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Hypothalamic paraventricular 5-hydroxytryptamine: Receptor-specific inhibition of NPY-stimulated eating and energy metabolism

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Abstract

The feeding effects of 5-hydroxytryptamine (5-HT)$_1$ and 5-HT$_2$ receptor agonists injected into the hypothalamic paraventricular nucleus (PVN) immediately prior to PVN administration of neuropeptide Y (NPY) were examined. The impact of these same compounds on NPY-induced alterations in energy metabolism was also assessed in an attempt to characterize further the potential interactive relationship of PVN NPY and 5-HT on feeding and whole body calorimetry. Specifically, several experiments examined the effect of various 5-HT receptor agonists on NPY-stimulated eating and alterations in energy substrate utilization [respiratory quotient (RQ)]. This included the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT, the 5-HT$_{1B/1A}$ agonist RU 24969, the 5-HT$_{1D}$ agonist L-694,247, the 5-HT$_{2A/2C}$ agonist DOI, the 5-HT$_{2B}$ agonist BW 723C86 and the 5-HT$_{2C}$ agonist mCPP. In feeding tests conducted at the onset of the dark cycle, drugs were administered 5 min prior to PVN injection of NPY and food intake was measured 2 h postinjection. The metabolic effects of NPY following a similar pretreatment were monitored using an open-circuit calorimeter measuring the volume of oxygen consumed (VO_2), carbon dioxide produced (VCO_2) and RQ (VCO_2/VO_2). PVN injection of NPY (100 pmol) potentiated feeding and evoked reliable increases in RQ. Only DOI (2.5–5 nmol) pretreatment antagonized NPY-induced eating and blocked the peptide's effect on energy substrate utilization. Direct PVN pretreatment with spiperone (SPRN), a 5-HT$_{2A}$ receptor antagonist, and ketanserin (KTSN), a 5-HT$_{2A/2C}$ antagonist, but not SDZ SER 082, a 5-HT$_{2B/2C}$ antagonist, or the 5-HT$_{2C}$ antagonist RS 102221, blocked the effect of DOI in both feeding and metabolic tests providing additional evidence that activation of PVN 5-HT$_{2A}$ receptors inhibits NPY's action on feeding and substrate utilization. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Food intake; Neuropeptide Y; Paraventricular nucleus; Respiratory quotient; Serotonin; 5-HT agonists; 5-HT antagonists

1. Introduction

There is now extensive evidence to indicate that 5-hydroxytryptaminergic (5-HT) innervation of the medial hypothalamus is involved in the control of ingestive behavior. Autoradiographic and immunofluorescence studies have identified 5-HT-containing neurons in the medial region of the hypothalamus as well as dense numbers of 5-HT$_{1/2}$ receptors (Hoyer, 1988; Leibowitz and Jhanwar-Uniyal, 1989; Sawchenko et al., 1983), which are reported to mediate the satiety-inducing effect of 5-HT (Currie and Coscina, 1996a; Curzon, 1990). The serotonergic innervation of the medial hypothalamus arises from 5-HT projections of the midbrain raphe nuclei (Steinbusch, 1981). Numerous studies have demonstrated that the paraventricular nucleus (PVN) is sensitive to the feeding inhibitory effect of 5-HT, particularly when the monoamine is administered at the onset of the nocturnal cycle (Currie, 1996; Currie and Coscina, 1996a; Leibowitz et al., 1989). Direct PVN injections of 5-HT dose-dependently suppress food intake (Currie, 1996; Currie and Coscina, 1996a; Leibowitz et al., 1989). This effect is reproduced following PVN infusion of various 5-HT receptor agonists and reuptake inhibitors (Currie, 1996; Currie and Coscina, 1996a; Curzon, 1990; Dourish, 1992). However, it is generally acknowledged that the impact of various 5-HT compounds is not mediated

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exclusively by the PVN, as lesions of this nucleus fail to modify the anorectic action of systemically administered 5-HT agonists (Fletcher et al., 1993).

In contrast to the feeding suppression elicited by PVN 5-HT, local injection of neuropeptide Y (NPY) elicits robust hyperphagia (Currie and Coscina, 1995; Leibowitz and Alexander, 1991; Stanley and Leibowitz, 1985). NPY is a 36-amino acid peptide and is synthesized in neurons of the arcuate nucleus projecting to the PVN (DeQuidt and Emson, 1986; Jhanwar-Uniyal et al., 1993). Bilateral PVN infusion of anti-NPY γ-globulin suppresses feeding (Shibasaki et al., 1993) and chronic NPY administration induces rapid weight gain and increased body fat deposition (Stanley et al., 1986, 1989). While NPY administration into either the PVN or perifornical region of the hypothalamus (PFH) elicits eating, PVN but not PFH injections of NPY evoke a concomitant hypothermia (Currie and Coscina, 1995). Further evidence shows that PVN but not PFH NPY alters energy substrate utilization as indicated by increases in respiratory quotient (RQ; Currie and Coscina, 1996b). This suggests that PVN NPY modulates integrative and regulatory mechanisms of feeding, thermogenesis and energy metabolism.

Recently, we reported that pretreatment with DOI, a 5-$HT_{2A/2C}$ receptor agonist, microinjected into the PVN, inhibited feeding stimulated by PVN NPY infusion (Currie and Coscina, 1997, 1998) as well as NPY-induced alterations in energy substrate utilization (Currie and Coscina, 1998). A similar effect was not found following DOI pretreatment in the PFH or ventromedial nucleus (VMN). In the present study, we investigated the effects of several receptor-specific 5-HT agonists on NPY eating and energy metabolism. The results of this study indicate that, of the various $5HT_1$ and 5-HT_2 receptor agonists administered, only PVN DOI, injected at the start of the dark cycle, inhibited the increases in eating and RQ elicited by PVN NPY. Moreover, blockade of 5-HT_{2A} but not 5-HT_{2B} or 5-HT_{2C} receptors antagonized the inhibitory action of DOI.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Charles River) weighing 275–300 g at the time of surgery were used. Rats were housed individually in polypropylene cages with ad libitum access to standard laboratory chow pellets and water. The animal colony room was maintained on a 12-h light/dark cycle (lights on at 04:00 h) and at a temperature of 22 ± 2 °C.

2.2. Apparatus

Oxygen consumption (O_2) and carbon dioxide (CO_2) production were measured using an Oxyscan open-circuit indirect calorimeter (AccuScan Instruments, Columbus, OH). Detectors measured O_2 and CO_2 sequentially across each acrylic test chamber. The flow rate was set at 1500 ml/min. Concentrations of the gases were recorded in ml/kg body weight min. RQ was calculated as the volume of CO_2 produced (VCO_2) divided by the volume of O_2 consumed (VO_2). The analyzers were frequently calibrated using primary gas standards of high purity (Matheson, New York, NY).

2.3. Drugs

The following 5-HT receptor agonists (Research Biochemicals/Sigma or Tocris) were administered as a pretreatment prior to NPY injection. This included the 5-HT_{1A} receptor agonist 8-OH-DPAT HBr, the 5-$HT_{1B/1A}$ agonist RU 24969, the 5-HT_{1D} agonist L-694,247, the 5-$HT_{2A/2C}$ agonist DOI HCl, the 5-HT_{2B} agonist BW 723C86 HCl and the 5-HT_{2C} agonist mCPP HCl. The 5-HT antagonists used included spiperone HCl (SPRN), a 5-HT_{2A} antagonist, the 5-$HT_{2A/2C}$ antagonist ketanserin tartrate (KTSN), the 5-$HT_{2B/2C}$ antagonist SDZ SER 082 fumarate and the 5-HT_{2C} antagonist RS 102221 HCl. Compounds were dissolved in sterile water vehicle with the exception of L-694,247 and BW 723C86, which were dissolved in DMSO. NPY (Peninsula) was dissolved in sterile water. All drugs were injected in a volume of 0.3 μl into the PVN using a microinjector extending 4 mm beyond the permanent guide cannula. Drugs and their respective doses were selected based on receptor binding properties, pilot data and previous reports examining 5-HT effects on ingestive behavior and energy metabolism (Currie and Coscina, 1997, 1998; Dryden et al., 1996; Fletcher et al., 1992; Grignaschi et al., 1996).

2.4. Design and procedure

Rats were anesthetized with sodium pentobarbital (50 mg/kg ip) and placed in a Kopf stereotaxic frame with the incisor bar set 3.6 mm below the interaural line. PVN coordinates for the guide cannula relative to bregma were AP – 1.5 mm, L – 0.3 mm and V – 4.5 mm (Paxinos and Watson, 1998). Guide cannulae (22 G; Plastics One, Roanoke, VA) were implanted 4 mm dorsal to the PVN. Implants were secured with acrylic cement and stainless-steel screws penetrating the skull. A 28-G stainless-steel inner stylet maintained cannula patency. Following a postoperative recovery period of 1 week, rats were acclimated to the metabolic chambers and received several mock injections.

In feeding tests, separate groups of rats ($n = 10$ per group) were used to examine the effects of the various 5-HT receptor agonists on NPY-stimulated eating. Serotonergic agonists or their respective vehicles were administered 5 min prior to 100 pmol of NPY, which itself was infused just before the start of the nocturnal cycle. Compounds were

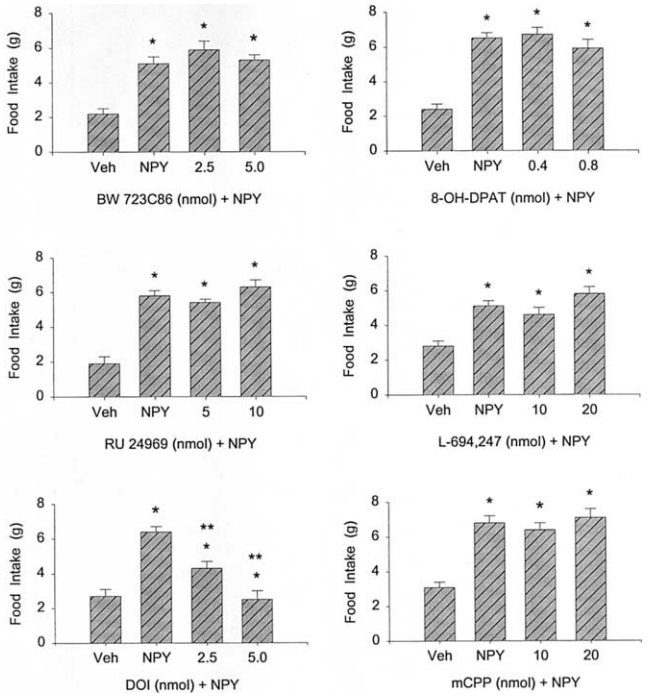

Fig. 1. Food intake after NPY injection into the PVN following 5-HT receptor agonist pretreatment. DOI significantly reduced feeding elicited by PVN NPY while other 5-HT agonists were ineffective. Values represent mean intakes (±S.E.M.) measured over 2 h. Data were analyzed by repeated-measures ANOVA and post hoc Tukey tests. * $P<.05$ compared to Veh; ** $P<.05$ compared to NPY.

injected at the following doses: BW 723C86 (2.5–5 nmol), RU 24969 (5–10 nmol), DOI (2.5–5 nmol), 8-OH-DPAT (0.4–0.8 nmol), L-694,247 (10–20 nmol) and mCPP (10–20 nmol). Under control conditions, two consecutive vehicle injections were administered. Food intake was measured 2 h postinjection. While separate groups of animals were used to examine the effects of each 5-HT agonist on NPY eating, within a particular group, all rats received each dose of the agonist paired with NPY in a randomized order. At least 4 days separated successive testing.

In metabolic testing, similar injection procedures and treatments were followed as outlined above. Again, the 5-HT agonist ($n=8$ per group) was administered 5 min prior to NPY (100 pmol). O_2 consumption and CO_2 production were recorded every 5 min for 2 h postinjection. Food and water were not available during testing. Respiratory exchange was monitored over 2 h.

The above studies demonstrated that only DOI pretreatment antagonized NPY-stimulated eating and blocked the peptide's effect on RQ. Consequently, a final series of studies examined the impact of direct PVN injections of several 5-HT receptor antagonists administered immediately prior to PVN DOI and NPY. Specifically, separate groups of rats were injected with either KTSN (15–30 nmol), RS 102221 (0.25–0.5 nmol), SDZ SER 082 (10–20 nmol) or SPRN (5–10 nmol) followed by DOI (5 nmol) and NPY (100 pmol). Equal numbers of vehicle injections were administered under control conditions. The feeding ($n=10$ per group) and RQ ($n=8$ per group) effects were measured as described in the previous experiments.

2.5. Histological and statistical analyses

Cannulae placements were confirmed via histological examination as described previously (Currie and Coscina, 1995, 1996b). Sections were viewed relative to the stereotaxic atlas of Paxinos and Watson (1998). All rats reported here were found to have injector tracts extending into the PVN.

Data were analyzed by separate one- or two-way analyses of variance (ANOVA) for repeated measures. Specific comparisons between means were evaluated using post hoc Tukey tests. The criterion for statistical significance was $P<.05$.

3. Results

Fig. 1 illustrates the effect of PVN administration of various 5-HT receptor agonists on NPY-stimulated eating at the onset of the nocturnal cycle. One-way ANOVA indicated that PVN injection of NPY elicited a significant increase in 2-h feeding, while pretreatment with DOI (2.5–5 nmol) antagonized this effect [$F(3,27)=289.5$, $P<.0001$]. However, NPY hyperphagia was unaffected by PVN BW 723C86, RU 24969, 8-OH-DPAT, L-694,247 or mCPP. The inhibitory effect of DOI on NPY feeding was, in turn, blocked by KTSN [15–30 nmol; $F(4,36)=212.9$, $P<.0001$] and SPRN [5–10 nmol; $F(4,36)=342.1$, $P<.0001$] but not by RS 102221 or SDZ SER 082, as

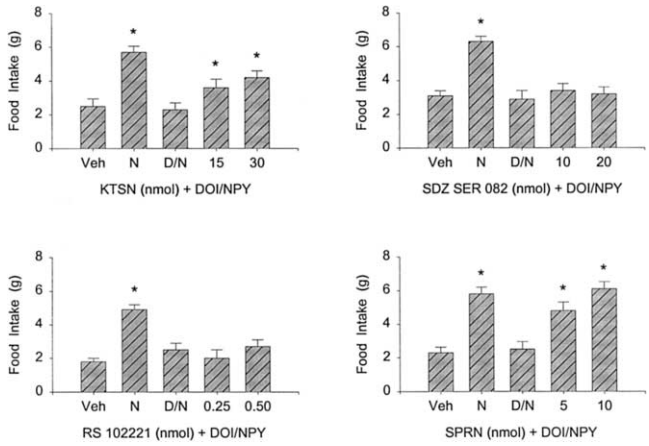

Fig. 2. Administration of the 5-HT receptor antagonists SPRN and KTSN, but not RS 102221 or SDZ SER 082, reversed the inhibitory action of DOI (5 nmol) on NPY (100 pmol)-stimulated eating. Values are represented as mean intakes (±S.E.M.) over 2 h. Data were analyzed by ANOVA and post hoc Tukey tests. * $P<.05$ compared to Veh.

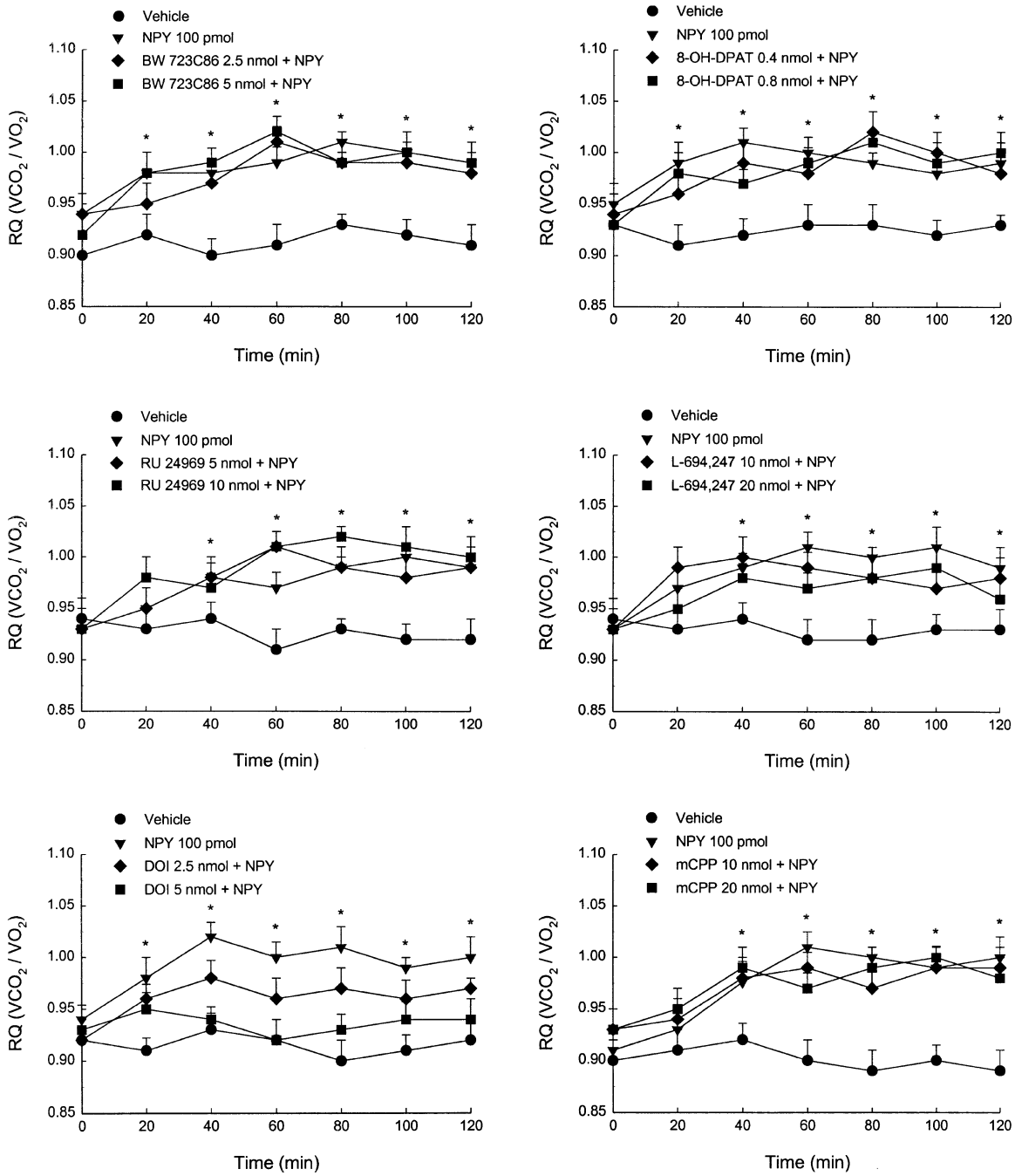

Fig. 3. Mean (±S.E.M.) RQ following PVN 5-HT receptor agonist pretreatment in NPY (100 pmol)-treated rats. Only DOI (2.5–5 nmol) attenuated NPY's effects on RQ. Statistical differences between treatments were determined by repeated-measures ANOVA and post hoc Tukey tests. * $P<.05$ compared to Veh.

shown in Fig. 2. In fact, SPRN completely antagonized DOI's action.

Fig. 3 illustrates the effects of NPY on RQ after injection into the PVN. Data are presented as mean RQ values during the initial 2 h of the dark period. Values are shown in 20-min intervals. Two-way ANOVA for repeated measures showed that both doses of DOI attenuated NPY's reliable effect on RQ [Treatment × Time interaction; $F(72,504)=108.3$, $P<.00001$]. This effect persisted throughout the entire 2-h test. In contrast, all other 5-HT receptor agonists tested failed to alter NPY metabolism. The attenuation by DOI of NPY's effect on RQ was reversed by KTSN [$F(96,672)=276.8$, $P<.00001$] and SPRN [$F(96,672)=419.3$, $P<.00001$], as demonstrated in Fig. 4. RS 102221 and SDZ SER 082 were ineffective. For clarity, only the higher dose of the antagonist paired with DOI/NPY is depicted graphically. However, both

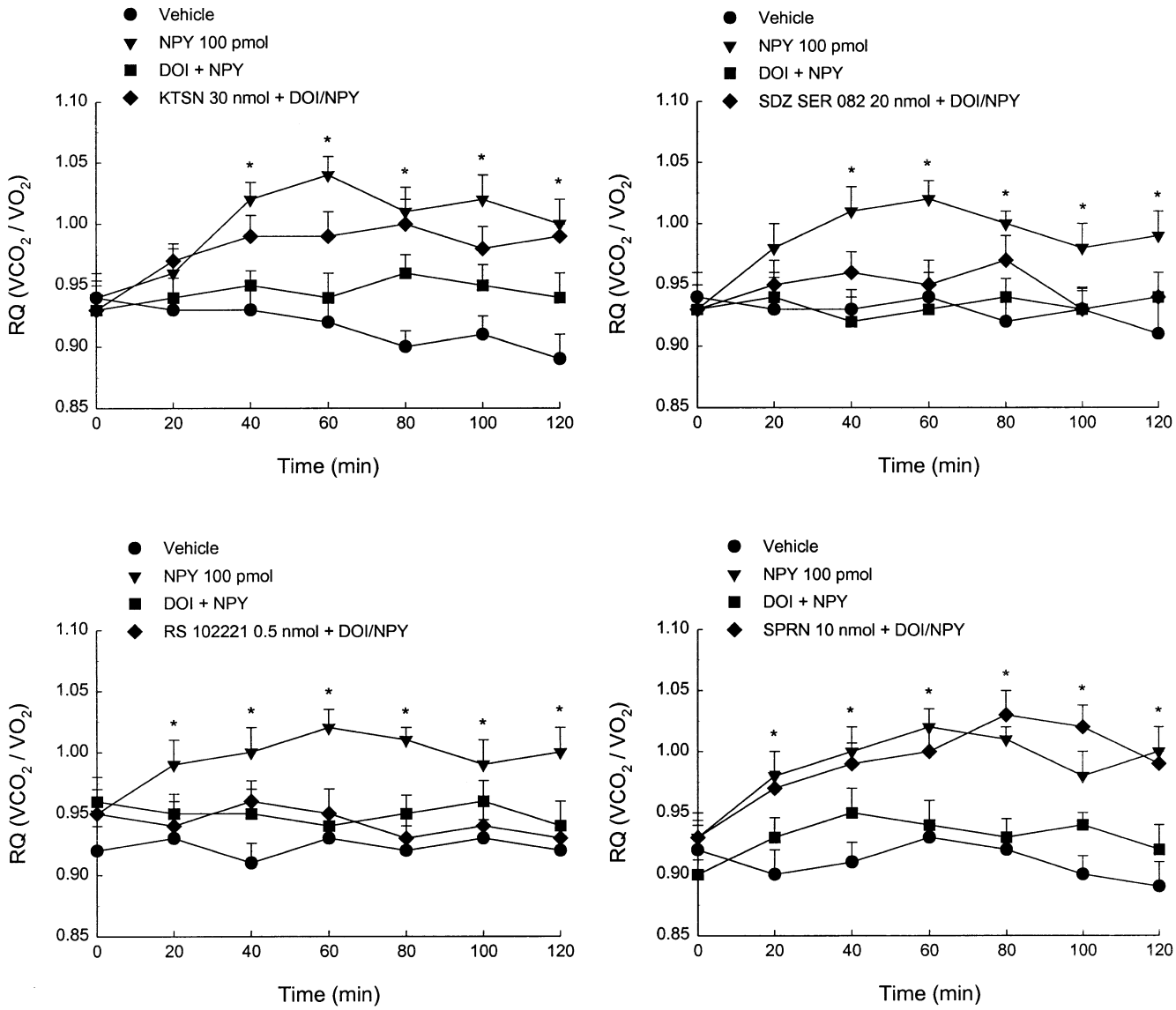

Fig. 4. PVN injection of SPRN and KTSN blocked the inhibitory effect of DOI (5 nmol) on NPY (100 pmol)-induced changes in energy substrate utilization (RQ). RS 102221 and SDZ SER 082 were ineffective in altering DOI's action. RQs (shown here as mean ± S.E.M.) were monitored over the initial 2 h of the dark cycle. Data were analyzed by repeated-measures ANOVA and Tukey tests. * $P < .05$ compared to Veh.

doses of SPRN and KTSN attenuated the inhibitory effect of DOI within 20 and 40 min of treatment, respectively.

4. Discussion

Previous reports suggest that hypothalamic NPY and 5-HT interact antagonistically in the control of ingestive behavior. NPY is found in high concentrations within medial hypothalamic neurons where it coexists with 5-HT (DeQuidt and Emson, 1986; Hokfelt et al., 1987). In contrast to the marked hyperphagia evoked by NPY (Currie and Coscina, 1995, 1996b, 1997; Leibowitz and Alexander, 1991; Stanley and Leibowitz, 1985; Stanley et al., 1986, 1989), 5-HT and its agonists suppress food intake (Currie, 1996; Currie and Coscina, 1996a; Curzon, 1990; DeQuidt and Emson, 1986; Dourish, 1992; Fletcher et al., 1992; Grignaschi et al., 1995; Leibowitz et al., 1989; Vickers et al., 2000). More recent evidence illustrates that the direct 5-$HT_{2A/2C}$ receptor agonist DOI injected into the PVN but not the PFH or VMN inhibits the effects of NPY on energy intake and metabolism (Currie and Coscina, 1997; Grignaschi et al., 1996). Because DOI pretreatment in hypothalamic sites other than the PVN fails to alter NPY's effects on eating (Currie and Coscina, 1997), and given that NPY's potentiation of RQ is mediated within the PVN (Currie and Coscina, 1996b), it is argued that the PVN mediates the effects of NPY and 5-HT on feeding and metabolism.

The results of the present study are in agreement with previous work illustrating the interactive action of PVN

5-HT and NPY on food intake and substrate utilization. Our findings clearly indicate that only 5-$HT_{2A/2C}$ receptor stimulation effectively antagonizes the action of NPY. That is, DOI pretreatment alone inhibited NPY's actions. However, PVN injections of the 5-HT_{1A} receptor agonist 8-OH-DPAT, the 5-$HT_{1B/1A}$ agonist RU 24969, the 5-HT_{1D} agonist L-694,247, the 5-HT_{2B} agonist BW 723C86 and the 5-HT_{2C} agonist mCPP all failed to alter the effect of NPY on feeding and RQ. Moreover, SPRN, a 5-HT_{2A} antagonist, and KTSN, a 5-$HT_{2A/2C}$ antagonist, reversed the inhibitory action of DOI, while the 5-$HT_{2B/2C}$ antagonist SDZ SER 082 and the 5HT_{2C} antagonist RS 102221 were ineffective.

As indicated above, in one previous report, PVN injections of DOI attenuated NPY-stimulated eating (Currie and Coscina, 1997). However, DOI injections into either the PFH or the VMN were not found to alter feeding after subsequent administration of NPY during the mid-light cycle. The present results are consistent with a recent study confirming that DOI pretreatment antagonizes NPY hyperphagia, specifically at the start of the active cycle (Currie and Coscina, 1998). While DOI antagonized NPY-stimulated eating, pretreatment with the 5-$HT_{1B/2C}$ against TFMPP was ineffective (Currie and Coscina, 1998). Given that TFMPP binds to 5-HT_{2C} receptors, its inability to suppress NPY feeding suggests that DOI's antagonism is mediated by PVN 5-HT_{2A} receptors. This is consistent with the findings of the current study in which mCPP also failed to alter NPY's action. A similar effect of DOI was observed on alterations in RQ induced by PVN NPY infusion. Therefore, microinjection of DOI but not TFMPP inhibited the marked increases in RQ evoked by NPY administration into the PVN (Currie and Coscina, 1998). Collectively, our findings suggest that PVN 5-HT_{2A} receptors modulate the action of 5-HT on NPY-induced feeding and substrate utilization, specifically at the onset of the nocturnal period.

In addition to its orexigenic action, NPY activates the endocrine pancreas to alter insulin secretion (Abe et al., 1989; Moltz and MacDonald, 1985), decreases sympathetic nerve activity to interscapular brown adipose tissue (Billington et al., 1991; Dryden et al., 1994; Egawa et al., 1991) and stimulates the hypothalamic–pituitary–adrenal axis (HPA; Hanson and Dallman, 1995; Wahlestedt et al., 1987). PVN NPY injections stimulate adrenocorticotropin hormone secretion as well as hypothalamic corticotropin-releasing hormone (CRH) immunoreactivity (Harfstrand et al., 1987; Hass and George, 1987; Wahlestedt et al., 1987). The NPY-induced reduction in thermogenesis is consistent with recent anatomical evidence dissociating the feeding and hypothermic effects of NPY (Currie and Coscina, 1995). While these findings further implicate NPY in metabolic regulation, other work has shown that hypothalamic NPY levels decrease in response to treatment with 5-HT agonists or increase after administration of 5-HT antagonists (Dryden et al., 1993; Rogers et al., 1991; Smialowska and Legutko, 1991). Systemic administration of D-fenfluramine attenuates NPY-stimulated eating (Bendotti et al., 1987; Brown and Coscina, 1995), an effect mediated by extrahypothalamic PVN 5-HT_{1B} receptors (Grignaschi et al., 1995). Also, acute injection of the 5-HT_{1A} agonist flesinoxan stimulates eating and increases NPY levels in the PVN and arcuate nucleus (Dryden et al., 1996). Our data suggest that 5-HT_{2A} receptors, within the PVN, exert a modulatory role over NPY, specifically in relation to NPY's feeding and metabolic action. Several recent findings support such a relationship. For example, decreases in the density of striatal NPY immunoreactive neurons have been detected after partial 5-HT lesions (Compan et al., 1996). While no data are yet available on the 5-HT receptor subtype expressed by PVN NPY neurons, in one report, NPY immunoreactivity and 5-$HT_{2A/2C}$ binding in the cortex were detected following 5-HT depletion (Compan et al., 1998). No differences in 5-HT_{1A} and 5-$HT_{1B/1D}$ binding were observed. While it is likely that NPY/5-HT relationships are influenced by anatomical site, cellular milieu and neurotransmitter colocalization, these findings continue to support a potential interactive relationship between NPY and 5-HT mechanisms.

In the present study, the increases in RQ to values exceeding 1.0 reflect an increase in carbohydrate oxidation in favor of fat storage (Bray, 1989; Kleiber, 1975). The diversion of metabolism toward carbohydrate utilization and fat synthesis is consistent with the preferential increase in carbohydrate appetite elicited by PVN NPY (Jhanwar-Uniyal et al., 1993; Leibowitz et al., 1992). With respect to 5-HT, peripherally administered DOI and RU 24969 have been reported to evoke nocturnal hypophagia associated with an attenuation in the elevation of metabolic rate (VO_2) (Bovetto and Richard, 1995). In contrast, the 5-HT_{1A} agonist 8-OH-DPAT increased VO_2. These findings are consistent with other evidence implicating 5-HT in thermogenic and metabolic processes (LeFeuvre et al., 1991; Rothwell and LeFeuvre, 1992; Rothwell and Stock, 1987; Sakaguchi and Bray, 1989). Moreover, recent work indicates that DOI-induced hypophagia is mediated via 5-HT_{2A} activation of the HPA (Raghavendra and Kulkarni, 2000) and that α-helical-CRF^{9-41} attenuates the antagonism of DOI on NPY-stimulated eating (Grignaschi et al., 1996). This in turn appears to implicate the HPA and CRH in the feeding and energetic effects of 5-HT and NPY.

In conclusion, the present results provide continued support for a unique role of 5-HT in relation to its interaction with NPY. Specifically, our findings indicate that 5-HT_{2A} receptors, within the medial hypothalamic PVN, modulate the action of NPY on ingestive behavior and energy metabolism.

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