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Involvement of neuropeptide Y Y_1 receptors in the acute, chronic and withdrawal effects of nicotine on feeding and body weight in rats

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ABSTRACT

We investigated the role of neuropeptide Y Y_1 receptors in acute, chronic and withdrawal effects of nicotine with reference to feeding behavior. Rats were administered with nicotine, neuropeptide Y, neuropeptide Y Y_1 receptor agonist [Leu^{31},Pro^{34}]neuropeptide Y or antagonist BIBP3226 (N^2-diphenylacetyl)-N-[(4-hydroxyphenyl)-methyl]-D-arginine amide) via i.c.v. route, and food intake was measured at 2 and 6 h post-injection time-points. While acute nicotine or BIBP3226 reduced food intake, increase was observed following neuropeptide Y or [Leu^{31},Pro^{34}]neuropeptide Y. Nicotine-induced anorexia was antagonized by pre-treatment with neuropeptide Y or [Leu^{31},Pro^{34}]neuropeptide Y, and potentiated by BIBP3226. Furthermore, effects of chronic nicotine (i.p.) and its withdrawal, alone and in combination with BIBP3226 were evaluated with reference to feeding and body weight. Concurrent administration of BIBP3226 with nicotine prevented the development of tolerance to nicotine-induced anorexia, and withdrawal hyperphagia and weight gain. Moreover, acute BIBP3226 attenuated the hyperphagia following nicotine termination. Additionally, immunocytochemical profile of neuropeptide Y in the hypothalamus was studied following differential nicotine treatments. Acute nicotine treatment dramatically reduced neuropeptide Y immunoreactivity in the arcuate and paraventricular nuclei. Chronic nicotine administration decreased neuropeptide Y immunoreactivity in arcuate, but not in paraventricular nucleus. Nicotine withdrawal resulted in significant increase in the neuropeptide Y immunoreactivity in both the nuclei. Neuropeptide Y immunoreactivity in the lateral hypothalamus did not change following any of the treatments. The results suggest that neuropeptide Y in the arcuate and paraventricular nuclei of hypothalamus may be involved in acute, chronic and withdrawal effects of nicotine on the feeding behavior, possibly via neuropeptide Y Y_1 receptors.

1. Introduction

Nicotine is a well-known psychoactive constituent of tobacco. In laboratory animals, nicotine administration caused transient anorexia and persistent weight loss, whereas abstinence following chronic treatment produced hyperphagia and weight gain (Grunberg et al., 1984; Levin et al., 1987). While nicotine is known to affect multiple neurotransmitter systems that modulate feeding, various attempts have been made to define the role of major hypothalamic orexigenic peptide, neuropeptide Y. Endogenous neuropeptide Y is known to act via 6 types of receptors identified as neuropeptide Y Y_1-*y*6, and of these, neuropeptide Y Y_1 and Y5 receptors have been implicated in the regulation of food intake (Kanatani et al., 1996; Schaffhauser et al., 1997). However, overwhelming evidence suggest a predominant role of neuropeptide Y Y_1 receptors in the feeding behavior (Kanatani et al., 2000a,b; Turnbull et al., 2002; Beck, 2006). While neuropeptide Y-induced feeding was significantly suppressed in neuropeptide Y Y_1 receptor-knockout mice, the same was not altered in neuropeptide Y Y5 receptor-knockout animals (Kanatani et al., 2000a). Selective neuropeptide Y Y5 receptor antagonist did not alter normal feeding or neuropeptide Y-induced hyperphagia (Kanatani et al., 2000b; Turnbull et al., 2002). Moreover, low levels of neuropeptide Y Y5 receptors have been expressed in the hypothalamus (Widdowson et al., 1997; Dumont et al., 1998; Naveilhan et al., 1998).

Although the participation of neuropeptide Yergic system in the different actions of nicotine on feeding behavior is well documented, the reports are quite inconsistent. While Frankish et al. (1995) reported decreased neuropeptide Y levels in the hypothalamic arcuate and paraventricular nuclei following nicotine treatment in rats, opposite effects were reported by Li et al. (2000). Kramer et al. (2007) reported no change in the hypothalamic neuropeptide Y gene expression following nicotine administration. Treatment with nicotine decreased neuropeptide Y immunoreactivity in cells of arcuate nucleus and fibers of paraventricular nucleus (Jang et al., 2003). Following nicotine withdrawal, Fornari et al. (2007) reported increased hypothalamic neuropeptide Y mRNA. However, Jang et al. (2003) and Fornari et al. (2007) used fasted and obese animals respectively, and both conditions are known to elevate the hypothalamic neuropeptide Y expression (Davies and Marks, 1994; Levin,

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1999). Neuropeptide Y-induced feeding was inhibited by nicotine and that neuropeptide Y potentiated the nicotine withdrawal-produced hyperphagia (Bishop et al., 2002). However, this study used female rats, and as already noted by earlier workers, feeding and hypothalamic neuropeptide Y levels are influenced by the estrus cycle (Pelletier et al., 1992; Wingkar, 1992). Like native neuropeptide Y, neuropeptide Y Y_1 receptors have been studied following nicotine manipulations. However, these findings are also contradictory. For example, following nicotine treatment, while decreased hypothalamic neuropeptide Y Y_1 receptors population was reported by Kane et al. (2001), Kramer et al. (2007) observed no change in the receptors gene expression. In spite of these reports, the behavioral evidence pertaining to the involvement of neuropeptide Y Y_1 receptors in acute, chronic and withdrawal effects of nicotine on food intake and body weight have not yet been directly investigated.

Against this background, we were interested in clarifying the role of neuropeptide Y Y_1 receptors in the modulation of food intake and body weight during different conditions of nicotine treatment in sated male rats. To accomplish this, neuropeptide Y Y_1 receptor modulators were administered under the conditions of acute or chronic nicotine or withdrawal. We also investigated the response by the endogenous neuropeptide Y-containing elements in the arcuate, paraventricular and lateral hypothalamic nuclei employing immunocytochemistry coupled with morphometric analysis in these nicotine treated rats.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats weighing between 240 and 260 g were group housed in acrylic cages under constant room temperature (25 ± 2 °C), relative humidity (50–70%), and maintained on 12 h dark/light cycle, advanced by 6 h (lights on at 0000 h). Rat chow food (contains 3.30 kcal/g, with 72.1% carbohydrate, 23.4% protein and 4.5% fat) and drinking water were available *ad libitum*. All procedures employed in the present study were approved and carried out under strict compliance with Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, MS, India.

2.2. I.c.v. surgery and administration

The detailed procedure of the cannulation, i.c.v. drug administration and post-surgical care has been described earlier (Rao et al., 2003; Kokare et al., 2006). Briefly, a 24-gauge stainless steel guide cannula (C316G/Spc; Plastics One, Roanoke, VA, USA) was implanted into the right lateral ventricle using stereotaxic coordinates, −0.8 mm posterior, +1.3 mm lateral to midline and 3.5 mm ventral with respect to bregma (Paxinos and Watson, 1998). A stainless steel dummy cannula (C316DC/Spc) was used to maintain patency of guide cannula. After cannulation, animals were housed individually and allowed to recover for 7 days. During this period, those rats showing any neurological or motor deficits like impairment of locomotion, grooming, social interaction or occurrence of aggressiveness, handling-induced hyper-excitability and stereotype behavior were not included in the study (Goyal et al., 2006). Also, those animals losing more than 10% of their body weight and not regaining it during the recovery period were excluded from the study. I.c.v. injection of 5 µl volume was given to each rat using microliter syringe (Hamilton, Nevada) connected by polyethylene tubing to a 31-gauge internal cannula (C316I/Spc, inner diameter 0.12 mm and outer diameter 0.25 mm; Plastics One) that projected 0.5 mm below the guide cannula.

2.3. Drugs

All drugs used in this study were procured from Sigma, St. Louis, MO. Nicotine hydrogen tartrate (Cat. No. N5260), neuropeptide Y (Cat. No. N5017), neuropeptide Y Y_1 receptor agonist [Leu^{31},Pro^{34}]neuropeptide Y (Cat. No. N7768) or selective neuropeptide Y Y_1 receptor antagonist BIBP3226 (N^2-diphenylacetyl)-N-[(4-hydroxy-phenyl)-methyl]-D-arginine amide) (Cat. No. B174) were dissolved in double distilled water. The final dilutions were made in 0.9% saline or artificial cerebrospinal fluid (aCSF) for i.p. or i.c.v. administration respectively. The method of preparation of aCSF has already been described (Kokare et al., 2005, 2006).

2.4. Experimental design

Since rodents showed peak feeding activity during the dark phase (Brown et al., 1998), feeding studies were conducted in this phase. Before experimentation, cannulated rats were acclimatized to the testing environment for 7 days. During this period, they were kept individually in the experimental cages. Daily, the animals were weighed, restrained on platform, dummy cannula was removed gently and i.c.v. aCSF (5 µl) was administered slowly over the period of 1–2 min, and the dummy cannula was replaced. The same platform was used for the administration of drugs. The injections were given 10 min prior to the onset of the dark phase, and immediately the pre-weighed food pellets (approximately 30 g) were placed inside the hopper of cage. The food consumed by individual rat was quantified by weighing leftover food in the hopper. Weighing of the food at each time-point helped to minimize the nonspecific stress. Animals that showed the stable baseline food intake were selected. This procedure has already been standardized in our laboratory (Rao et al., 2003; Kokare et al., 2006; Meena et al., 2009). For acute and chronic studies, the rats were divided into different groups ($n = 8$ in each), and the experiments were conducted according to protocols given below.

2.4.1. Acute studies

Earlier studies employed parenteral route for the acute application of nicotine and reported anorexia in 24/48 h time frame (Frankish et al., 1995; Bishop et al., 2002). In the present study, i.c.v. route was used for the quick onset of action, and the range of doses, for nicotine, neuropeptide Y, [Leu^{31},Pro^{34}]neuropeptide Y or BIBP3226, were comparable to those used in earlier reports (Iitake et al., 1986; Kalra et al., 1991; Hla-Hla-Aye et al., 1998; Kask et al., 1998; Lambert et al., 1998).

In the first series of studies, different groups of rats were administered with aCSF (5 µl/rat, $n = 8$), nicotine (5–20 µg/rat, $n = 7$ per group), neuropeptide Y (0.04–0.16 ng/rat, $n = 6$ per group), [Leu^{31},Pro^{34}]neuropeptide Y (0.06–0.2 ng/rat, $n = 7$ per group) or BIBP3226 (0.001–0.01 ng/rat, $n = 8$ per group) by the i.c.v. route. Based on the results obtained in these experiments, subeffective and effective doses were determined. In combination studies, separate groups of animals were administered with neuropeptide Y (0.04 ng/rat, i.c.v., $n = 7$), [Leu^{31},Pro^{34}]neuropeptide Y (0.06 ng/rat, i.c.v., $n = 7$) or BIBP3226 (0.001 ng/rat, i.c.v., $n = 8$) 10 min prior to nicotine (5 or 10 µg/rat, i.c.v.). After the injections, rats were returned to their home cages and the cumulative food intake was measured at 2 and 6 h post-injection time-points.

2.4.2. Chronic studies

Administration of nicotine (4 mg/kg/day, i.p.) was confined to the dark phase, subdivided in four equal doses with an interval of 3 h between two consecutive injections. Parallel to nicotine treatments, control rats were given saline (0.25 ml, i.p.). Nicotine injected to each rat was calculated on the basis of its initial body weight. This strategy was also adopted in some recent studies (Bellinger et al., 2003; Kramer et al., 2007). There have been other compelling reasons as detailed below. It has been argued that people smoke in a constant range as a part of nicotine addiction or to maintain the body weight (Albanes et al., 1987; Fulkerson and French, 2003). Researchers have attempted to simulate this condition in the rodents by exposing them to a constant dose of cigarette smoke over a period of several days, and

observed anorexia and weight loss (Wager-Sradar et al., 1984; Chen et al., 2005, 2007). Rats kept on self-administration acquired nicotine in a similar range over a period of study (Valentine et al., 1997). Moreover, nicotine treatment significantly reduced the food intake in dark, but not in the light phase, and there was no compensatory rebound feeding during the light phase (Bellinger et al., 2003).

All animals were habituated to the 5 µl of aCSF (i.c.v.) at the onset of the dark phase and 0.25 ml of saline (i.p.) four times during the dark phase, for a period of 4 days. Treatments were continued for 3 more days to obtain the baseline food intake and body weight. Thereafter, different groups of rats were administered daily for 12 days with (1) aCSF + saline ($n=8$), (2) aCSF + nicotine (4 mg/kg, i.p., $n=7$), (3) BIBP3226 (0.001 ng/rat, i.c.v.) + saline ($n=8$), and (4) BIBP3226 (0.001 ng/rat, i.c.v.) + nicotine (4 mg/kg, i.p., $n=7$). The food intake and body weight were measured daily before the subsequent administrations.

2.4.3. Withdrawal studies

Different groups of rats were subjected to the following treatments to study the nicotine abstinence effects on feeding and body weight for 7 days, and their modulation by BIBP3226. Animals were administered daily with (1) aCSF + saline ($n=8$) throughout the length of the experiment (19 days), (2) aCSF + nicotine (4 mg/kg/day, i.p., $n=7$) for 12 days and then subjected to nicotine deprivation, but continued on aCSF + saline for a period of subsequent 7 days (these rats showed maximum hyperphagia and weight gain on day 4 following nicotine withdrawal), (3) BIBP3226 (0.001 ng/rat, i.c.v.) + saline ($n=8$) or nicotine (4 mg/kg/day, i.p., $n=7$) for 12 days, thereafter the treatment was terminated, but the rats continued to receive aCSF + saline for a period of subsequent 7 days, (4) aCSF + saline for 15 days and on day 16, they received BIBP3226 (0.001 or 0.004 ng/rat, i.c.v., $n=6$ per group) + saline (this time-point was chosen since it coincides with the withdrawal-induced peak hyperphagia and weight gain), and (5) aCSF + nicotine (4 mg/kg/day, i.p.) for the period of 12 days and then subjected to nicotine deprivation, but continued on aCSF + saline up to day 15 and injected with BIBP3226 (0.001 or 0.004 ng/rat, i.c.v., $n=6$–7) + saline on day 16.

At the end of all experiments, placement of the guide cannula was tested for accuracy (Kokare et al., 2005; Dandekar et al., 2008). Dilute India ink (5 µl) was injected by i.c.v. route and animals were euthanized by overdose of thiopentone sodium (60 mg/kg, i.p.; Abbott Pharmaceuticals, Mumbai, India). Immediately the brains were dissected out, and cut in coronal plane to verify the placement of guide cannula and distribution of ink in the ventricle. The data of animals with incorrect placement were excluded from the study.

2.4.4. Immunocytochemistry

Brain sections of different groups of rats receiving acute (2 days) and chronic (12 days) nicotine (4 mg/kg/day, i.p.), as well as its withdrawal (day 4 following termination of chronic nicotine), and saline injected control animals were employed for immunostaining ($n=6$ per group). Briefly, rats were anesthetized with thiopentone sodium, perfused transcardially with heparinized phosphate-buffered saline (PBS; pH 7.4) for 30 s followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 10–15 min. The brains were post-fixed in the same fixative overnight, cryoprotected in 30% sucrose solution and serially sectioned on a cryostat (Leica, model CM1850) at 30 µm thickness in the coronal plane and collected in PBS. Sections were processed for neuropeptide Y immunolabeling using the streptavidin–biotin-peroxidase method standardized earlier in our laboratory (Sakharkar et al., 2005; Dandekar et al., 2008). Briefly, tissue sections were washed with PBS for 15 min and incubated in polyclonal primary antibodies against neuropeptide Y (Sigma, Cat. No. N9528) diluted in PBS containing 2% normal horse serum, 0.2% Triton X-100, 0.2% Kodak PhotoFlo solution and 0.08% sodium azide at 1:5000 dilution for 2 days at 4 °C. The sections were washed in PBS for 10 min and incubated with biotinylated goat anti-rabbit IgG (Sigma, Cat. No. B6648) for 2 h at room temperature. The sections were then washed in PBS for 10 min, incubated in streptavidin peroxidase (Sigma, Cat. No. E8386) for 2 h at room temperature and again washed with PBS. For visualization of the antigen–antibody complex, the sections were incubated for 2–3 min in a solution containing 0.03% hydrogen peroxide that served as substrate and 3-amino-9-ethyl-carbazole as chromogen (Sigma, Cat. No. AEC101). Reddish-brown precipitate indicated the presence of antigen in the sections. Finally, sections were washed with double distilled water and mounted in glycerol-gelatin.

To ensure reliable comparisons among different groups and maintain stringency in tissue preparation and staining conditions, sections from the brains of various groups were processed at the same time under identical conditions. Omission of primary antibody and replacement with bovine serum albumin produced no immunoreaction. In preadsorption controls, application of 1 ml diluted antibody pre-incubated with neuropeptide Y at 10^{-5} M for 24 h completely blocked the immunoreaction. This procedure has already been standardized in our laboratory (Sakharkar et al., 2005).

2.4.5. Morphometric analysis

Brain sections showing neuropeptide Y immunoreactivity in the arcuate, paraventricular and lateral hypothalamic nuclei were subjected to the analyses. The images (×480) were analyzed using Leitz-LaborLux S microscope, CCD video camera system (JVC, Japan) and Leica-Qwin Standard software (version 3). The method has already been standardized in our laboratory (Dandekar et al., 2008). The area (μm^2) covered by neuropeptide Y immunoreactive product was estimated from the transverse sections passing through the arcuate, paraventricular and lateral hypothalamic nuclei of the acute and chronic nicotine treated, and withdrawal rats. The images of neuropeptide Y immunoreactive area were digitized, the background was considered as threshold, and area occupied by immunostained cells and/or fibers were measured based on individual pixel intensity in the control, acute and chronic nicotine treated, and nicotine withdrawal rats. The neuropeptide Y immunoreactive product above the threshold was filled with overlaid color, and the area of the color overlay was automatically obtained using Leica QWin Standard software. The measurements were taken from predetermined fields for each subregion on either side from each brain. The data from all animals in each group were pooled separately and the mean ± standard error of mean (S.E.M.) was calculated.

2.5. Statistical analyses

The data are presented as mean ± S.E.M. Data obtained from acute studies, withdrawal protocols and morphometric analyses were determined using one-way analysis of variance (ANOVA) followed by post-hoc analyses of significance with Student–Newman–Keuls test. The data from chronic studies were analyzed by two-way ANOVA and individual means were compared by post-hoc Bonferroni test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Effect of acute treatments of nicotine or neuropeptide Yergic agents on food intake

While nicotine (10–20 µg/rat, i.c.v.) or BIBP3226 (0.004–0.01 ng/rat, i.c.v.) reduced, neuropeptide Y (0.08–0.16 ng/rat, i.c.v.) or [Leu^{31},Pro^{34}] neuropeptide Y (0.1–0.2 ng/rat, i.c.v.) increased the cumulative food intake (Table 1). The effects of these agents were found to be significant at 2 and 6 h time-points following the injections. Nicotine at 10 and 20 µg dose significantly inhibited food intake at 2 h [$F(3,28)=7.40$, $P<0.001$] and 6 h [$F(3,28)=4.66$, $P<0.01$]. As compared to aCSF-treated control rats, nicotine at the dose of 10 µg, decreased food intake by 1.46 g

Table 1
Effect of acute nicotine or neuropeptide Yergic agents on the cumulative food intake.

Treatment (i.c.v./rat)	Food intake (g ± S.E.M.)	
	2 h	6 h
aCSF	4.14 ± 0.36	8.31 ± 0.52
Nicotine		
5 μg	3.50 ± 0.35	7.63 ± 0.39
10 μg	2.68 ± 0.33[a]	6.73 ± 0.29[a]
20 μg	2.12 ± 0.23[b]	6.29 ± 0.41[a]
Neuropeptide Y		
0.04 ng	4.54 ± 0.35	8.95 ± 0.31
0.08 ng	5.71 ± 0.42[a]	9.93 ± 0.34[a]
0.16 ng	6.95 ± 0.42[c]	10.91 ± 0.38[b]
[Leu^{31},Pro^{34}]neuropeptide Y		
0.06 ng	4.92 ± 0.22	9.18 ± 0.33
0.1 ng	5.50 ± 0.49[a]	10.21 ± 0.23[a]
0.2 ng	6.35 ± 0.33[b]	10.72 ± 0.53[b]
BIBP3226		
0.001 ng	3.61 ± 0.24	7.32 ± 0.33
0.004 ng	3.03 ± 0.24[a]	6.57 ± 0.38[b]
0.01 ng	2.56 ± 0.21[b]	6.09 ± 0.27[b]

Different groups of rats were administered with aCSF (5 μl/rat), nicotine (5–20 μg/rat), neuropeptide Y (0.04–0.16 ng/rat), [Leu^{31},Pro^{34}]neuropeptide Y (0.06–0.2 ng/rat) or BIBP3226 (0.001–0.01 ng/rat) by i.c.v. route at the onset of dark cycle. The cumulative food intake was measured at 2 and 6 h post-injection time-points. The data represent mean ± S.E.M. for each group at respective time-points. [a]$P<0.05$, [b]$P<0.01$ or [c]$P<0.001$ vs. aCSF.

and 1.58 g at 2 and 6 h respectively, while at 20 μg dose, the reduction of 2.02 g was observed at both the time-points. Similarly, BIBP3226 (0.004 and 0.01 ng) significantly decreased food intake at 2 h [$F(3,31)=6.26$, $P<0.002$] and 6 h [$F(3,31)=6.07$, $P<0.002$] time-points. BIBP3226, at the dose of 0.004 ng reduced food intake from 4.14 g and 8.31 g to 3.03 g and 6.57 g at 2 and 6 h respectively, while at 0.01 ng BIBP3226, food intake was reduced to 2.56 g and 6.09 g at 2 and 6 h time-point respectively. However, at lower dose, nicotine (5 μg) or BIBP3226 (0.001 ng) did not show any significant effect ($P>0.05$) at both the time-points.

On the other hand, administration of neuropeptide Y (0.08–0.16 ng/rat, i.c.v.) or [Leu^{31},Pro^{34}]neuropeptide Y (0.1–0.2 ng/rat, i.c.v.) resulted in a dose dependent stimulation of food intake. Neuropeptide Y at the dose of 0.08 and 0.16 ng significantly increased food intake at 2 h [$F(3,25)=10.29$, $P<0.0002$] and 6 h [$F(3,25)=7.24$, $P<0.001$] time-points. As compared to food intake in control rats, neuropeptide Y at the dose of 0.08 ng triggered an increase of 1.57 g and 1.62 g, whereas 0.16 ng dose by 2.81 g and 2.6 g at 2 and 6 h respectively. Likewise, administration of [Leu^{31},Pro^{34}]neuropeptide Y at 0.1 and 0.2 ng dose significantly increased food intake at 2 h [$F(3,28)=6.50$, $P<0.002$] and 6 h [$F(3,28)=6.28$, $P<0.002$] time-points. In terms of net food intake, 0.1 ng [Leu^{31},Pro^{34}] neuropeptide Y increased food intake by 1.36 g and 1.9 g at 2 h and 6 h time-points, while at higher dose (0.2 ng), an increment of 2.21 g and 2.41 g was observed at both the time-points, respectively. The lower dose of neuropeptide Y (0.04 ng) or [Leu^{31},Pro^{34}]neuropeptide Y (0.06 ng) failed to exhibit significant effect ($P>0.05$) at both the time-points.

3.2. Effect of acute treatment of neuropeptide Y, [Leu^{31},Pro^{34}]neuropeptide Y or BIBP3226 on the anorectic action of nicotine

The neuropeptide Yergic agents at 2 and 6 h post-injection time-points influenced the anorexia following acute nicotine. The anorectic effect of nicotine was prevented by 10 min prior treatment with neuropeptide Y or [Leu^{31},Pro^{34}]neuropeptide Y (Fig. 1). Nicotine (10 μg/rat, i.c.v.)-induced anorexia was significantly reversed by microinjection of subeffective neuropeptide Y (0.04 ng/rat, i.c.v.) at 2 h [$F(3,27)=4.57$, $P<0.01$] and 6 h [$F(3,27)=4.72$, $P<0.009$] time-points, as neuropeptide Y in combination with nicotine elevated food

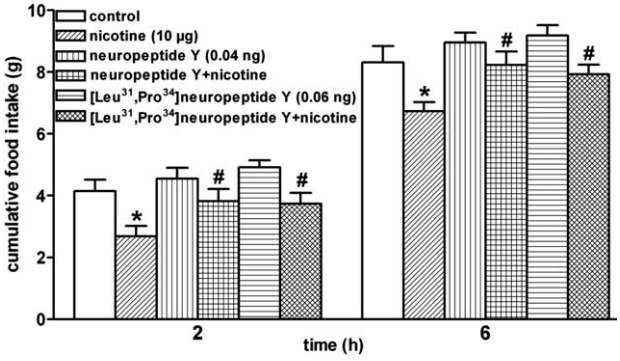

Fig. 1. Effect of neuropeptide Y and [Leu^{31},Pro^{34}]neuropeptide Y on the anorectic action of nicotine. Different groups of rats were administered with aCSF + aCSF (control), aCSF + nicotine (10 μg/rat, i.c.v.), neuropeptide Y (0.04 ng/rat, i.c.v.) + aCSF or nicotine (10 μg/rat, i.c.v.) and [Leu^{31},Pro^{34}]neuropeptide Y (0.06 ng/rat, i.c.v.) + aCSF or nicotine (10 μg/rat, i.c.v.) at the onset of the dark phase. The cumulative food intake (g) was measured at 2 and 6 h post-injection time-points. Each column and bar represents mean ± S.E.M. *$P<0.05$ vs. control and #$P<0.05$ vs. nicotine.

consumption by 1.14 g and 1.5 g respectively. Similarly, [Leu^{31},Pro^{34}] neuropeptide Y (0.06 ng/rat, i.c.v.) antagonized the anorectic effect of nicotine at 2 h [$F(3,28)=7.68$, $P<0.0008$] and 6 h [$F(3,28)=6.49$, $P<0.002$] by 1.05 g and 1.19 g respectively.

The nicotine-induced anorexia was greatly potentiated by prior administration of BIBP3226 (Fig. 2). Lower dose of nicotine (5 μg/rat, i.c.v.) or BIBP3226 (0.001 ng/rat, i.c.v.) was devoid of inhibitory effect on food intake ($P>0.05$). However, on prior treatment of BIBP3226 (0.001 ng), nicotine (5 μg) treated rats showed decrease food intake by 1.11 g at 2 h [$F(3,30)=5.72$, $P<0.003$] and 1.81 g at 6 h [$F(3,30)=7.12$, $P<0.001$] time-points. Also, the anorexia following higher dose of nicotine (10 μg), was potentiated by BIBP3226 (0.001 ng) at 2 h [$F(3,30)=16.61$, $P<0.0001$] and 6 h [$F(3,30)=9.41$, $P<0.0001$], as BIBP3226 in combination with nicotine reduced food intake by 1.15 g and 1.29 g respectively.

3.3. Effect of concomitant BIBP3226 on nicotine-produced tolerance to anorexia, and withdrawal hyperphagia

During chronic nicotine (4 mg/kg/day) treatment for 12 days, significant reduction in the food intake was observed from days 1 to 6 as compared to that in the control rats (Fig. 3). Two-way ANOVA revealed a significant interaction between variables nicotine treatment and duration in days [$F(21,286)=8.80$, $P<0.0001$], and treatment [$F(1,286)=14.96$, $P<0.0001$] and days [$F(21,286)=14.53$,

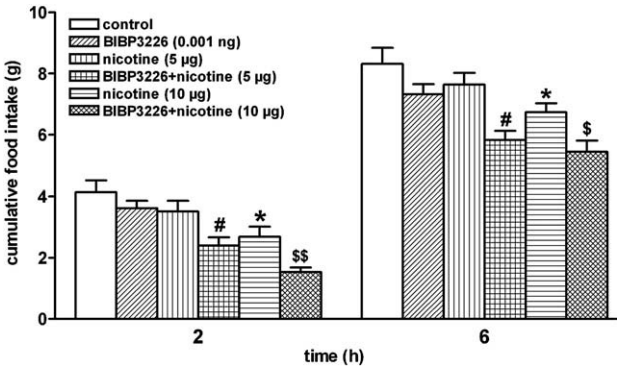

Fig. 2. Effect of BIBP3226 on the anorectic action of nicotine. Different groups of rats were treated with aCSF + aCSF (control), BIBP3226 (0.001 ng/rat, i.c.v.) + aCSF, aCSF or BIBP3226 (0.001 ng/rat, i.c.v.) + nicotine (5 or 10 μg/rat, i.c.v.) at the onset of the dark phase. The cumulative food intake (g) was measured at 2 and 6 h post-injection time-points. Each column and bar represents mean ± S.E.M. *$P<0.05$ vs. control; #$P<0.05$ vs. nicotine 5 μg and $$P<0.05$, $$$P<0.01$ vs. nicotine 10 μg.

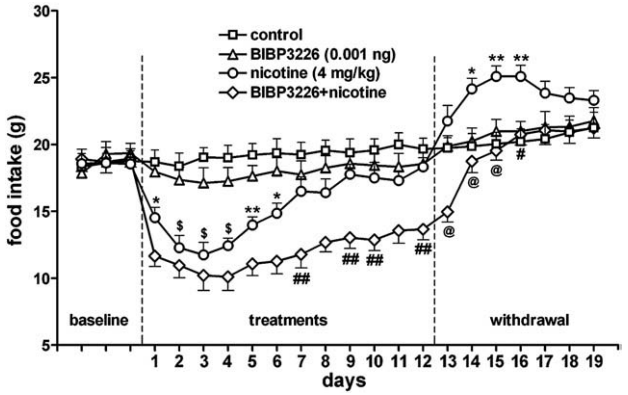

Fig. 3. Effect of concomitant BIBP3226 on the development of tolerance to anorectic action of nicotine and withdrawal hyperphagia. Initially, different groups of rats were injected daily with aCSF + saline, for 3 days, to obtain baseline food intake. Thereafter, rats were administered daily with aCSF + saline (control), BIBP3226 (0.001 ng/rat, i.c.v.) + saline, aCSF + nicotine (4 mg/kg, i.p.), or BIBP3226 (0.001 ng/rat, i.c.v.) + nicotine (4 mg/kg, i.p.) at the onset of the dark phase. Nicotine (4 mg/kg/day, i.p.) was administered in four equally divided doses throughout the dark phase. Food intake (g) was measured daily for 12 days. Following cessation of all the treatments, animals were treated with aCSF + saline and food intake was measured daily for the next 7 days. Each line and bar represents mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, $^{\$}P<0.001$ vs. control and $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{@}P<0.001$ vs. nicotine.

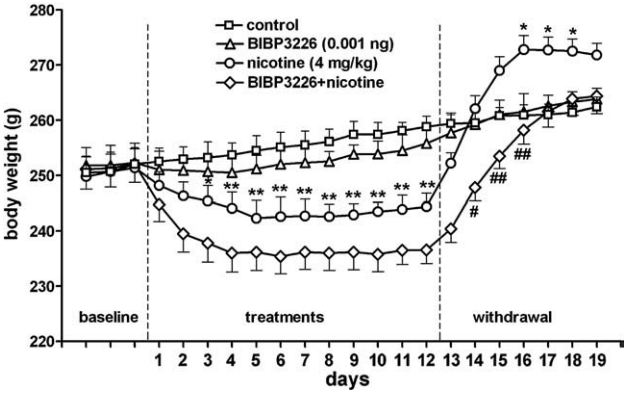

Fig. 4. Effect of concomitant BIBP3226 on the body weight lowering action of nicotine and withdrawal weight gain. Initially, different groups of rats were injected daily with aCSF + saline, for 3 days, to obtain baseline body weight. Thereafter, rats were administered daily with aCSF + saline (control), BIBP3226 (0.001 ng/rat, i.c.v.) + saline, aCSF + nicotine (4 mg/kg, i.p.), or BIBP3226 (0.001 ng/rat, i.c.v.) + nicotine (4 mg/kg, i.p.) at the onset of the dark phase. Nicotine (4 mg/kg/day, i.p.) was administered in four equally divided doses throughout the dark phase. Body weight (g) was measured daily for 12 days. After termination of all treatments, animals were treated with aCSF + saline and body weights were measured daily for the next 7 days. Each line and bar represents mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$ vs. control and $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. nicotine.

$P<0.0001$]. Application of post-hoc Bonferroni test showed maximum anorexia between days 2 and 4 ($P<0.001$). However, rats progressively developed tolerance to the anorectic effect of nicotine from day 7 onwards, which is indicated by non-significant difference in food intake between control and nicotine treated rats ($P>0.05$). Cessation of nicotine administration on day 12 produced significant hyperphagic effect during the withdrawal session from days 2–4.

Daily injection of BIBP3226 (0.001 ng/rat, i.c.v.) for 12 days, did not influence the food intake (Fig. 3). However, at the same dose, BIBP3226 prevented the development of tolerance to the anorectic action of nicotine. Application of two-way ANOVA revealed a significant interaction between BIBP3226 + nicotine treatment and duration in days [$F(21,264) = 2.44$, $P<0.0001$], and treatment [$F(1,264) = 140.6$, $P<0.0001$] and days [$F(21,264) = 42.95$, $P<0.0001$]. The post-hoc Bonferroni test revealed significant reduction in food intake in BIBP3226 + nicotine treated rats as compared to that in nicotine treated animals on days 7, 9, 10 and 12 ($P<0.05$). Moreover, termination of BIBP3226 or BIBP3226 + nicotine treatment did not induce withdrawal hyperphagia.

3.4. Effect of concomitant BIBP3226 on weight reducing effect of nicotine, and withdrawal-induced weight gain

The pattern of changes obtained in the experiment of food intake, were not entirely reflected on the body weight data (Fig. 4). While chronic nicotine administration showed persistent weight loss, cessation of treatment produced weight gain. Two-way ANOVA showed a significant interaction between variables nicotine treatment and duration in days [$F(21,286) = 7.4$, $P<0.0001$], and treatment [$F(1,286) = 30$, $P<0.0001$] and days [$F(21,286) = 17$, $P<0.0001$]. As compared to control rats, significant reduction in body weight was observed from day 3 onwards during chronic nicotine treatment, and maximum weight loss was recorded from day 4 ($P<0.01$). Termination of nicotine administration on day 12 produced significant weight gain from days 4–6 during the withdrawal period.

BIBP3226 (0.001 ng/rat/day, i.c.v.) per se did not alter body weight throughout chronic treatment and withdrawal session (Fig. 4). The concomitant treatment of BIBP3226 failed to alter the effect of nicotine on body weight significantly, but prevented the nicotine withdrawal weight gain. Application of two-way ANOVA revealed a significant interaction between BIBP3226 + nicotine treatment and duration in days [$F(21,264) = 1.2$, $P<0.0001$], and treatment [$F(1,264) = 73$, $P<0.0001$] and days [$F(21,264) = 28$, $P<0.0001$]. Though concomitant administration of BIBP3226 potentiated the weight lowering effect of nicotine, the difference was not statistically significant ($P>0.05$) during 12 days treatment period. Moreover, termination of the BIBP3226 + nicotine treatment on day 12, did not produce weight gain throughout the withdrawal session.

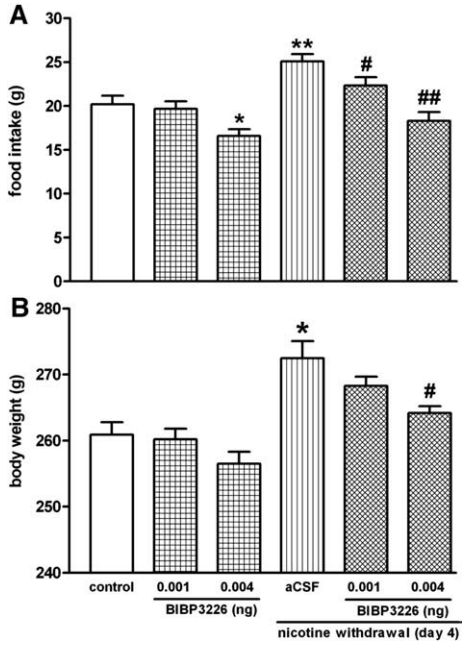

Fig. 5. Effect of BIBP3226 on nicotine withdrawal-induced hyperphagia (A) and weight gain (B). Control rats were injected with aCSF + saline throughout the length of the experiment (19 days). Different groups of animals were administered aCSF + saline for 15 days, and on day 16, rats received acute BIBP3226 at 0.001 or 0.004 ng dose. In other groups, rats received aCSF + nicotine (4 mg/kg/day, i.p.) for 12 days and BIBP3226 (0.001 or 0.004 ng/rat, i.c.v.) was administered on day 4 following nicotine withdrawal. Each column and bar represents the mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.001$ vs. control and $^{\#}P<0.05$, $^{\#\#}P<0.001$ vs. aCSF.

3.5. Effect of acute BIBP3226 on nicotine withdrawal-induced hyperphagia and weight gain

Fig. 5 depicts the effect of acute BIBP3226 (0.001 and 0.004 ng/rat, i.c.v.) on food intake (A) and body weight (B) following nicotine withdrawal. Cessation of chronic nicotine produced maximum hyperphagia and weight gain ($P<0.001$) on day 4 as compared to that in the control rats. Administration of vehicle for 15 days, followed by acute BIBP3226 (0.004 ng/rat, i.c.v.) on day 16, significantly suppressed the food intake ($P<0.05$), without altering the body weight ($P>0.05$). At lower dose (0.001 ng/rat, i.c.v.), BIBP3226 did not affect both the variables ($P>0.05$). BIBP3226 at 0.001 or 0.004 ng dose reversed the nicotine withdrawal hyperphagia [$F(5,40)=10.65$, $P<0.0001$], whereas only higher dose of BIBP3226 (0.004 ng) attenuated the weight gain [$F(5,40)=10.28$, $P<0.0001$].

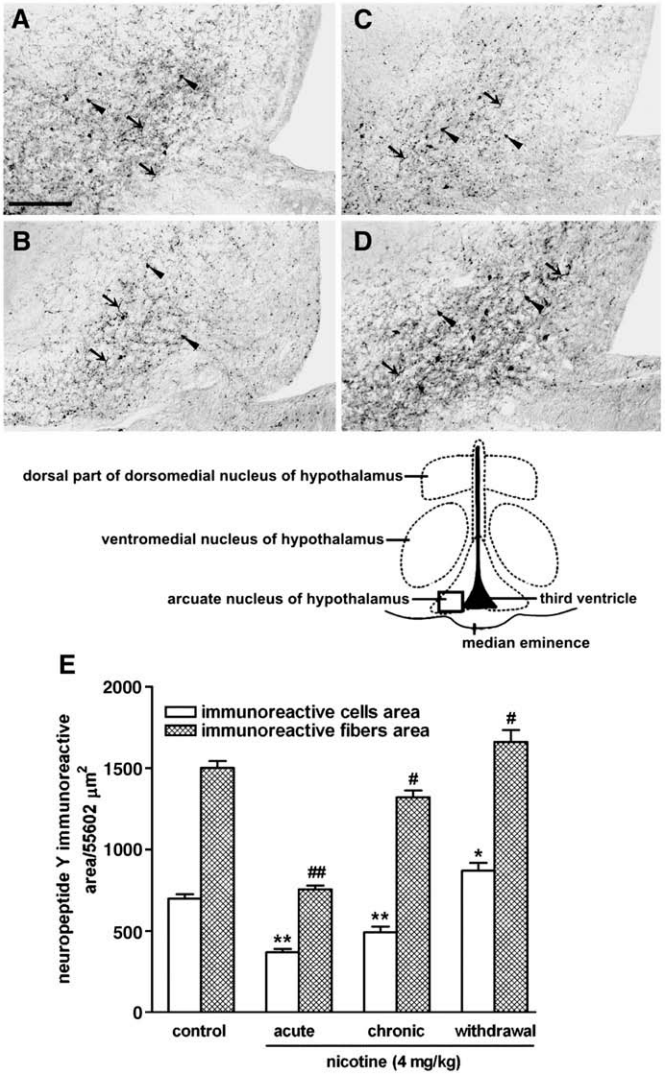

Fig. 6. Photomicrographs showing neuropeptide Y immunoreactive cells (arrowheads) and fibers (arrows) in the hypothalamic arcuate nucleus of control (A), acute nicotine treated (B), chronic nicotine treated (C) and nicotine-withdrawn (D) groups. The outline of the transverse section through the brain (coordinates: −2.56 mm with reference to bregma, Paxinos and Watson, 1998) indicates the region of the arcuate nucleus (square, not to scale) from which the measurements were collated. Semiquantitative morphometric analysis of neuropeptide Y immunoreactivity is summarized in diagram E. Each column and bar represents mean ± S.E.M. $^{\#}P<0.05$, $^{*}P<0.01$, $^{\#\#}P<0.001$ and $^{**}P<0.001$ vs. respective control. Scale bar = 200 µm.

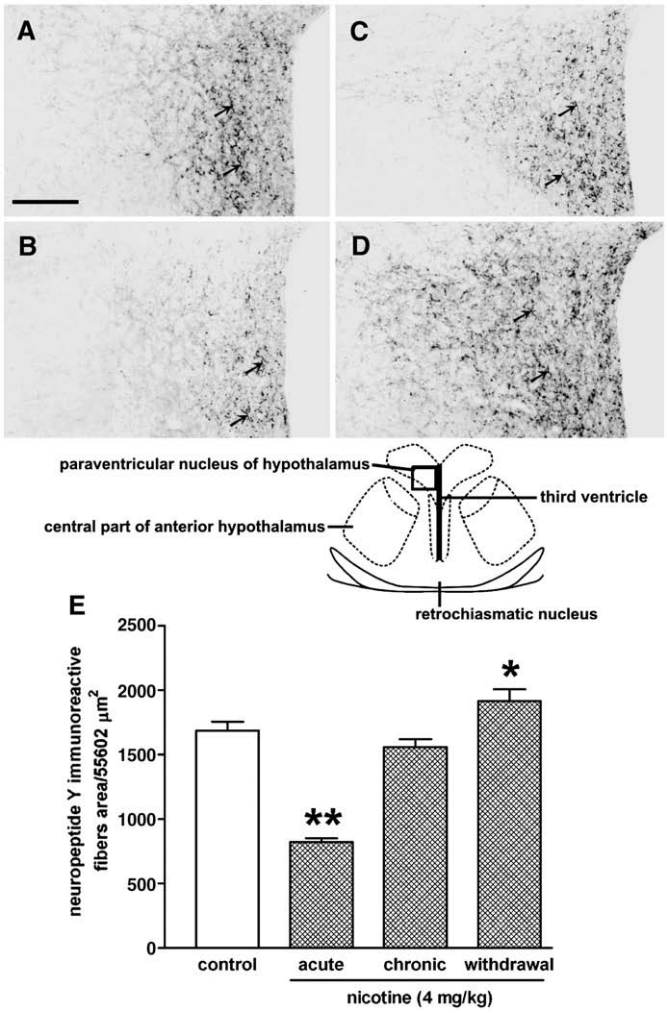

Fig. 7. Photomicrographs showing neuropeptide Y immunoreactive fibers (arrows) in the hypothalamic paraventricular nucleus of control (A), acute nicotine treated (B), chronic nicotine treated (C) and nicotine-withdrawn (D) groups. The outline of the transverse section through the brain (coordinates: bregma −1.80 mm, Paxinos and Watson, 1998) indicates the region of the paraventricular nucleus (square, not to scale) from which the measurements were collated. Semiquantitative morphometric analysis of neuropeptide Y immunoreactivity is summarized in diagram E. Each column and bar represents mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.001$ vs. control. Scale bar = 200 µm.

3.6. Effect of acute and chronic nicotine treatment, and withdrawal on neuropeptide Y immunoreactivity

3.6.1. Arcuate nucleus of hypothalamus

The arcuate nucleus in the brain of control rats showed several neuropeptide Y immunoreactive cells and fibers (Fig. 6A). Acute treatment with nicotine (4 mg/kg/rat, i.p., 2 days) resulted in dramatic reduction in the neuropeptide Y immunoreactive cells by 47% and fibers by 50% ($P<0.001$) (Fig. 6A, B, E). Similarly, chronic nicotine treatment at the same dose for 12 days, decreased the neuropeptide Y immunoreactive cells by 30% and fibers by 12% as compared to those in control animals ($P<0.05$) (Fig. 6A, C, E). A comparison of the neuropeptide Y immunoreactive area in the acute and chronic nicotine treated rats was also undertaken. Following chronic nicotine treatment, the immunoreactivity in the cells and fibers was increased by 34% and 75% respectively, as compared to those in rats on acute treatment (Fig. 6B, C, E). On day 4 following nicotine withdrawal, the neuropeptide Y immunoreactive area of cells and fibers showed an increase of about 77% and 26% respectively, as compared to those in the chronic nicotine treated rats (Fig. 6C–E).

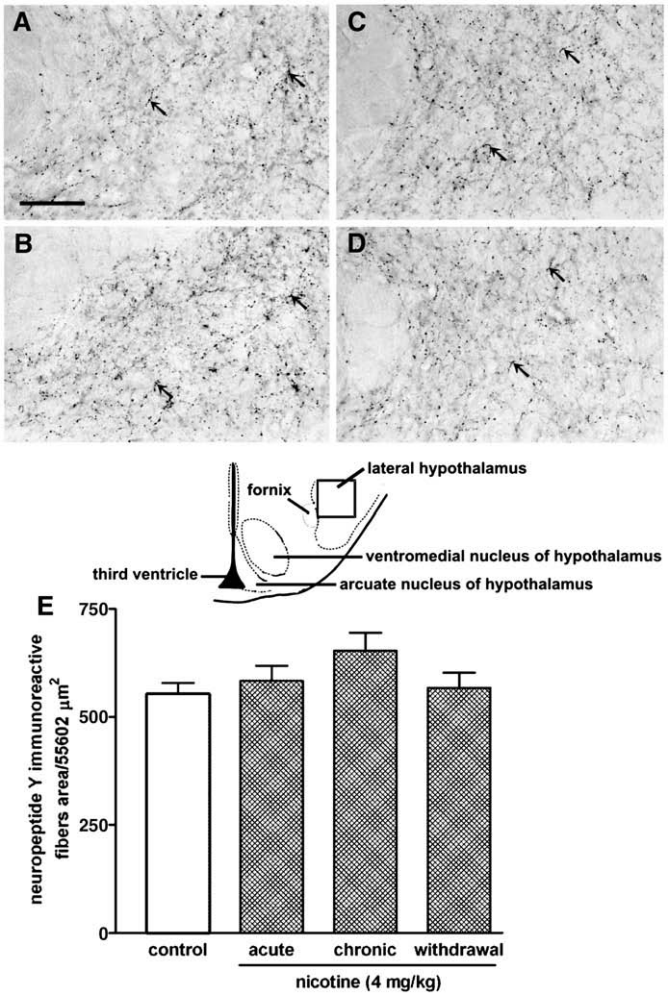

Fig. 8. Photomicrographs showing neuropeptide Y immunoreactive fibers (arrows) in the lateral hypothalamus of control (A), acute nicotine treated (B), chronic nicotine treated (C) and nicotine-withdrawn (D) groups. The outline of the transverse section through the brain (coordinates: bregma −2.56 mm, Paxinos and Watson, 1998) indicates the region of the lateral hypothalamus (square, not to scale) from which the measurements were collated. Semiquantitative morphometric analysis of neuropeptide Y immunoreactivity is summarized in diagram E. Each column and bar represents mean ± S.E.M. Scale bar = 50 µm.

3.6.2. Paraventricular nucleus of hypothalamus

The paraventricular nucleus of hypothalamus in the brain of control rats showed several neuropeptide Y immunoreactive fibers (Fig. 7A). The population of neuropeptide Y immunoreactive fibers was drastically decreased by 50% following acute nicotine treatment ($P<0.001$) (Fig. 7B, E). In chronic nicotine (12 days) treated rats, the population of neuropeptide Y immunoreactive fibers was similar to that in the control group ($P>0.05$) (Fig. 7C, E). However, nicotine withdrawal (day 4) increased neuropeptide Y immunoreactive fibers by 30% as compared to those in the chronic nicotine treated rats (Fig. 7C–E).

3.6.3. Lateral hypothalamus

The lateral hypothalamus in the brain of control rats showed moderate population of neuropeptide Y immunoreactive fibers (Fig. 8A). Acute or chronic nicotine treatment or withdrawal conditions did not influence the neuropeptide Y immunoreactivity in the fibers ($P>0.05$) and which remained similar to that in the respective control rats (Fig. 8B–E).

4. Discussion

The information on neuroanatomical substrate within which nicotine might modulate the neuropeptide Y system, leading to feeding-related outputs, is limited. Several studies reported the presence of nicotine binding sites in the appetite-controlling nuclei of hypothalamus like arcuate and paraventricular (Sharp et al., 1987; Okuda et al., 1993; Huang and Winger-Serhan, 2007). Moreover, the importance of neuropeptide Yergic input from arcuate to paraventricular nucleus in the regulation of feeding behavior is well established (Pedrazzini et al., 2003). Therefore, in this study, immunocytochemical profile of neuropeptide Y in the arcuate and paraventricular nuclei was evaluated following different nicotine treatment conditions.

The present investigation for the first time provides functional evidence pertaining to the involvement of neuropeptide Y Y_1 receptors in the modulation of feeding behavior by nicotine, or its withdrawal in sated rats. Acute nicotine administration suppressed the food ingestion at 2 and 6 h post-injection time-points. While treatment with neuropeptide Y or [Leu^{31},Pro^{34}]neuropeptide Y increased, BIBP3226 reduced the food intake in a dose dependent manner. Although nicotine and neuropeptide Yergic agents showed profound effects on the feeding over a period of 2 h, the effects were noticed at longer durations by others (Clark et al., 1984; Frankish et al., 1995; Kask et al., 1998; Beck, 2006; Kramer et al., 2007), and also in the present study. While nicotine-induced anorexia was prevented by neuropeptide Y and [Leu^{31},Pro^{34}]neuropeptide Y, BIBP3226 potentiated the same at 2 and 6 h post-injection time-points. These results suggest that, the acute anorectic action of nicotine may be mediated via neuropeptide Y Y_1 receptors.

While the data from different laboratories on the acute nicotine generated anorexia are quite consistent (Frankish et al., 1995; Chen et al., 2005; Kramer et al., 2007), there is some controversy regarding the response of hypothalamic neuropeptide Y-containing system. Reduced neuropeptide Y levels in the arcuate and paraventricular nuclei were reported following acute nicotine treatment in sated and food-deprived rats (Frankish et al., 1995; Jang et al., 2003). We also observed marked reduction (about 50%) in the neuropeptide Y immunoreactivity in the cells and/or fibers of arcuate and paraventricular nuclei following acute nicotine, however, Chen et al. (2005) reported no change in the hypothalamic neuropeptide Y content, and the discrepancy may be attributed to the differences in doses, regimen and routes of administration. These authors exposed the mice to cigarette smoke daily at three time-points in a 6 h duration that provided <1.2 mg of nicotine. This dose of nicotine is much lower as compared to that used in the present study (4 mg/kg/day). Furthermore, they exposed animals to smoke during the light phase when the feeding activity is at the minimum. On the other hand, nicotine administration in the present study was undertaken during the dark phase when the rodents show highest food consumption (Brown et al., 1998). Since the effects of nicotine on feeding are mainly noticed in the dark phase (Bellinger et al., 2003), it is possible that smoke exposure given by Chen et al. (2005) might be inadequate to exert its influence on the hypothalamic neuropeptide Y. Moreover, it has been reported that high dose of nicotine (12 mg/kg/day) decreased neuropeptide Y mRNA expression in the arcuate and paraventricular nuclei (Frankish et al., 1995). While Kramer et al. (2007) reported no change in the neuropeptide Y gene expression following acute nicotine (4 mg/kg/day), the same treatment in the present study caused reduction in the neuropeptide Y immunoreactivity in the arcuate and paraventricular nuclei. Taken together, it seems that acute nicotine treatment at high dose might suppress the transcription of hypothalamic neuropeptide Y gene, however, lower dose may promote the loss of the peptide from cells and fibers, which in turn might contribute to anorexia.

Several studies have reported the development of tolerance to the anorectic effect of nicotine during chronic treatment (Grunberg et al., 1984; Levin et al., 1987; Bishop et al., 2002; Bellinger et al., 2003). In the present study also, chronic nicotine treatment reduced food intake for the initial 6 days, which eventually returned to control level

indicating tolerance. Co-administration of BIBP3226 prevented the development of tolerance to the anorexic action of nicotine. Since BIBP3226 is a selective neuropeptide Y Y_1 receptor antagonist, the neuropeptide Y Y_1 receptors may be involved in this phenomenon. Neuropeptide Y Y_1 receptors are abundantly present on the neurons of paraventricular nucleus and mediate feeding-related information borne by the neuropeptide Y input from the arcuate nucleus (Pedrazzini et al., 2003). The observations on feeding, following acute as well as chronic nicotine treatments, appear directly correlated with the immunocytochemical data. Initially there was drastic reduction in the neuropeptide Y immunoreactivity in the arcuate and paraventricular nuclei, however, a trend towards return to control was noticed during chronic nicotine treatment. Neuropeptide Y immunoreactivity following chronic nicotine was increased by 34% and 75% in the cells and fibers of arcuate nucleus respectively, as compared to that in the acute treatment. Moreover, population of neuropeptide Y immunoreactive fibers in the paraventricular nucleus of chronic nicotine treated rats appeared similar to that in the control animals. This suggests that tolerance to anorexia during chronic nicotine may be due to the disinhibition of neuropeptide Y system in arcuate and paraventricular nuclei, which resulted in restoration of food intake to the normal level.

Data on the neuropeptide Y levels and gene expression following chronic nicotine treatment are inconsistent. Following chronic nicotine (12 mg/kg/day) treatment (Frankish et al., 1995) or 12 weeks smoke exposure (Chen et al., 2007), hypothalamic neuropeptide Y content was decreased. On the other hand, Frankish et al. (1995) reported increased neuropeptide Y mRNA in arcuate and paraventricular nuclei following chronic nicotine administration. Similar increase was reported by Li et al. (2000) following application of low dose (4 mg/kg/day) of nicotine during the light phase. On the contrary, nicotine (4 mg/kg/day) injected intermittently during the dark phase, did not influence the neuropeptide Y expression (Kramer et al., 2007). In the present study also, chronic administration of nicotine, in a similar dose regimen, resulted in the similar neuropeptide Y immunoreactivity profile as that in the control. It seems that low nicotine dose may have no effect on the hypothalamic expression of neuropeptide Y mRNA particularly during the dark phase. However, it may trigger a loss of the peptide initially, which may be restored to the normal during chronic treatment. This in turn may be reflected in the initial anorexia followed by restoration of normal food intake.

The pattern of changes on food intake observed during chronic nicotine administration was not entirely reflected on the body weight. In the present study, animals showed persistent body weight reduction from day 2 onwards during the chronic nicotine regimen. Similar observations have also been reported from earlier studies and attributed to the decreased body weight set-point (Frankhama and Cabanaca, 2003) or caloric intake (Chajek-Shaul et al., 1987), and increased energy expenditure (Frankish et al., 1995; Jensen et al., 1995; Bishop et al., 2004). Several studies have reported that, neuropeptide Y inhibits energy expenditure (Billington et al., 1991, 1994), increases carbohydrate intake and fat deposition, and promotes the weight gain (Stanley et al., 1986, 1989; Menéndez et al., 1990; Currie and Coscina, 1996). The present immunocytochemistry data suggest that the inhibitory effect of nicotine on the hypothalamic neuropeptide Y appears to be transient. Therefore, it seems that neuropeptide Y may contribute to the onset of the weight reducing effect of nicotine, and persistent weight loss could be attributed to the increased energy expenditure by nicotine as already mentioned.

In agreement with the previous reports (Grunberg et al., 1984; Levin et al., 1987), we observed increased food intake and body weight between 2 and 6 days after the termination of chronic nicotine treatment. Fornari et al. (2007) demonstrated increased hypothalamic neuropeptide Y mRNA expression following nicotine withdrawal. In the present study also, withdrawal of nicotine considerably increased neuropeptide Y immunoreactive cells and/or fibers in the arcuate as well as paraventricular nucleus. Comparisons of the neuropeptide Y immunoreactive cells/fibers population in arcuate and paraventricular nuclei in the chronic nicotine vs. nicotine-withdrawn rats permit some interesting conclusions. While there was 77% increase in the neuropeptide Y immunoreactivity in arcuate cells population, increase of about 25% was observed in the fibers of arcuate and paraventricular nuclei. The data suggest that nicotine withdrawal might eliminate the inhibitory effect of nicotine on neuropeptide Y system and this in turn might increase the neuropeptide Y input to paraventricular nucleus from arcuate nucleus. Thus, earlier proposed mechanism that chronic nicotine treatment might result in the adaptive changes in neuropeptide Y system, whose rebound may lead to hyperphagia and weight gain following withdrawal stands confirmed. This has also been supported by the report demonstrating potentiation of nicotine withdrawal hyperphagia following injection of neuropeptide Y in the paraventricular nucleus of hypothalamus (Bishop et al., 2002). Moreover, as mentioned above, decreased body weight set-point or caloric intake, and increased energy expenditure during nicotine treatment attributed to the weight loss. These effects might be diminished following nicotine withdrawal, and contribute to the weight gain. However, cessation of combination treatment of BIBP3226 and nicotine did not result in the withdrawal hyperphagia and weight gain. Furthermore, acute administration of BIBP3226 reversed the peak hyperphagia and weight gain induced by nicotine withdrawal. Taken together, these results indicate that neuropeptide Y Y_1 receptors may be involved in the neuropeptide Y mediated hyperphagia and weight gain following nicotine withdrawal within the framework of arcuate and paraventricular nuclei.

Neuropeptide Y is prominently seen in the arcuate, paraventricular and lateral hypothalamic nuclei. Since these areas also show nicotine binding sites (Clarke et al., 1985; Sharp et al., 1987; Broussolle et al., 1989; Leutje et al., 1990; Okuda et al., 1993; Huang and Winzer-Serhan, 2007), it is possible that nicotine may directly influence neuropeptide Y in the framework of these nuclear groups. Available data suggest that neuropeptide Y interrelates with other systems like anorectic peptide cocaine- and amphetamine-regulated transcript (CART) and leptin, mainly at the level of arcuate and paraventricular nuclei to regulate the energy homeostasis. CART peptide and leptin receptors are colocalized with neuropeptide Y in the paraventricular or arcuate nucleus (Satoh et al., 1997; Lambert et al., 1998; Baskin et al., 1999; Ur et al., 2002). Moreover, CART and leptin have an inverse association with neuropeptide Y with reference to the feeding (Wang et al., 1997; Lambert et al., 1998; Ahima et al., 1999; Jang et al., 2003). It is reported that, these systems are influenced by nicotine treatment at the level of arcuate and paraventricular nuclei (Jang et al., 2003; Kramer et al., 2007). However, neuropeptide Y content did not alter in the lateral hypothalamus following acute and chronic nicotine administrations (Frankish et al., 1995; Li et al., 2000). In the present study also, following differential exposure of nicotine, the profile of neuropeptide Y immunoreactive fibers in lateral hypothalamus did not change. Thus it can be argued that, neuropeptide Y in the lateral hypothalamus may not be involved in the actions of nicotine associated with the feeding behavior.

We suggest that, neuropeptide Y in the arcuate and paraventricular nuclei of hypothalamus, acting via neuropeptide Y Y_1 receptors, may serve as an important neuroanatomical substrate that processes the nicotine triggered responses on feeding behavior.

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