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Y1 Receptor Activation is Involved in the Effect of Exogenous Neuropeptide Y on Pup Growth and the Early Termination of Lactational Diestrus in the Postpartum Rat

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Abstract

The effect of chronic administration of exogenous neuropeptide Y (NPY) and specific NPY receptor agonists and antagonists on reproductive function was examined in lactating rats. As previously demonstrated in our laboratory, chronic (7-day) intracerebroventricular (i.c.v.) NPY infusion (6 μg/day) from days 8–15 postpartum (pp) caused a significant decrease in milk production and an early termination of lactational diestrus. Similar application of the mixed Y1/Y4/Y5 receptor agonist (Leu^{31}, Pro^{34}) NPY (at 3, 6 and 9 μg/day) reproduced the effect of chronic NPY infusion on milk production in a dose-independent manner. Consistent with this effect, the potent Y1 antagonist/Y4 agonist, 1229U91, given concomitantly with NPY eliminated the decline in milk production. The Y2 receptor agonist, $NPY_{13–36}$, had no effect on milk production at any of the doses used. Length of lactational diestrus was reduced following administration of the Y2 agonist at 18 μg/day but not at 9 μg or 27 μg/day whereas (Leu^{31}, Pro^{34}) NPY infusion had no effect on this parameter at any of the doses used. However, the group that was treated with NPY plus 1229U91 exhibited the usual length of lactational diestrus, indicating that there is at least some Y1 involvement in the effects of NPY on lactational infertility. To test the possibility that the effects of NPY infusion are mediated through changes in circulating prolactin and progesterone, plasma concentrations of these hormones were measured on day 15 pp in NPY-, (Leu^{31}, Pro^{34}) NPY- and vehicle-treated females. NPY-infused females had lower plasma prolactin concentrations than vehicle-infused dams but progesterone concentrations were similar across groups. Overall, these data indicate that chronic exogenous NPY-infusion in lactating females disrupts milk production and shortens lactational diestrus, most likely through reducing prolactin secretion, and that this effect is mediated via Y1 receptor activity.

Neuropeptide Y (NPY), a 36-amino-acid member of the pancreatic polypeptide family, is the most widely distributed neuropeptide in the mammalian nervous system (1). Among its repertoire of functions, NPY is involved in the modulation of energy balance and in the control of the hypothalamic-pituitary-gonadal axis.

NPY administration stimulates increases in food intake (2), reduces the thermogenesis of brown adipose tissue, while causing concomitant increases in white fat storage (3), and attenuates sympathetic activation (4). In this way, NPY acts to replenish available energy stores and reduce energy expenditure. Accordingly, physiological states that produce heavy demands on energy resources, such as the reduced availability of food or the production of milk during lactation, are accompanied by significant elevations in central NPY (5–8). In particular, these increased energy demands cause elevated levels of NPY mRNA in the arcuate nucleus of the hypothalamus, along with increased NPY peptide in the terminal fields projecting from the arcuate nucleus to such areas as the paraventricular and the medial preoptic nuclei (MPOA) of the hypothalamus (9, 10). Interestingly, the elevated levels of hypothalamic NPY induced by changes in metabolic energy demands are additive. For example, when the energy demands of lactation are combined with food restriction, the increased NPY levels usually observed in the lactating rat are further exaggerated (8, 11).

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NPY has both stimulatory and inhibitory effects on the reproductive axis depending on background steroid levels. For example, when oestrogen and progesterone are elevated, as on the afternoon of proestrus, a bolus injection of NPY facilitates lutenizing hormone (LH) release. However, when gonadal steroids are low, as in ovariectomized females or castrated males, NPY inhibits the release of LH (12–14). In addition, chronic NPY infusion disrupts cyclicity in mature female rats and delays the onset of puberty in prepubescent rats of both sexes, suggesting that continuous high levels of NPY act to suppress the release of LH (15–17).

Previous studies in our laboratory have demonstrated that lactating rats restricted to 50% of *ad libitum* food levels for the first 2 weeks postpartum (pp) show a significant increase in the length of lactational diestrus (18). This extended period of lactational infertility, which is accompanied by decreased secretion of LH, and increased circulating progesterone, but no changes in prolactin or ACTH secretion (19), is also associated with high levels of hypothalamic NPY. These data are consistent with the hypothesis that lactation-induced increases in NPY, exacerbated by food restriction, play a role in extending lactational diestrus in food-restricted lactating rats. However, subsequent experiments showed that chronic exogenous NPY administration, over a period of 7 days, to lactating females fed *ad libitum* results in a profound decline in milk production, accompanied by a significantly shorter period of lactational diestrus.

In the present set of experiments we replicate our previous results with chronic NPY infusion in lactating females and begin the identification of the particular NPY receptor subtype(s) responsible for these effects. To achieve this, NPY and two NPY receptor agonists: a mixed Y1/Y4/Y5 receptor agonist, (Leu^{31}, Pro^{34}) NPY, and a selective Y2 receptor agonist, ($NPY_{13–36}$), or a combination of NPY and the mixed Y1 antagonist/Y4 agonist 1229U91 were chronically infused into the lateral ventricle of lactating rats from days 8–15 pp. The effect of these treatments on pup litter growth and the length of lactational diestrus was measured. Additionally, given the role of prolactin in milk production (20), as well as its involvement in maintaining lactational diestrus (21), we tested the hypothesis that chronic NPY infusion in lactating dams reduces litter weight gain by inhibiting suckling-induced prolactin release and reducing serum progesterone levels.

Materials and methods

Animals

Virgin female Wistar rats, weighing 220–250 g, were obtained (Charles River, St Constant, Quebec, Canada) and group housed at our facility. Rats were maintained on a 12 : 12 h light/dark schedule (lights on at 08.00 h) at a constant room temperature of 20 ± 2 °C, with *ad libitum* access to food and water. Females were mated with males at our facility. Three weeks later, pregnant females were placed in individual plexiglass cages. The day of birth was designated as day 0. On day 1 pp, females were placed in one of the experimental groups and all litters were culled to eight pups. All manipulations were carried out according to the guidelines of the Canadian Council of Animal Care.

Compounds

Human/rat $NPY_{2–36}$, $NPY_{13–36}$ (Bachem, Torrence, CA, USA) (Leu^{31}, Pro^{34}) NPY (Sigma Chemical Co., St Louis, MO, USA), and 1229U91 (a generous gift from Dr A. Daniels, of Glaxo Wellcome, North Carolina, USA) were individually dissolved in a 0.04 M phosphate buffer vehicle containing 0.15 M NaCl, 0.01% ascorbic acid and 0.2% bovine serum albumin. Drugs were administered using Alzet osmotic minipumps (model 2001, Alza Co., Palo Alto, CA, USA) with an infusion rate of 1 µl/h. Minipumps and plastic tubing (inner diameter 0.69 mm, outer diameter 1.14 mm) were connected to 22-gauge L-shaped cannulae (Plastic One, Richmond, VA, USA) and primed by being placed in a tube containing sterile saline solution in a Haake incubator at 37 °C for 6 h before implantation.

Intracerebraventricular (ICV) minipump implantation

On day 8 pp dams were anaesthetized (6 mg ketamine/1.1 mg xylazine per 100 g of body weight) and cannulae were inserted into the right lateral ventricle (coordinates: anterior–posterior 0.02; lateral 0.16; vento–medial 0.5; nose bar set at 5 mm above interaural line). Cannulae were secured to the skull with jeweler's screws and dental cement. Minipumps were inserted subcutaneously in the neck region and the antibiotic powder, Cicatrin (Burroughs Wellcome inc., Kirkland, Quebec, Canada) was applied before suturing in order to prevent infection. Seven days after surgery, minipumps were removed under Metophane (Janssen Pharmaceuticals, Mississauga, Ontario, Canada) anaesthesia. Cicatrin was applied and the wound was closed using staples.

Data collection

In experiments 1 and 2, food intake, dam and litter weights were recorded daily beginning on day 1 pp. Food intake was measured until day 17 pp when pups started to feed independently and the other measures taken for 26 days. Vaginal smears were obtained daily beginning on day 4 pp. The smears were rated for the number of cornified epithelial cells by two independent judges. When 70% of the cells present on the slide were of the cornified type, the female was considered to be in oestrus. This day marked the end of lactational diestrus. Observations of maternal behaviour were made before and after data collection on each day of the experiment. In experiment 3, dam and litter weight data were collected for 16 days beginning on day 1 pp.

Plasma prolactin and progesterone concentrations

On day 13 pp (5 days after minipump implantation) one group of females was anaesthetized as described above, and implanted with jugular catheters (Silastic, Dow Corning Corp., Midland, MI, USA). After implantation, each rat was given a bolus injection (350 µl) of 0.9% saline solution containing 1% heparin (stock is 1000 IU/ml). On the day of testing, day 15 pp, the jugular catheter was extended using 60–70 cm of PE50 tubing (Clay Adams, Parsippany, NJ, USA), and following a 60-min waiting period, blood samples (300 µl) were taken every 10 min for a period of 1 h. Blood volume was replaced after each sample with 275 µl of a 0.9% saline solution (without heparin). Blood samples were kept on ice, centrifuged at 12 000 r.p.m. for 3 min, and stored at -20 °C before prolactin, and progesterone hormone assays.

Hormone assays

Plasma prolactin and progesterone concentrations were measured using specific radioimmunoassays from Amersham (Baie d'Urfe, Quebec, Canada) and ICN (Montreal, Canada).

Cannulae placement

Following completion of the experiment, females were given an overdose of sodium pentobarbital and perfused transcardially with saline followed by 10% formol saline. The brains were sliced into 50-µm sections and the tips of the cannulae located. Rats in which the cannulae were found to be misplaced were removed from all subsequent analyses.

Statistical analysis

Data for length of lactational diestrus were analysed by one-way between groups ANOVA. To obtain a measure of litter growth the average litter weight gain was calculated for each dam for each day of treatment. A mixed ANOVA with group as the independent measure and day as the repeated measure was used to assess the effects of peptide administration on litter growth. For maternal food intake and body weight change, a mixed ANOVA was also used with group as the independent measure and day of treatment as the repeated measure. For these analyses, the day of cannula implantation and the day of pump removal were omitted to eliminate the effects of surgery on these

parameters. These analyses were followed by post-hoc tests (Fisher's PLSD) where appropriate. Plasma prolactin and progesterone concentrations were compared between groups using one-way ANOVA. $P<0.05$ was considered statistically significant for all tests.

Experimental design

Experiment 1

The purpose of this experiment was to replicate the effect of chronic exogenous NPY infusion on the length of lactational infertility, and begin the determination of receptor subtype involved. Thus, lactating rats on day 8 pp were assigned to one of four groups: NPY (6 μg/day); (Leu^{31}, Pro^{34}) NPY (6 μg/day); $NPY_{13–36}$ (18 μg/day), or vehicle, and were infused for 7 days. A separate group of nonoperated controls (Noc) was included in this experiment to assess the effect of the surgical procedure. Doses of the (Leu^{31}, Pro^{34}) NPY and $NPY_{13–36}$ for this experiment were determined based on the affinity of these agonists for their respective receptors in comparison with that of the natural ligand NPY. Groups did not differ with respect to litter weight gain, food intake, or body weight before the onset of treatment.

Experiment 2

To determine the dose–response relationship for the effects of (Leu^{31}, Pro^{34}) NPY and $NPY_{13–36}$, additional studies were undertaken. In Study A, lactating females were chronically infused with (Leu^{31}, Pro^{34}) NPY (3 or 9 μg/day) or vehicle. An additional group of lactating females chronically infused with a cocktail containing NPY and the mixed Y1 antagonist/Y4 agonist, 1229U91 (at 6 μg/day and 20 μg/day, respectively), was also included in this study. There were no differences among the groups with respect to body weight or litter weight gain before treatment, but subsequent analyses revealed that females in the 1229U91/NPY group ate more on days 1–7 compared to those in the other three groups; thus, the effect of treatment on food intake in this study was assessed by analysing change in food intake from the pretreatment baseline. Females in Study B were infused with $NPY_{13–36}$ (0, 9 or 27 μg/day). As in experiment 1, groups did not differ with respect to litter weight gain, or body weight before the onset of treatment.

Experiment 3

The effect of chronic NPY and (Leu^{31}, Pro^{34}) NPY infusion on serum prolactin and progesterone levels was measured in lactating females on the morning of day 15 pp, after 7 days of drug infusion. This study comprised three groups of lactating females assigned to one of three treatment groups: two groups infused with either NPY (6 μg/day) or (Leu^{31}, Pro^{34}) NPY (6 μg/day) and a vehicle-infused group. Blood samples were taken every 10 min for a period of 1 h. Prolactin concentrations were measured for each sample and then averaged over the six samples collected for statistical analysis. Progesterone concentrations were measured in samples pooled from the six collected.

Results

Experiment 1

Figure 1 shows the average daily weight gain (g) during treatment of litters nursed by females infused with NPY (6 μg/day), the Y1/Y4/Y5 receptor agonist (Leu^{31}, Pro^{34}) NPY (6 μg/day), the Y2 receptor agonist $NPY_{13–36}$ (18 μg/day) or vehicle together with nonoperated controls. Analysis of these data showed a significant group effect [$F(4,31)=4.99$; $P<0.05$] but neither a significant effect of days nor a significant group–time interaction. Post-hoc pairwise analyses showed that pups nursed by females in the NPY-treated group gained less weight than those nursed by females in the $NPY_{13–36}$, vehicle or nonoperated control groups ($P<0.05$). Pups nursed by females in the (Leu^{31}, Pro^{34}) NPY-treated group gained less weight than those nursed by females in the vehicle or nonoperated control groups ($P<0.05$).

As can be seen in Fig. 2, overall there was a significant effect of treatment on length of lactational diestrus [$F(4,31)=7.80$; $P<0.05$]. Post-hoc analysis showed NPY-infused females had a significantly shorter length of lactational diestrus than any other group. The length of lactational diestrus was also significantly shorter in Y2 agonist-infused females than in vehicle-infused females ($P<0.05$).

Table 1 shows average daily food intake and weight change during treatment for all experimental groups. As expected, all

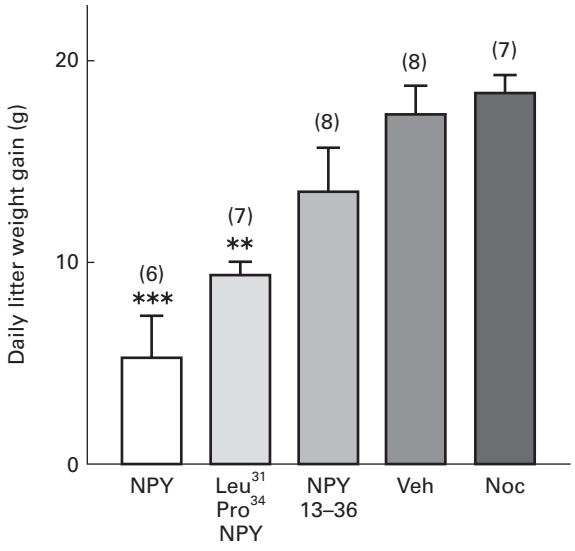

FIG. 1. Average daily litter weight gain for litters nursed by females treated with either neuropeptide Y (NPY) (6 μg/day) (Leu^{31}, Pro^{34}) NPY (6 mg/day), $NPY_{13–36}$ (18 μg/day), or vehicle. Noc, nonoperated controls. ***Significantly different from both vehicle and Noc; **significantly different from vehicle; *significantly different from Noc.

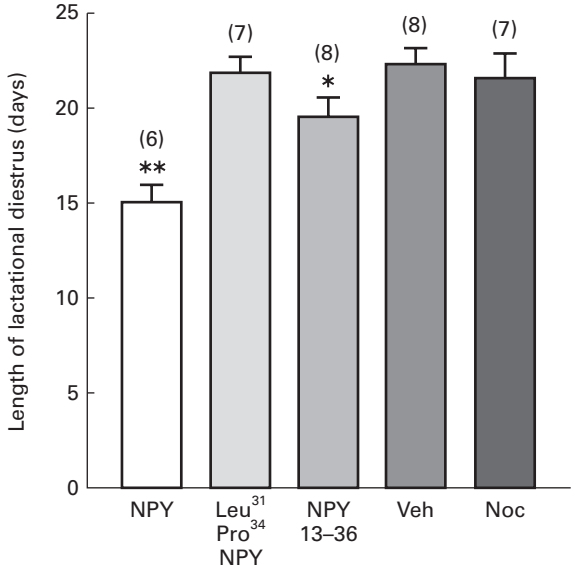

FIG. 2. Length of lactational diestrus in lactating females treated with either neuropeptide Y (NPY) (6 μg/day) (Leu^{31}, Pro^{34}) NPY (6 μg/day), $NPY_{13–36}$ (18 μg/day), or vehicle. Figure includes nonoperated controls (Noc). **Significantly different from vehicle and Noc; *significantly different from vehicle.

TABLE 1. The Effect of Treatment on Daily Food Intake (Days 9–14 pp) and Daily Maternal Weight Change (Days 9–14 pp) in Experiment 1.

	NPY (6 μg/day)	(Leu^{31}, Pro^{34}) NPY (6 μg/day)	NPY$_{13–36}$ (18 μg/day)	Vehicle	Nonoperated controls
Food intake ± SE (g)	54.97 ± 0.96	57.04 ± 1.65	52.97 ± 2.55	57.04 ± 2.42	57.64 ± 1.90
Weight change ± SE (g)	–0.04 ± 1.23	0.58 ± 1.14	3.68 ± 1.07	1.22 ± 1.07	1.04 ± 1.14
n	6	7	8	8	7

females ate more over the second week postpartum resulting in a significant main effect of days [$F(5,155) = 9.15$; $P < 0.05$]. Neither the main effect for treatment nor the treatment–days interaction reached statistical significance. When daily maternal weight change was analysed, there were no significant effects of treatment, days or the treatment–days interaction.

Experiment 2

Study A

Daily weight gain of litters nursed by females infused with either 3 or 9 μg/day of the mixed NPY receptor agonist (Leu^{31}, Pro^{34}) NPY, or a combination of NPY (6 μg/day) and 1229U91 (20 μg/day), and vehicle-infused females is shown in Fig. 3. ANOVA showed a significant group effect [$F(3,20) = 11.90$; $P < 0.05$] that was modified by a significant day–group interaction [$F(3,21) = 4.71$, $P < 0.05$]. Post-hoc analysis revealed that, overall, litters from dams infused with both doses of (Leu^{31}, Pro^{34}) NPY gained less weight than those of vehicle-infused dams and those infused with NPY/1229U91. Average daily weight gain did not differ between litters nursed by NPY/1229U91 and vehicle-infused dams. These effects were apparent on all days except day 9 pp.

There was no difference in the length of lactational diestrus among treatment groups (Table 2). However, there was a significant effect of both treatment and days on food intake [$F(3,20) = 3.69$, $P < 0.05$; $F(5,105) = 14.00$, $P < 0.05$, respectively] and post-hoc analyses revealed that vehicle-treated rats increased their food intake above pretreatment levels more than rats treated with the low dose of the Y1 agonist or with the combination of NPY and 1229U91 (Table 2). Analysis of the maternal body change data yielded no significant main effect of treatment but a significant effect of days and of the interaction between group and days [$F(5,105) = 4.12$, $P < 0.05$; $F(5,15) = 2.72$, $P < 0.05$, respectively]. Further investigation of these effects showed that groups differed in their weight gain on days 9 and 12 pp. On day 9, females in the vehicle group gained more weight than those in the group receiving the high dose of the Y1 agonist or the combination of NPY and 1229U91. On day 12 pp, the females infused with either the vehicle or the low dose of the Y1 agonist gained more weight than those infused with a combination of NPY and 1229U91.

Study B

Results from this study are shown in Table 3. Daily litter weight gain for pups nursed by females treated with two doses (9 or 27 μg/day) of the Y2 receptor agonist NPY$_{13–36}$, or vehicle showed no effect of treatment, days or their interaction. Similarly, there was no effect of treatment on length of lactational diestrus daily maternal weight change or food intake. As expected, however, females did increase there food intake over the course of treatment, resulting in a significant effect of days on the latter parameter [$F(5,100) = 13.14$, $P < 0.01$].

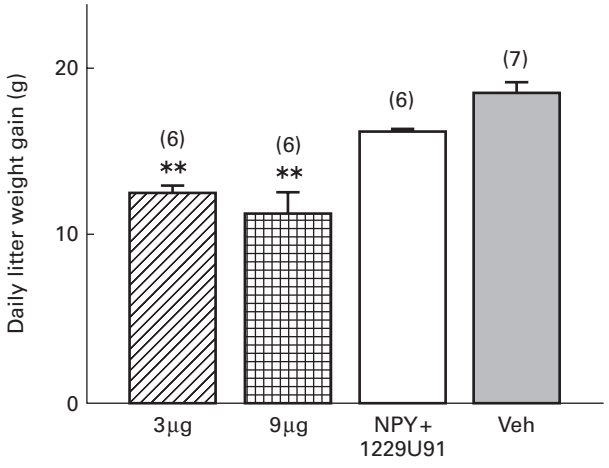

FIG. 3. Average daily litter weight gain for litters nursed by females treated with 3 μg or 9 μg/day of (Leu^{31}, Pro^{34}) neuropeptide Y (NPY), or a mixture of NPY/1229U91 (6 and 24 μg/day, respectively), or vehicle. **Significantly different from vehicle, and from NPY/1229U91.

Experiment 3

Mean plasma prolactin and progesterone concentrations for each treatment group are shown in Fig. 4. The average plasma prolactin concentrations over a period of 1 h were significantly lower in NPY- than in vehicle-infused dams ($P < 0.05$). Plasma progesterone concentrations did not differ among the groups.

No differences in maternal behaviour were observed in any of the experiments.

Discussion

In agreement with earlier studies from our laboratory, the results of experiment 1 demonstrate that chronic i.c.v. infusion of exogenous NPY to lactating rats fed *ad libitum* results in a profound reduction in litter weight gain together with a significant reduction in length of lactational diestrus. Administration of the Y2 agonist NPY$_{13–36}$ had no significant effect on litter weight gain over the range of doses used here,

TABLE 2. The Effect of Treatment on Length of Lactational Diestrus, Daily Food Intake (Days 9–14 pp) and Daily Maternal Weight Change (Days 9–14 pp) in Experiment 2A.

	(Leu^{31}, Pro^{34}) NPY (3 μg/day)	(Leu^{31}, Pro^{34}) NPY (9 μg/day)	1229U91/NPY	Vehicle
Lactational diestrus ± SE (days)	22.0 ± 1.53	20.0 ± 1.15	21.9 ± 1.08	21.8 ± 1.01
Food intake ± SE (g) (change from pretreatment)	20.9 ± 1.63	23.8 ± 1.93	19.3 ± 1.84	26.9 ± 1.70
Weight change ± SE (g)	4.1 ± 0.76	4.0 ± 1.29	4.5 ± 1.21	2.7 ± 0.86
n	6	6	6	7

TABLE 3. The Effect of Treatment on Length of Lactational Diestrus, Daily Litter Weight Gain, Daily Food Intake (Days 9–14 pp) and Daily Maternal Weight Change (Days 9–14 pp) in Experiment 2B.

	$NPY_{13–36}$ (9 μg/day)	$NPY_{13–36}$ (24 μg/day)	Vehicle
Lactational diestrus ± SE (days)	20.0 ± 0.93	20.8 ± 1.08	21.9 ± 0.67
Daily litter weight gain ± SE (g)	17.9 ± 1.20	16.4 ± 1.18	16.8 ± 1.30
Food intake ± SE (g)	58.0 ± 1.90	58.2 ± 1.79	57.2 ± 2.03
Weight change ± SE (g)	5.6 ± 0.89	4.5 ± 1.19	3.6 ± 0.87
n	8	8	7

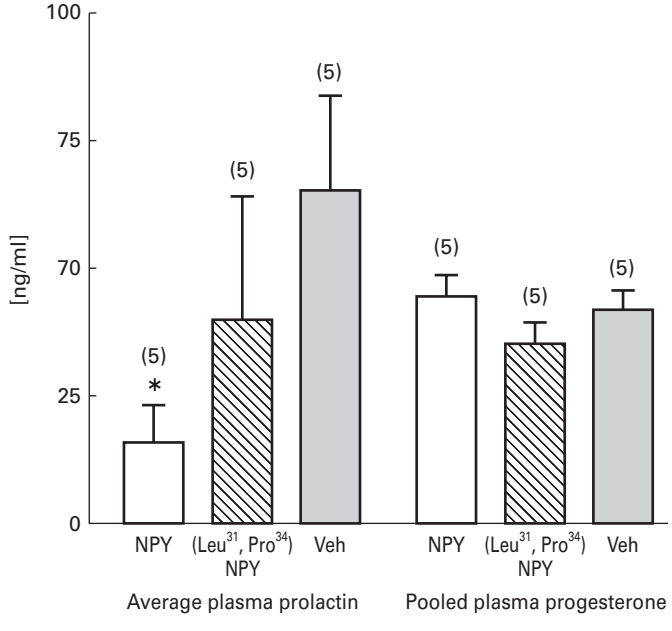

FIG. 4. Average plasma prolactin and pooled progesterone concentrations of neuropeptide Y (NPY) (Leu^{31}, Pro^{34}) NPY- and vehicle-infused lactating females. *Significantly different from vehicle.

whereas treatment with (Leu^{31}, Pro^{34}) NPY, a mixed Y1/Y4/Y5 receptor agonist, reduced pup growth in a dose-dependent fashion. Moreover, the NPY-mediated decrease in pup growth was blocked when NPY was given in combination with 1229U91, a mixed Y1 antagonist/Y4 agonist. Given that NPY has little affinity for the Y4 receptor and 1229U91 has no action at the Y5 receptor, these data indicate that Y1 activation is necessary to produce the suppression of litter growth elicited by NPY infusion. The lack of any observed effect of these manipulations on maternal behaviour suggests that the effect of Y1 activation on litter growth results from a decrease in milk production and/or delivery rather than a change in mother–litter interaction.

The release of prolactin, elicited by the suckling stimulus, initiates and maintains the synthesis of milk, and serum prolactin concentrations remain high throughout most of lactation, as do progesterone levels (21, 22). Thus, the mechanism through which Y1 receptor activation by exogenous NPY and (Leu^{31}, Pro^{34}) NPY causes a decrease in milk production is likely related to the specific hormones involved in the biosynthesis of milk. Indeed, when we examined plasma prolactin and progesterone concentrations on day 15 pp in NPY and (Leu^{31}, Pro^{34}) NPY-infused females, we found a significant reduction in plasma prolactin concentrations in NPY- compared to vehicle-infused dams, and no difference in progesterone concentrations between treatment groups (Fig. 4). Although serum prolactin concentrations did not significantly differ from vehicle in (Leu^{31}, Pro^{34}) NPY-infused females, there was a trend towards lower levels in this group also.

In accordance with our present results, previous reports indicate that prolactin release can be inhibited through NPY Y1 receptor activation. A recent study found that bilateral infusions of antisense to the Y1 receptor applied directly into the MPOA of ovariectomized rats resulted in a significant increase in plasma prolactin release with no concomitant effect on either LH or follicle-stimulating hormone release (23). Another study examined the effect that tuberoinfundibular (TIDA) NPY, synthesized in this area only during the lactational period, had on prolactin release from the pituitary. Results indicated that NPY acts through Y1 receptors at the level of the lactotroph to inhibit prolactin release by interfering with intracellular Ca^{2+} mobilization (24). In agreement with these studies, a new report demonstrated that central administration of NPY or (Leu^{31}, Pro^{34}) NPY stimulates TIDA neuronal activity while concurrently depressing prolactin release (25). Together, these findings suggest that NPY can act centrally and at the level of the pituitary to inhibit prolactin release.

As well as having a role in milk production, prolactin has also been implicated in the maintenance of lactational infertility (26). Suppression of prolactin release with the D_2-like agonist bromocryptine results in a fall in progesterone concentrations and termination of lactational diestrus (19).

Hence the reduction of prolactin concentrations by NPY administration is one mechanism through which NPY administration might induce an early termination of lactational diestrus, although no effects of NPY on progesterone concentrations were observed in experiment 3.

Although the results of the studies described here point to a clear contribution of Y1 activation to the modulation of milk production, they give a less clear picture of the means through which chronic NPY infusion induces termination of lactational diestrus. Administration of the Y2 agonist only shortened the length of lactational diestrus at the mid-dose used, suggesting that this effect should be interpreted with caution. The fact that when NPY was coadministered with 1229U91, the early termination of length of lactational diestrus was eliminated, indicates that Y1 receptor activation also contributes to this facet of the effects of NPY infusion. However, the length of lactational diestrus was not affected by any of the doses of (Leu^{31}, Pro^{34}) NPY administered in these studies.

The inability of (Leu^{31}, Pro^{34}) NPY to mimic the effect of exogenous NPY on lactational diestrus might be because an insufficient dose of this ligand was used in these experiments. Another possibility might be the mixed receptor affinity of the ligand itself. Originally thought to be selective for the Y1 receptor, (Leu^{31}, Pro^{34}) NPY was later shown to activate both the Y5 and Y4 receptor subtypes (27–29). Importantly, whereas it has been shown that NPY binds to the Y1 and Y5 receptor with a similar affinity to that of (Leu^{31}, Pro^{34}) NPY, it has little affinity for the Y4 receptor (27). Thus (Leu ^{31}, Pro^{34}) NPY can be expected to activate the Y4 receptor to a much greater extent than NPY. There is evidence to support a role for Y1 receptor activation in the stimulatory effect of NPY on LH surge and it has also been suggested that activation of the Y4 receptor might inhibit the surge while stimulating basal LH secretion (30). The differential effect of NPY and (Leu^{31}, Pro^{34}) NPY administration, then, might reflect differences in balance of activation of the Y1 and Y4 receptors. Indeed, the restoration of the duration of lactational diestrus to control levels, which was observed when NPY was combined with 1229U91, could be the result of chronic Y4 stimulation as well as, or distinct from, antagonism of the Y1 receptor.

Another explanation for the disparate effects of (Leu^{31}, Pro^{34}) NPY and NPY on lactational diestrus is that activation of the Y2 receptor occurs only with NPY infusion. Evidence indicates that Y2 receptors are situated primarily at the presynaptic terminals of NPY neurones where they are responsible for short-term inhibitory feedback (31). The activation of Y2 receptors by exogenous NPY infusion would be expected to reduce endogenous release of NPY. Conversely, infusion of (Leu^{31}, Pro^{34}) NPY does not act at the Y2 receptor to modulate endogenous NPY release; hence, infusion of this ligand might result in an additive effect, coupling the activation of the Y1/Y4/Y5 receptors by (Leu^{31}, Pro^{34}) NPY with that of the endogenous ligand. Again, this could result in sustained stimulation of receptor subtypes that interfere with the facilitation of the LH surge and the termination of diestrus. Therefore, maintaining synaptic NPY levels within a particular range might be required to facilitate the early return to oestrus in NPY-infused females.

The involvement of Y5 receptor stimulation by exogenous NPY on milk production and lactational diestrus is difficult to determine in these present studies. NPY (Leu^{31}, Pro^{34}) NPY and 1229U91, are all Y5 receptor agonists, although 1229U91 only has weak affinity for this receptor subtype. The restoration of milk production and length of lactational diestrus following 1229U91 administration probably does not involve the Y5 receptor. Nevertheless, a role for Y5 receptor activation in lactational infertility cannot be ruled out and studies examining the effect of chronic Y5 stimulation are currently underway in our laboratory.

Neither the administration of NPY itself, nor of the Y1 agonist, increased maternal food intake and body weight relative to vehicle-treated dams or nonoperated controls. Given the well-documented effects of NPY on food intake and the putative involvement of the Y1 receptor in this effect, these data initially are surprising. It should be noted, however, that the effect of NPY and the Y1 agonist to decrease milk production means that the energetic demand placed on females treated with these peptides is markedly reduced. That the food intake of these females is not reduced relative to vehicle-treated and nonoperated controls suggests that NPY and the Y1 agonist have some effect on food intake which is masked by differences in milk production among the groups.

Overall, data from the first two experiments in the present study suggest that the suppression of milk production by NPY and (Leu^{31}, Pro^{34}) NPY is mediated through Y1 receptor activation. We suggest that the effect of chronic NPY-infusion seen in the present investigation is a function of Y1 receptor activation causing the inhibition of prolactin release and thereby repressing the production of milk. The effect of NPY on the termination of lactational diestrus might also be mediated through Y1 receptor activation, but a clear demonstration of this effect requires more specific agonists and antagonists than those employed here.

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