ABSTRACT: The synthetic met-enkephalin syndyphalin-33 (SD-33) increases feed intake in sheep and transiently increases circulating GH concentrations in sheep, rats, and pigs. Two experiments were performed to evaluate the effects of SD-33 on recently weaned pigs. In a preliminary experiment, pigs were administered SD-33 (0.5 µmol/kg, given intramuscularly) or saline immediately before a 3-h transport and subsequent placement into group pens. Treatment with SD-33 increased \( P = 0.01 \) daily feed intake; cumulatively, pen intake over 7 d postweaning tended \( P = 0.06 \) to be greater than in control pens. In Exp. 2, pigs were weaned and fitted with jugular catheters. The following day, pigs were treated with SD-33 or saline as described above. Transient increases \( P < 0.05 \) in circulating concentrations of GH (at 1 and 1.5 h postinjection) and cortisol (at 3.5 and 4 h postinjection) were observed in pigs treated with SD-33 relative to controls. No difference in feed intake was observed between treatments over 4 d postinjection. Increased \( P < 0.05 \) numbers of circulating neutrophils, lymphocytes, and monocytes were observed in both treatment groups over 4 d postinjection, and treatment with SD-33 tended \( P = 0.07 \) to selectively increase monocyte numbers. Although SD-33 has potential to be used to increase feed intake and decrease the negative effects of stress during weaning in pigs, further investigation is needed to better understand the timing of effect and to rule out possible immunosuppressive effects.

Key words: appetite, opioid, pig, stress, weaning

INTRODUCTION

Syndyphalin-33 (SD-33; Tyr-D-Met(O)-Gly-N-methylphenethylamide) is a synthetic enkephalin with prolonged analgesic activity (Kiso et al., 1981). In pigs, rats, and sheep, administration of SD-33 via oral, subcutaneous (s.c.), or intravenous routes resulted in transient increases in circulating concentrations of GH (Buonomo et al., 1991). Recently, SD-33 was found to increase feed intake in adult sheep 48 h after intravenous administration, but this effect was lost when the animals were challenged with lipopolysaccharide (Obese et al., 2007). In these 3 studies, the effects of SD-33 relating to analgesia, circulating GH concentrations, and appetite were all blocked by naloxone, indicating that they were mediated at least in part through the µ-opioid receptor. The discrepancy in the timing of effects (immediate analgesia and stimulation of GH, but 48 h before significant increases in feed intake) is not yet understood.

The recently weaned pig often exhibits decreased feed intake, increased susceptibility to disease, and poor growth (Matteri et al., 2000; Kojima et al., 2007, 2008). These responses to the stress of weaning often manifest as a growth lag but can be manifested as more severe morbidity and even mortality, particularly in conditions of suboptimal herd health or management. Through its actions on appetite, SD-33 may offer some protection during the weaning process, increasing overall health and well-being during this critical period.

Two experiments were conducted to investigate the potential for SD-33 to ameliorate the postweaning
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growth decline in recently weaned pigs. A preliminary experiment focused on the effects of SD-33 on feed intake and growth, whereas the second experiment investigated acute effects of SD-33 on the growth, stress, and immune axes.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the University of Tennessee Animal Care and Use Committee.

Animals and Diets

Crossbred pigs (Landrace, Duroc, Hampshire) were farrowed in standard farrowing pens and processed according to usual University of Tennessee Experiment Station practice at 4 to 7 d of age. Procedures included needle teeth clipping, tail docking, iron supplementation, ear tagging, and castration of males. Pigs were kept in farrowing pens with their dams until weaning, with creep feed (Diet 554PE, Tennessee Farmers Cooperative, LaVergne, TN) available 10 d after birth.

Experimental Design

Exp. 1. On d 0, 12 pigs (6 barrows and 6 gilts, 24 ± 1 d of age, 7.08 ± 0.22 kg) were weighed and allocated by sex and BW to the following treatment groups: SD-33, receiving 0.5 µmol/kg of SD-33 (Bachem, Torrance, CA) in saline by a single intramuscular (i.m.) injection of 0.5 mL or less; or vehicle (VEH), receiving a single i.m. injection of 0.5 mL of saline. Pigs were removed from their sows, administered their respective treatments, mixed, and subjected to a 3-h transport according to usual University of Tennessee Experiment Station practice. Procedures included needle teeth clipping, tail docking, iron supplementation, ear tagging, and castration of males. Pigs were kept in farrowing pens with their dams until weaning, with creep feed (Diet 554PE, Tennessee Farmers Cooperative, LaVergne, TN) available 10 d after birth.

Blood Collection and Analyses

For hormone assays, 3 mL of blood during the serial sampling period was collected into heparinized (90 USP sodium heparin) tubes and centrifuged at 2,000 × g for 10 min at 4°C and the plasma stored at –20°C until analyzed for GH and cortisol. For WBC count and differential, blood was collected in tubes spray-coated with 5.4 mg of K2 EDTA and immediately shipped on ice to a commercial clinical laboratory (Vet Path Labs, Tulsa, OK). Reported are total WBC concentration (WBC/µL); the concentration of neutrophils, lymphocytes, and monocytes; and the percentage of neutrophils, lymphocytes, and monocytes relative to total WBC concentration.

Plasma total cortisol concentration was determined by RIA as previously reported (Scroggs et al., 2002). Cortisol concentration was expressed as nanomoles per liter. Intra- and interassay CV were 6.6 and 12.9% for low (110 nmol/L), 8.3 and 4.8% for medium (325 nmol/L), and 5.5 and 7.3% for high (772 nmol/L) cortisol quality control standards (Con6 Multivalent Control Module, Diagnostic Products, Los Angeles, CA), and 4.5 and 9.0% for a pooled pig plasma sample. The sensitivity for the assay was 5.5 nmol/L. Plasma GH concentration (ng/mL) was determined by a commercially available RIA specific for porcine GH (Linco, St. Charles, MO). Intra- and interassay CV were 17.6 and 19.8% for low (4.2 ng/mL), and 14.1 and 17.9% for high (28.9 ng/mL) GH quality control standards provided within the assay kit. The sensitivity of the assay was 1 ng/mL.

Statistical Analyses

Variables were analyzed with mixed model ANOVA, using a model for a randomized block design. For Exp. 1, pen was the experimental unit for feed intake, and pig was the experimental unit for BW. In Exp. 2, pig was the experimental unit. For all variables except cumulative feed intake and BW gain, the model included treatment as a main effect with repeated measures. Square root transformations were performed on GH and cortisol data to preserve homogeneity of variance. For GH and cortisol, only animals with a complete set of data were included in the analysis (VEH: n = 7; SD-33: n = 4) because several cannulae failed during the sampling period. Least squares means were compared using Fisher’s protected LSD. A significance level of P < 0.05 was used for all testing; trends in which P < 0.10 were also reported. All graphical and textual descriptions of results are reported as raw means and SE. For Exp. 2, preliminary analysis detected no replicate effect, so replicate was removed from the model.
RESULTS

Feed Intake and Growth

Exp. 1. Treatment of pigs with a single injection of SD-33 at the time of weaning resulted in increased \( P < 0.01 \) daily feed intake (Figure 1A). Cumulatively, a trend was observed \( P = 0.06 \) such that pen intake over 7 d postweaning in SD-33 pens \( (10.68 \pm 0.58 \text{ kg}) \) was greater than in VEH pens \( (7.93 \pm 0.41 \text{ kg}; \text{data not shown}) \). Although weaning resulted in a loss of BW on d 1 for both treatment groups, BW did not differ between the 2 treatment groups at any time (Figure 1B).

Exp. 2. No increase in daily feed intake was noted in SD-33-treated animals through d 4 postinjection relative to VEH pigs (Figure 2A). Cumulative feed intake over this same period was not different between SD-33 pigs \( (1.14 \pm 0.16 \text{ kg}) \) and VEH pigs \( (0.89 \pm 0.11 \text{ kg}; \text{data not shown}) \). There were no differences in BW due to treatment at any time (Figure 2B). Cumulative BW gain over 4 d postinjection did not differ between SD-33 pigs \( (0.81 \pm 0.15 \text{ kg}) \) and VEH pigs \( (0.96 \pm 0.09 \text{ kg}; \text{data not shown}) \).

Plasma GH and Cortisol

A transient increase \( P < 0.05 \) in plasma concentrations of GH was observed at 1 and 1.5 h postinjection in SD-33 pigs relative to VEH (Figure 3A). By 2 h postinjection, GH concentrations were not different between treatment groups and remained so for the re-
Circulating WBC Populations

Overall WBC concentrations, as well as individual populations of neutrophils, lymphocytes, and monocytes, were elevated on d 1 to 4 postinjection relative to preinjection concentrations in SD-33 and VEH pigs (Figure 4A to D). A trend \((P = 0.07)\) was noted in which monocyte numbers were increased in SD-33 pigs relative to VEH pigs on d 1 to 4 postinjection (Figure 5D). This increase in monocytes resulted in a decrease \((P < 0.05)\) in the percentage of WBC that were lymphocytes in SD-33 pigs \((31.88 \pm 4.76)\) relative to controls \((47.87 \pm 5.49)\) at 2 d postinjection (Figure 5B).

DISCUSSION

The inhibition of piglet growth rate caused by weaning is well recognized and clearly associated with reduced feed intake (Bark et al., 1986; McCracken et al., 1995). In our preliminary experiment (Exp. 1), a clear and robust increase in feed intake was seen in weaned and transported pigs that received a single injection of SD-33 at weaning. In agreement with observations made in adult sheep (Obese et al., 2007), this increase did not manifest until 2 d after administration. The continued increase in feed intake throughout the experiment is intriguing. Although it is possible that this may arise out of a long-term stimulatory action of SD-33 on appetite, the mechanism behind the prolonged effect is unclear. In Exp. 2, no significant increase in feed intake was noted in treated animals, although a numerical pattern was evident. The pigs in Exp. 2 were administered SD-33 the day after they were weaned and cannulated, and these pigs were not subjected to transportation. The amount, duration, and type of stress experienced by the pig may all modulate the physiological response to SD-33.

As a µ-opioid agonist, SD-33 may display a range in the amplitude and duration of effect based on the level of stress present when the treatment is given. Obese et al. (2007) observed that the increase in feed intake by SD-33 was blocked by naloxone, indicating that the effect was mediated through µ-opioid receptors. Appetite is stimulated by µ-opioid agonists in many ways. They upregulate activity of the appetite stimulators agouti-related peptide (AGRP; Hagan et al., 2001; Brugman et al., 2002) and neuropeptide Y (NPY; Kotz et al., 1993; Pomonis et al., 1997; Dodo et al., 2005), and decrease synthesis of the appetite suppressor α-melanocyte stimulating hormone (α-MSH), a product of the pro-opiomelanocortin gene (Wardlaw et al., 1996). Opioids also act to decrease expression of the receptor, which binds α-MSH and AGRP, the melanocortin 4 receptor (Chaki and Okuyama, 2005). Stress also regulates the expression of many of these regulators of appetite. Kas et al. (2005) observed that in rats, AGRP mRNA expression in the arcuate nucleus is decreased after a stressful event, contributing to increased sensitivity for α-MSH and subsequent decreased feed intake. Liu et al. (2007) observed that forced swimming and restraint increased pro-opiomelanocortin gene expression and suppressed feeding, but pretreatment with an MC4R antagonist blocked the anorectic and anxiogenic effects of these stressors, indicating that the melanocortinergic pathway is heavily involved in stress-related anorexia. Taken together, these observations indicate that the effectiveness of SD-33 in increasing feed intake may depend on the severity of the stress the animal is experiencing at that time. Experiments are currently underway to determine if a better orexigenic response may be observed if pigs are treated with SD-33 some days before weaning.
Although feed intake was increased by SD-33 in Exp. 1, there was no accompanying increase in BW; similarly, no difference in BW due to treatment was observed in Exp. 2. Increased energy from enhanced feed intake may be partitioned toward some physiological activity other than growth because opioids have actions on many systems, including increased lipolysis in the human (Vettor et al., 1993) and rabbit (Richter et al., 1983) and increased metabolism through enhanced release of triiodothyronine and thyroxine in rats (Tal et al., 1984; Baumgartner et al., 1998). In the case of the newly weaned pig, feed efficiency is not as much of an issue as is health and general well-being, particularly for the first few days after weaning.

Circulating concentrations of GH were transiently elevated in pigs given a single i.m. injection of SD-33. Previously, Buonomo et al. (1991) observed immediate increases in GH concentrations in wethers (given 0.05, 0.10, or 0.20 µmol of SD-33/kg intravenously), rats (given 0.5, 1.0, or 2.0 µmol of SD-33/kg s.c., and 50-kg barrows (given 0.50 µmol of SD-33/kg s.c.). This effect was also observed when barrows were given SD-33 orally, but the increases were less intense and were delayed (the peak was seen 30 to 90 min postinjection, depending on the dose). In the present experiment GH concentrations in pigs given SD-33 were markedly elevated at 1 and 1.5 h postinjection. The amplitude of the increase was similar to that reported for barrows given SD-33 s.c., but the timing of the effect was more similar to that observed with an oral dose. Clearly, route of administration may alter the profile of acute response.

Opioids are known to stimulate GH secretion in rats when given centrally (Chihara et al., 1978) or peripherally (Kriskich et al., 1986). Vaccarino and Taub (1997) confirmed that this was through a central mechanism involving the activation of hypothalamic neurons containing GHRH. Interestingly, GHRH functions not only at the pituitary gland to drive GH secretion, but also centrally to increase feed intake (as reviewed by Vaccarino and Taub, 1997). There is an anatomical relationship between GHRH- and NPY-containing neurons in the median eminence, indicating that GHRH may regulate NPY synthesis or release (Deltondo et al., 2008).

Cortisol is the primary glucocorticoid released during times of stress in the pig. Cortisol concentrations are normally increased for at least the first 24 h postweaning, presumably due to the stress associated with transportation, social mixing, and maternal separation (Kojima et al., 2008; Cooper et al., 2009). The baseline cortisol values in the current study (1 d postweaning) represent that elevated stress status. Treatment with

![Figure 4](image-url)
A single i.m. injection of SD-33 resulted in a transient increase in circulating cortisol concentrations 3 to 4 h postinjection. It is unclear why the increases in cortisol were observed 2 h later than when the effects of SD-33 on GH became apparent.

The effects of exogenous opioids on the hypothalamic-pituitary-adrenal (HPA) axis appear to be species-dependent. In humans (Pechnick, 1993) and monkeys (Pascoe et al., 2008), µ-opioid receptor agonists inhibit or have no effect on HPA activity, but in rodents they will stimulate the HPA axis (Nikolarakis et al., 1987). In monkeys, κ-opioid receptor agonists stimulate HPA activity (Pascoe et al., 2008), possibly through central regulation of corticotropin-releasing hormone (CRH; Buckingham and Cooper, 1986; Nikolarakis et al., 1987). Cortisol concentrations were elevated in postpubertal gilts after treatment with the µ-opioid receptor antagonist naloxone, indicating that µ-opioid receptor agonists act to suppress HPA activity in pigs (Barb et al., 1986). Administration of morphine and fentanyl (both µ-opioid receptor agonists) during a surgical procedure resulted in decreased postoperative cortisol concentrations relative to controls in young (20 kg) pigs (Malavasi et al., 2006), but it is unclear if this was due to a direct effect of the opioids or a response to differing levels of postoperative pain (an indirect effect due to the analgesic properties of the opioids). In our study, SD-33 increased circulating cortisol concentrations for approximately 1 h, but the effect was not observable until 3 h postinjection. This may be a reflection of the time needed to translate the stimulatory effect of an opioid on CRH in the hypothalamus (Yamauchi et al., 1997) through an increase in adrenocorticotropic hormone from the pituitary gland before the end result, stimulation of cortisol release from the adrenal glands, can be observed.

Glucocorticoids can have a stimulatory effect on feed intake, particularly if chronically overexpressed as in Cushing’s disease. Di et al. (2003) postulated the existence of a rapid negative feedback of glucocorticoids on CRH-containing neurons involving the release of endocannabinoids, which act to increase feed intake (reviewed by Dallman, 2003). This may indicate yet another possible mechanism by which SD-33 acts to increase feed intake.

We observed increases in WBC, neutrophils, lymphocytes, and monocytes over time in syndyphalin and VEH treatment groups. Previously, we have shown that circulating immune cell populations increase at 1 d postweaning and return to preweaning levels by 7 d postweaning (Kojima et al., 2008; Cooper et al., 2009), but we had not monitored these populations at any time in between. It would appear from our current data that immune cell populations continue to increase for several days postweaning. This may indicate prolonged inhibition of chemotaxis by cortisol and a progressive accumulation of cell numbers.

Opioids are known to have immunomodulatory functions, and cells of the immune system (neutrophils,
monocytes, and lymphocytes) express opioid receptors, as reviewed by Finley et al. (2008). Grimm et al. (1998) reported that endogenous met-enkephalin and morphine induced monocyte chemotaxis, but inhibited chemokine-induced chemotaxis of human neutrophils; these responses were blocked by naloxone. Recent evidence (Finley et al., 2008) indicates that activation of κ-opioid receptors may induce an anti-inflammatory response, whereas activation of μ-opioid receptors induces a proinflammatory response. Naloxone, although primarily known as a μ-opioid receptor agonist, also antagonizes κ- and δ-opioid receptors. Although most of the published data indicate that SD-33 is a μ-opioid, the possibility that this molecule may also bind one of the other opioid receptor subtypes must be considered.

The synthetic enkephalin SD-33 has potential to be used as an agent to increase feed intake and decrease the negative effects of stress during weaning in pigs, but further investigation is needed to better understand the timing of the effect and to rule out any immunosuppressive effects that would be detrimental to the well-being of the animal.

LITERATURE CITED


