Anorectic actions of prolactin-releasing peptide are mediated by corticotrophin-releasing hormone receptors

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Prolactin-releasing peptide (PrRP) reduces food intake and body weight, and modifies body temperature when administered centrally in rats, suggesting a role in energy homeostasis. However, the mediators of PrRP's actions are unknown. The present study, therefore, firstly examined the possible involvement of the anorectic neuropeptides, corticotrophin-releasing hormone (CRH) and the melanocortins (e.g. α-melanocyte stimulating hormone) in PrRP’s effects on food intake and core body temperature, and secondly determined if PrRP affects energy expenditure by measuring oxygen consumption (VO₂). Intracerebroventricular (icv) injection of PrRP (4 nmol) to 24h fasted male Sprague-Dawley rats decreased food intake and modified body temperature. Blockade of central CRH receptors by icv co-administration of the CRH receptor antagonist, astressin (20 µg), reversed the PrRP-induced reduction in feeding. However, astressin’s effect on PrRP-induced changes in body temperature was complicated, since the antagonist itself caused a slight rise in body temperature. In contrast, icv co-administration of the melanocortin receptor-3/4 antagonist, SHU9119 (0.1 nmol), had no effect on any of PrRP’s actions. Finally, icv injection of PrRP (4 nmol) caused a significantly greater VO₂ over a 3 h test period compared with vehicle-treated rats. These results show that the anorectic actions of PrRP are mediated by central CRH receptors, but not by melanocortin receptors-3/4, and that PrRP can modify VO₂.

**Keywords:** prolactin-releasing peptide, food intake, corticotrophin-releasing hormone, astressin, SHU9119.
Prolactin-releasing peptide (PrRP) was first identified to be the endogenous ligand for the human orphan receptor, GPR10/hGR3 (or UHR-1 in the rat), and was reported to display specific prolactin-releasing properties (15). PrRP mRNA and immunoreactive cell bodies are located exclusively in three brain regions, the dorsomedial hypothalamus (DMH), and in the nucleus tractus solitarius (NTS) and the ventrolateral medulla of the brainstem (2, 7, 18, 26, 30, 33, 39, 60). UHR-1 receptor (PrRP-R) mRNA is also found in the rat brain, and is expressed most highly in the reticular nucleus of the thalamus, the periventricular hypothalamic nucleus, paraventricular hypothalamic nucleus (PVN), DMH, NTS and the area postrema (7, 19, 22, 39). PrRP-immunoreactive fibers are detected in several brain regions that express PrRP-R mRNA, including the PVN and the periventricular hypothalamic nucleus (18, 30, 60).

We have proposed a role for PrRP in energy homeostasis, since mRNA for the peptide is reduced, typically for an anorectic peptide, in states of negative energy balance (fasting and lactation) and in the obese Zucker rat (5, 22). Intracerebroventricular (icv) administration of PrRP reduces both fast-induced and spontaneous feeding, and body-weight gain in rats (5, 22, 23, 47). Furthermore, PrRP does not appear to affect feeding via non-specific or non-homeostatic actions, as it does not support conditioned taste aversion or disrupt the behavioral satiety sequence (23). Pair-feeding experiments suggest that the reduction in body weight after icv injection of PrRP is not due entirely to its anorectic actions (22), implying that PrRP may have additional effects, possibly on energy expenditure. In support of this idea, PrRP affects core body temperature, and hyperthermia is observed typically 2-8 h after icv injection (5, 22). However, an increase in body temperature is only an
indicator that PrRP is increasing energy expenditure, and more direct evidence based on oxygen consumption needs to be determined.

The mechanisms of PrRP’s action on appetite and body temperature are unknown. It is unlikely that prolactin itself is a mediator as several groups have demonstrated that PrRP does not, or can only weakly, cause prolactin release both in vivo and in vitro (19, 32, 42-44, 48, 54). One possible downstream mediator is corticotropin-releasing hormone (CRH). Like PrRP, central administration of CRH reduces food intake and body weight [see (13)], and modifies energy expenditure in rodents [see (3, 12, 41)]. PrRP-immunoreactive fibres appear to make synaptic contact with CRH-containing cell bodies in the rat PVN, providing morphological evidence for a relationship between the two neuropeptides (31). Furthermore, icv injection of PrRP increases neuronal activation (measured by induction of c-Fos expression) in the PVN of the rat (23), including in approximately 80% of CRH-containing neurons (31), and in vitro PrRP increases hypothalamic CRH release (46). In addition, central administration of PrRP in rats stimulates plasma levels of ACTH, an effect that is dependent on CRH receptor activation (31, 46), and corticosterone (42). In view of the above, we hypothesized that the actions of PrRP on energy balance are mediated via CRH receptors.

Therefore, the main aim of this study was to test the hypothesis that central CRH receptors mediate the effects of PrRP on food intake and core body temperature. Another anorectic pathway, the melanocortin system, was considered since it partially mediates the effects of modulators such as leptin (11, 16, 45, 49), and because the acute actions of leptin and PrRP are very similar (5). Additionally, the effect of PrRP on energy metabolism was tested more directly by measuring oxygen consumption using closed-circuit calorimetry.
METHODS

**Materials.** Rat PrRP (Peptide Institute Inc., Osaka, Japan), the melanocortin receptor-3/4 antagonist, SHU9119 \([(Ac-Nle\textsuperscript{4}-c(Asp\textsuperscript{5},D-2'Nal\textsuperscript{7},Lys\textsuperscript{10})\textalpha-MSH-(4-10)-NH\textsubscript{2}\textsuperscript{13})]; Bachem, Saffron Walden, UK\], and the CRH receptor antagonist, astressin (Bachem), were all dissolved in 0.9% sterile saline.

**Animals and Surgery.** Male, Sprague-Dawley rats (body weight 250-300 g, Charles River, Sandwich, UK) were used in all experiments and were housed at a constant ambient temperature of 21 ± 1 °C on a 12 h:12 h light-dark cycle (lights on from 0800-2000 h). Standard pelleted rat chow (Beekay International, Hull, UK) and tap water was provided *ad libitum* unless stated otherwise. All procedures conformed to the requirements of the UK Animals (Scientific Procedures) Act, 1986. Five to seven days before icv injections animals were anaesthetized with 2.5-3% halothane (Fluothane; AstraZeneca, Macclesfield, UK) in oxygen and a guide cannula was inserted stereotaxically into the lateral ventricle [0.8 mm posterior, 1.5 mm lateral and 3.5 mm ventral (1 mm above injection site) to bregma], according to the atlas of Paxinos and Watson (35), and the placement checked histologically post experiment.
Measurement of food intake and core body temperature. To allow remote measurement of core body temperature in undisturbed animals, radiotransmitters (TA10TA-F40, Data Sciences, Minneapolis, MN) were implanted into the peritoneum at the same time as lateral ventricle cannulations.

A week after surgery, and 24 h before the start of the experiment, animals were housed individually, without food, in cages over receiver pads that monitored output frequency (temperature-dependent radio signals) emitted from the transmitters. The signals were then converted (to ºC) via a peripheral processor (BCM 100; Data Sciences, Minneapolis, MN).

Icv injections were performed in the conscious, unrestrained, 24 h-fasted animals, 2 h after the beginning of the light phase (i.e. 1000 h). Immediately after injections, a pre-weighed amount of food was presented to the animals. Food consumption was measured 1 h and 2 h later and core body temperature was monitored continuously over an 8 h period.

Measurement of oxygen consumption. Oxygen consumption (VO₂) was determined in closed-circuit respirometers maintained at the thermoneutral temperature for rats (29 ºC). The system allows eight rats to be tested individually at one time (52). All animals were accustomed to the respirometers and procedures on two occasions the week before the experiments. A week after lateral ventricle cannulation animals were housed individually, without food, in the respirometers and VO₂ was then recorded every 5 min. After a 90 min measurement of baseline VO₂, animals were taken out, lightly restrained and icv injections were performed approximately 2 h after the beginning of the light phase (i.e. 1000 h). Following
injections rats were placed back into the respirometers and measurement of VO$_2$ was continued for a further 3 h.

**Experiment 1: Effect of a CRH receptor antagonist on PrRP actions on food intake and core body temperature.** Groups of rats (n = 10-12) were infused icv with or without PrRP (4 nmol in 2 µl) and astressin (20 µg in 2 µl). The total volume injected was always made up to 4 µl with saline vehicle. The dose of PrRP was selected on the basis a previous studies demonstrating a hyperthermic and anorectic action in *ad libitum* fed or fasted rats (5, 22). In addition, icv injections of astressin, at doses between 10-100µg, have been shown to inhibit CRH-induced actions, including effects on food intake (1, 20)

**Experiment 2: Effect of a melanocortin receptor-3/4 antagonist on PrRP actions on food intake and core body temperature.** To assess the effect of a melanocortin receptor antagonist on PrRP’s actions, separate groups of rats (n = 15-18) were treated as in Experiment 1, but with the astressin being replaced by SHU9119 (0.1 nmol in 2 µl). This dose of SHU9119 has been shown previously to inhibit reductions in feeding induced by icv injection of the melanocortin receptor agonist, MTII in rats, but does not effect feeding alone (24).

**Experiment 3: Effect of a PrRP on core body temperature in satiated rats.** To investigate the actions of PrRP on core body temperature in the absence of possible confounding effects of feeding and activity (seen in fasted rats; Experiments 1 and 2), PrRP was given to non-fasted satiated rats. Icv injection of PrRP (4 nmol in 2 µl, n = 8) or vehicle (2 µl saline, n = 8) was administered to satiated rats at 1000 h.
Core body temperature was monitored continuously for 8 h and food intake was measured at 6 h.

**Experiment 4: Effect of PrRP on oxygen consumption.** In order to test the thermogenic effect of PrRP, rats were injected icv with either vehicle (2 µl sterile saline; n = 13) or PrRP (4 nmol in 2 µl; n = 13). On another occasion a separate group of rats (n = 5 per group) were administered with PrRP (4 nmol in 2 µl) with or without astressin (20 µg in 2 µl).

**Data and statistical analyses.** All data are presented as mean ± SEM. Body temperatures were plotted as the mean change (Δ) from the point of injection (time zero) and were analyzed by calculating the integrated temperature response for 2-8 h after injection [area under the curve (AUC), °C h] for each animal by the trapezoidal method. Average AUC values were then determined for each treatment group. VO\(_2\) is expressed as ml oxygen per min per kg metabolic body size (i.e. ml/min/kg\(^{0.75}\)). Thirty minute averages of VO\(_2\) were calculated, and the time course of the change (Δ) in VO\(_2\) was determined by comparing the mean of the last 30 min of the baseline readings with the mean values obtained for the post-injection period. The mean change in VO\(_2\) from baseline over the 3 h period was calculated for statistical analysis.

Statistical comparisons were performed using an unpaired Student's t-test for two groups (Experiment 3, and Experiment 4, Fig. 5B), and all other experiments were analyzed using a one-way ANOVA, followed by a Tukey-Kramer comparisons *post hoc* test. Statistical significance assumed a two tailed probability with a *P* < 0.05.
RESULTS

Experiment 1: Effect of a CRH receptor antagonist on PrRP actions on food intake and core body temperature. Central injection of the CRH receptor antagonist, astressin (20 µg), alone had no effect on food intake at the 0-1 h and 1-2 h time points compared with vehicle-treated animals (Fig. 1). Administration of PrRP (4 nmol) suppressed food intake at 1 h (67%) after injection when compared with vehicle-injected animals, but there was no difference in the second hour. Astressin reversed the effects of PrRP on food intake at 1 h, and values for the PrRP plus astressin co-injected group was not significantly different from control animals.

Remote radiotelemetry revealed that fasted animals, injected icv with vehicle, reliably displayed an initial hyperthermia (over approximately 2 h) probably due to increased feeding activity when food was returned. Astressin alone had no significant effect on core body temperature during the experimental period, although the graphs suggest that there was a steady, but non-significant, rise (over 2-8 h) in body temperature in this group of animals (AUC for 2-8 h: vehicle, 2.82 ± 0.36 °C h versus astressin, 4.06 ± 0.83 °C h, P > 0.05, Fig. 2A). Injection of PrRP caused an initial hypothermic response followed by a significant increase in core body temperature over 2-8 h compared with vehicle injection (AUC for 2-8 h: vehicle, 2.82 ± 0.36 °C h versus PrRP, 5.58 ± 0.88 °C h, P < 0.05, Fig. 2B). Co-injection of astressin prevented the initial hypothermic response induced by PrRP (Fig. 2C). However, core body temperature observed during the 2-8 h after PrRP plus astressin was also significantly greater compared with vehicle injection (AUC for 2-8 h: vehicle, 2.82 ± 0.36 °C h versus PrRP + astressin, 5.84 ± 0.99 °C h, P < 0.05, Fig. 2C). There was
no significant difference between the AUC for the PrRP alone and PrRP plus astressin groups ($P > 0.05$).

**Experiment 2: Effect of a melanocortin receptor-3/4 antagonist on PrRP actions on food intake and core body temperature.** Icv injection of SHU9119 alone had no effect on 0-1 h or 1-2 h fast-induced feeding (Fig. 3). PrRP caused a significant reduction in 1 h (56%) food intake compared with vehicle-treated rats and as reported for Experiment 1, there was no effect on feeding during 1-2 h after injection (Fig. 3). However, co-injection of SHU9119 had no effect on the decrease in food intake observed after injection of PrRP. Food intake in this group of animals was reduced by 51% at 1 h versus vehicle-treated groups, and was not significantly different when compared with PrRP injection alone (Fig. 3). Food intake was also measured for up to 8 h after injections, and that confirmed that SHU9119 did not have any delayed effect on the PrRP response (data not shown).

SHU9119 alone also had no significant effect on the change in body temperature compared with vehicle-treated animals (AUC for 2-8 h: vehicle, $3.04 \pm 0.27 ^\circ C$ h versus SHU9119, $4.41 \pm 0.49 ^\circ C$ h, $P > 0.05$, Fig. 4A). As expected PrRP administration caused a characteristic drop in core body temperature (Fig. 4B). This hypothermic phase was followed by a period where body temperature settled above that of vehicle-injected animals, but failed to reach statistical significance in this experiment using *post-hoc* analysis (AUC for 2-8 h: vehicle, $3.04 \pm 0.27 ^\circ C$ h versus PrRP, $4.64 \pm 0.56 ^\circ C$ h, $P > 0.05$, Fig. 4B). Consistent with the effects on feeding, SHU9119 had no effect on the temperature response induced by PrRP, as the hypothermic phase was still observed. In addition, core body temperature for the 2-8 h period was not significantly different compared with PrRP alone (AUC for 2-8 h:
PrRP, 4.64 ± 0.56 °C h versus PrRP + SHU9119, 5.44 ± 0.87 °C h, \( P > 0.05 \), but was significantly increased compared with vehicle-treated animals (AUC for 2-8 h: vehicle, 3.04 ± 0.27 °C h versus PrRP + SHU9119, 5.44 ± 0.87 °C h, \( P < 0.05 \), Fig. 4C).

Experiment 3: Effect of a PrRP on core body temperature in satiated rats.

Normally-fed rats did not eat significant amounts of food during the light-phase (0800-2000 h), and there was no difference in food intake between vehicle and PrRP-treated rats at 6 h post injection (0-6 h food intake: vehicle, 1.4 ± 0.5 g versus PrRP, 1.7 ± 0.6 g, \( P > 0.05 \)). PrRP injection into satiated (non-fasted) rats caused the typical effect on core body temperature as reported previously. An initial rapid hypothermia was followed by a prolonged period of hyperthermia (AUC for 2-8 h: vehicle, 2.01 ± 0.33 °C h versus PrRP, 4.53 ± 0.81 °C h, \( P < 0.05 \)).

Experiment 4: Effect of PrRP on oxygen consumption. The effect of a single icv dose of PrRP (4 nmol) on VO₂ is shown if Fig. 5A as the change (\( \Delta \)) in VO₂ from baseline. The VO₂ in vehicle-treated animals declined slowly over the 3 h test period. This decline is normal, and probably reflects a decrease in the diet-induced thermogenesis resulting from the last few nocturnal meals consumed just before the start of the light phase (0800 h). This decline, however, was less in animals that received PrRP, and VO₂ was significantly greater than in vehicle-treated animals for the 3 h measurement period. Fig. 5B shows the mean change from baseline over 3 h, with the vehicle-treated animals showing an average 14% decrease of 1.87 ± 0.18 ml/min/kg^{0.75}, which was significantly greater (\( P < 0.001 \)) than the 4% decrease (0.63 ± 0.16 ml/min/kg^{0.75}) observed after PrRP treatment.
In a separate experiment, 4 nmol PrRP caused a similar effect on the 3 h change in VO$_2$ compared with controls (vehicle, 10% decrease of 1.39 $\pm$ 0.12 ml/min/kg$^{0.75}$; versus PrRP, 4% decrease of 0.61 $\pm$ 0.19 ml/min/kg$^{0.75}$; P < 0.01, Fig. 5C). However, 20 µg astressin also affected 3 h VO$_2$ when administered alone (5% decrease of 0.63 $\pm$ 0.15 ml/min/kg$^{0.75}$; P < 0.01 compared with control group), and although co-administration of astressin partially inhibited the effects of PrRP (7% decrease of 0.93 $\pm$ 0.10 ml/min/kg$^{0.75}$; P > 0.05 compared with other groups), this was not significantly different to the PrRP alone-treated animals.

**DISCUSSION**

We have shown previously that icv injection of PrRP reduces food intake and body weight, and modifies core body temperature in fasted and ad libitum fed rats (5, 22, 23). The acute effects of PrRP on food intake, which occur within 1 h of injection, have been confirmed by Seal and co-workers (47) and here in the present study. The CRH receptor antagonist, astressin, but not the melanocortin-3/4 receptor antagonist, SHU9119, blocked the effect of PrRP on food intake. Agonists of melanocortin receptor-3/4 (e.g., $\alpha$-melanocyte stimulating hormone, MTII) suppress food intake in rodents (6, 55). The dose of the antagonist, SHU9119, used in the present experiment (0.1 nmol) is effective as it blocks the anorectic actions of an equimolar injection of the melanocortin receptor agonist, MTII (24). Furthermore, it has its effect when it is co-administered at the same time with either MTII, leptin or d-fenfluramine (14, 24, 45). Since higher doses of SHU9119 can initiate food intake at later time points (9, 40), we confirmed also that it did not reverse the effect of PrRP on cumulative food intake over an 8 h period.
The effect of PrRP on core body temperature is two-fold, as after an initial, brief hypothermic period a hyperthermic phase is evident. Data presented here and previously (4, 5, 22) demonstrate that the temperature profile observed after PrRP is the same regardless of when injections are performed (light phase versus dark phase), and whether the animals are eating or not. Since astressin reversed both the initial drop in body temperature and the reduction in food intake observed after PrRP injection, it is possible that the hypothermic response is responsible for the ensuing effects on feeding. However, we observe similar acute hypothermic phases after icv injection of several other neuropeptides, which are conversely orexigenic, that is they stimulate feeding [e.g., growth hormone releasing peptide-6 and ghrelin; (25)]. Thus, the acute hypothermia is neither sufficient to cause a reduction in feeding, nor is it a result of reduced feeding. The subsequent increase in core body temperature was not significantly inhibited by astressin, though these results are complicated by the fact that astressin itself has a thermogenic effect.

Increases in core body temperature may be indicative of heightened energy expenditure and we now provide more direct evidence for this, as PrRP increased oxygen consumption, at least acutely. These results therefore demonstrate that PrRP’s effects on body weight are probably brought about by dual actions on food intake and energy expenditure. Astressin itself increased oxygen consumption significantly post-injection, and this correlated with the increase in core body temperature. The actions of astressin on these parameters highlights a need for caution in interpretation of experiments involving this compound and the role of CRH receptors in energy balance. Thus, the involvement of CRH receptors in mediating the metabolic response to PrRP may need further investigation, to establish whether
the PrRP-induced rise in energy expenditure is mediated by the same mechanism as the anorectic response.

There are several CRH receptor subtypes described to date; CRH receptor type 1 and CRH receptor type 2, which can exist as several splice variants, see (37, 56). The CRH receptor subtype involved in PrRP's anorectic effects cannot be ascertained from this study, as the antagonist, astressin, does not distinguish between the two (10, 36). Likewise, the actions of PrRP cannot be conclusively ascribed to CRH since there are several other endogenous ligands that act at CRH receptors. These include, urocortin (58), and the recently identified urocortin II (17, 38) and urocortin III (17, 27). Each of these neuropeptides affect feeding in rodents (17, 38, 51) and, thus, could potentially be involved in PrRP's anorectic actions. This stated, CRH's involvement is supported by the observation that 80% of CRH-containing neurons in the PVN are activated by icv injection of PrRP (31), and in vitro PrRP causes hypothalamic CRH release (46). However, actions of PrRP in the PVN may not be direct, since although PrRP-R mRNA is located in this area (19, 22, 39) and PrRP-immunoreactive fibers make synaptic contact with CRH cell bodies in the PVN (31), a recent study suggests that only a small percentage of PrRP-R expressing cells in the PVN contain CRH (28). Thus, the described effects of PrRP in the PVN may be indirect due to activation of neuronal pathways that connect with the PVN.

Both CRH and urocortin induce a range of behaviors, such as anxiety and hyperactivity, when administered centrally (21, 50). Thus, the significance of CRH and the urocortins in the physiological regulation of food intake is still debatable. Our own data demonstrate that, in addition to the PVN, increases in c-Fos after icv PrRP injection are observed in the amygdala (23), which is also a major site of CRH.
synthesis (53). Although, we propose a role for CRH receptors in the actions of PrRP on feeding, the caveat noted above might indicate that PrRP can affect appetite, possibly by causing anxiety. However, we have demonstrated that PrRP, at doses that decrease feeding, enhances the behavioral satiety sequence, and does not cause conditioned taste aversion (23), or affect water intake (22, 43). In addition, Seal and colleagues have demonstrated that PrRP, at comparable doses, does not affect overt behaviors significantly (47). We have demonstrated recently that repeated doses of PrRP lead to tolerance to the anorectic action (4). However, we do note that one study failed to reproduce the acute effects of PrRP on feeding at doses used in our own and the experiments of Seal and co-workers (59). These workers did report an effect with repeated central injections of PrRP at higher dose.

The acute effects of PrRP on feeding and body temperature are similar to those reported after central administration of leptin (5, 29), and leptin actions on food intake are partially blocked by either CRH receptor antagonists (8, 57) or an anti-CRH antibody (34). However, in contrast to PrRP, leptin also functions through melanocortin receptors (11, 16, 45, 49). We have shown previously that leptin and PrRP have an additive interaction when co-administered (5) and, thus, CRH receptors may be a point of convergence for these two catabolic factors.
ACKNOWLEDGEMENTS

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Reference List


Figure legends

Fig. 1. Effect of a CRH receptor antagonist, astressin, on PrRP-induced reduction in food intake. Vehicle (2 µl saline) or astressin (20 µg) were co-administered icv with either vehicle (2 µl saline) or PrRP (4 nmol) into 24 h-fasted rats at 1000 h. Food intake was measured at 1 h and 2 h after injection. Data are mean ± SEM for n = 10-12 rats per group. *** P < 0.001 versus vehicle; ### P < 0.001 versus astressin; φφ P < 0.01 versus PrRP + astressin.

Fig. 2. Effect of astressin on PrRP-induced changes in core body temperature in the same animals as in Fig 1. Core body temperature (in °C) was monitored continuously for 8 h and is expressed as the change (Δ) in temperature from time of injection. Data points at 30 min intervals are plotted as mean ± SEM for n = 10-12 rats per group. To aid comparisons, the vehicle group is plotted against each experimental group in the three panels, though all data are from the same study.

Fig. 3. Effect of a melanocortin-3/4 receptor antagonist, SHU9119, on PrRP-induced reduction in food intake. Vehicle (2 µl saline) or SHU9119 (0.1 nmol) were co-administered icv with either vehicle (2 µl saline) or PrRP (4 nmol) into 24 h-fasted rats at 1000 h. Food intake was measured at 1 h and 2 h after injection. Data are mean ± SEM for n = 15-18 rats per group. * P < 0.05, ** P < 0.01 versus vehicle; ## P < 0.01, ### P < 0.001 versus SHU9119.

Fig. 4. Effect of SHU9119 on PrRP-induced changes in core body temperature in the same animals as in Fig 3. Core body temperature (in °C) was monitored for 8 h and
is expressed as the change ($\Delta$) in temperature from time of injection. Data points at 30 min intervals are plotted as mean ± SEM for $n = 15-18$ rats per group. To aid comparisons, the vehicle group is plotted against each experimental group in the three panels, though all data are from the same study.

Fig. 5 Effect of PrRP on oxygen consumption ($ VO_2 $). Vehicle (2 $ \mu $l saline, $ n = 13 $) or PrRP (4 nmol, $ n = 13 $) were injected icv into rats at 1000 h. $ VO_2 $ was measured for 3 h after injections in closed-circuit respirometers and is expressed as (A) change ($\Delta$) in $ VO_2 $ from basal and (B) 3 h mean change in $ VO_2 $ from basal. From a separate experiment, graph (C) illustrates the effect of a CRH receptor antagonist, astressin, on PrRP effects on $ VO_2 $. Vehicle (2 $ \mu $l saline) or astressin (20 $ \mu $g) were co-administered icv with either vehicle (2 $ \mu $l saline) or PrRP (4 nmol) into rats ($ n = 5 $ per group) at 1000 h and $ VO_2 $ was measured as above and is expressed as the 3 h mean $\Delta$ in $ VO_2 $ from basal.
Figure 1

![Bar chart showing food intake in different conditions: Vehicle, Astressin, PrRP, and PrRP + Astressin.](chart)

- **0-1h**
  - Vehicle: [Bars with error bars]
  - Astressin: [Bars with error bars] **##**
  - PrRP: [Bars with error bars] **###**
  - PrRP + Astressin: [Bars with error bars] **###**

- **1-2h**
  - Vehicle: [Bars with error bars]
  - Astressin: [Bars with error bars]
  - PrRP: [Bars with error bars]
  - PrRP + Astressin: [Bars with error bars]

Food intake (g)
Figure 2

A. Vehicle
- Astressin

B. Vehicle
- PrRP

C. Vehicle
- PrRP + Astressin
Figure 3

![Bar chart showing food intake (g) over 0-1h and 1-2h periods for different treatments: Vehicle, SHU9119, PrRP, and PrRP + SHU9119. Significant differences are indicated with asterisks: ###, ##, **, and * for Vehicle, SHU9119, PrRP, and PrRP + SHU9119, respectively.]
Figure 4

A. 

- Vehicle
- SHU9119

B. 

- Vehicle
- PrRP

C. 

- Vehicle
- PrRP + SHU9119
Figure 5

A. 

![Graph showing the effect of PrRP and Vehicle on VO2 (ml/min/kg) with time post injection (h)].

B. 

![Bar chart showing 3h ΔVO2 (ml/min/kg) with vehicles and PrRP].

C. 

![Bar chart showing 3h ΔVO2 (ml/min/kg) with different treatments: Vehicle, Astressin, PrRP, PrRP + Astressin].