaded columns), and at intervals after (open columns), iv administration of saline or apomorphine (20 mg/kg). *p<0.05 (two-tailed) indicating a difference between treatments in the change from pre-injection levels.
**Discussion**

The present results provide new information regarding the mechanisms involved in the neuroendocrine events following iv administration of CCK to pigs. The vasopressin response to apomorphine represents a physiological correlate of nausea in species able to vomit (1) and, in the present study, both apomorphine and CCK caused emesis and produced similar effects on LVP release. Therefore, since apomorphine acts on central dopamine receptors (17), the effects of CCK on LVP release in the pig may also involve dopaminergic neurons.

The effects of apomorphine on vasopressin secretion in pigs differ from those previously described in sheep (7), whereas the effects of dopamine antagonists on the vasopressin response to CCK in sheep in a former study (8), and on pigs in the present investigation, are similar. The dopamine antagonist metoclopramide appeared to reduce the LVP response to CCK in pigs and significantly reduced the rise in plasma cortisol. However, the possibility that a higher dose of the drug might suppress the effects of CCK on LVP secretion was not investigated because the dose given in Ex-

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**Fig. 2.** Plasma concentrations (x ± SEM) of lysine vasopressin (LVP) in pigs before (shaded columns), and at intervals after (open columns), iv administration of CCK (1.3 µg/kg), metoclopramide (300 µg/kg) given at −10 min followed by saline at time zero, metoclopramide followed by CCK, and PD 117302 (20 mg) given orally at −25 min followed by CCK at time zero. *p<0.05 (two-tailed), indicating a difference from CCK in the change from pretreatment levels.

**Fig. 3.** Plasma concentrations (x ± SEM) of cortisol in pigs under the same experimental conditions described for Fig. 2. ***p<0.001, **p<0.01, *p<0.05 (two-tailed), indicating a difference from CCK, over the same time period, in the change from pre-injection levels.
experiment 2 produced arousal (vocalization) and temporarily disrupted operant feeding in pigs (n = 3) when given on its own. These effects are likely to be related to the atypical behavioural responses induced in this species by antidopaminergic neuroleptic drugs (9). It should also be noted that lower doses of metoclopramide than those given in this study are reported to increase vasopressin secretion in man (18), but no effect of this kind was observed in the present investigation. Moreover, although metoclopramide is considered to act on central dopamine receptors, some of its pharmacological effects may also be ascribed to an action on peripheral serotonin receptors (19), though whether this also applies to endocrine responses remains to be investigated.

In contrast to apomorphine, circulating CCK does not cross the blood-brain barrier (20). Therefore, the neuroendocrine effects of CCK must be produced by an action either, on the neurohypophysis, afferent neural pathways, or on a circumventricular organ such as the area postrema where the barrier is absent. In the rat, exogenous CCK stimulates oxytocin rather than vasopressin release (21), but CCK does not induce hormone secretion from posterior pituitary cells in vitro (22), suggesting that the peptide does not directly affect the neurohypophysis. Instead, it seems more probable that exogenous CCK may act via "A" type receptors in the gut and the area postrema (23), both of which are neuronally linked with the nucleus of the solitary tract and are involved in the control of emesis in response to noxious chemical stimuli (24); in this connection, it is also relevant that the area postrema contains dopamine (D2) receptors sensitive to apomorphine (25). Moreover, the nucleus of the solitary tract is indirectly connected to magnocellular serotory neurons (26) and, in cats, topical application of sodium chloride to the area postrema region stimulates vasopressin release (27). Therefore, it seems likely that dopamine (D2) receptors in the area postrema (27), the chemoreceptor trigger zone for the emetic reflex (24), may be involved in the LVP responses to both CCK and apomorphine.

Vasopressin release in rats induced by osmotic and non-osmotic stimuli is decreased by administration of an opioid agonist acting at the kappa receptor (28). Similarly, PD 117302 is a kappa agonist that is orally active and induces diuresis in mice (13). Hence, the inhibition of the LVP response to CCK following administration of this opioid to pigs is consistent with the concept that kappa agonists modulate neuroendocrine function. Since PD 117302 was given orally, this inhibition might be mediated by visceral afferents from the gut to the nucleus of the solitary tract, or might be due to an effect on the pituitary following absorption into the circulation. A central action, though, seems unlikely because the opioid did not prevent the CCK-induced decrease in feeding. However, this was only a small-scale experiment using 4 pigs and a larger dose-response analysis is required to substantiate this finding. Furthermore, it may also be deduced that the rise in plasma vasopressin is likely to be a consequence, and not a cause, of those aversive effects of CCK that are responsible for the suppression of feeding.

The adrenocortical effects of auditory stress in the dog are reduced by oral administration of PD 117302 (14) and, in like manner, a large oral dose of the opioid reduced the cortisol response of pigs to CCK in the present study. This supports previous findings of increased cortisol secretion in pigs following naloxone treatment (29). However since CCK stimulates secretory activity in pituitary corticotropes (11,30), these results may indicate that PD 117302 has effects on anterior, as well as pos-

Fig. 4.
Number of food reinforcements (x ± SEM) obtained by pigs in 5-min periods before (closed columns) and after (open columns) iv administration of saline or CCK (1.0 μg/kg) at time zero, or PD 117302 (20 mg) administered orally at -25 min followed by CCK at time zero. *p<0.05 (comparison with saline), two-tailed paired t-test.
terior, pituitary function in pigs. By contrast, the inhibitory action of metoclopramide on CCK-induced cortisol release is more likely to be mediated centrally by dopaminergic neurons.

The following conclusions may be drawn from this study. Firstly, the neuroendocrine responses of pigs to CCK are likely to be a consequence of the noxious properties of this peptide hormone when given as an iv bolus. Secondly, the similar effects of apomorphine and CCK on LVP release would seem to indicate that both may affect medullary (area postrema) dopaminergic neurons. However, because metoclopramide only partially antagonized this action of CCK, further studies are needed to test this hypothesis. Thirdly, the kappa agonist PD 117302 interfered with the endocrine responses to CCK in pigs while leaving its behavioural effect intact, indicative of peripheral opioid action. Fourthly, the release of LVP after CCK administration was not responsible for the subsequent increase in cortisol secretion because opioid blockade of the LVP response reduced, but did not eliminate, the rise in cortisol. Finally, it should be noted that although the present results are concerned with mechanisms involved in the response to exogenous CCK, further research is needed to determine how these findings relate to the physiological actions of the endogenous hormone.

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References


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