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Increases in Sucrose Consumption, But Not Ethanol Consumption, Following ICV NPY Administration

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SLAWECKI, C. J., M. BETANCOURT, T. WALPOLE, AND C. L. EHLERS. *Increases in sucrose consumption, but not ethanol consumption, following ICV NPY administration.* PHARMACOL BIOCHEM BEHAV **66**(3) 591–594, 2000.—Neuropeptide Y (NPY) is a centrally acting neuromodulator that influences both consummatory behaviors and anxiety. NPY's effects on feeding are primarily regulated through Y5 receptors in hypothalamic sites, whereas NPY-induced anxiolysis appears to be mediated by Y1 receptors in the amygdala. Recently, NPY has been postulated to play a role in the regulation of ethanol consumption. The present study assessed the influence of intracerebroventricular (ICV) administration of NPY on the consumption of 10% ethanol or 2% sucrose in rats. Male Wistar rats were trained to self-administer 10% ethanol using the sucrose-substitution procedure and then implanted with an intracerebroventricular (ICV) cannula. The effects of NPY (0–15 μg) on ethanol consumption and sucrose consumption were then examined. ICV NPY infusion had no significant effects on the consumption of 10% ethanol, however, NPY significantly increased the consumption of 2% sucrose, [$F(1, 11) = 6.18$, $p = 0.03$]. These data suggest that ethanol intake and sucrose intake are differentially regulated by NPY. It is hypothesized that ICV infusion of NPY may be affecting both Y1 and Y5 receptors producing increased consummatory drive and anxiolysis, two factors that have opposing effects on subsequent ethanol consumption. Therefore, additional studies including site specific injection of NPY will be necessary to provide further insight into the role of NPY on ethanol consumption. © 2000 Elsevier Science Inc.

Ethanol Sucrose Neuropeptide Y ICV

NEUROPEPTIDE-Y (NPY) is a well-characterized modulator within the central nervous system. NPY, as measured by immunoreactivity, has been shown to be present throughout the central nervous system with particularly high levels in the limbic system (2,17). Moderate to high levels of NPY are found in various nuclei of the hypothalamus, in the striatum, in the amygdala, and throughout the cortex (2,17). As this widespread distribution would suggest, NPY has been shown to play an important role in many behavioral and physiological processes including: feeding (6,22), anxiety (13,16,29), neuroendocrine responses of the hypothalamic–pituitary axis (7,21), and the regulation of circadian rhythms (1).

In previous studies we have assessed the common neurophysiological effects of ethanol and NPY (9,10). We have found that the effects of NPY, more than any other peptide thus far tested, resemble those of ethanol (9,10). However, whether NPY influences ethanol consumption directly is unclear. When administered intracerebroventricularly, NPY has been shown to increase feeding and drinking (6,22,23,27) and to decrease experimental measures of anxiety (14,16,29,36) as measured by the elevated plus maze and other behavioral paradigms. The consumption of ethanol is an ingestive behavior that one might expect to be regulated by central NPY mechanisms. Further, numerous studies have suggested that stress and anxiety influence the consumption of ethanol (3,4,24,30). Therefore, the dual action of NPY as a regulator of ingestive behavior and as an anxiolytic uniquely qualifies it to influence ethanol consumption.

There have been numerous reports that indirectly implicate NPY in the regulation of ethanol consumption or ethanol preference. Knockout mouse lines that lack NPY drink more ethanol and have a greater preference for ethanol than do wild-type mice (35). Quantative trait loci analyses in alcohol-preferring (P) and alcohol nonpreferring (NP) rats have re-

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vealed a locus with a LOD score greater than 8 encompassing a genomic region that holds the NPY gene (5). Our own studies suggest that P and NP rats differ in their regional concentrations of NPY in the brain (8,19) and in their neurophysiological responses to NPY (11). Taken together, these data suggest that differences in NPY activity may influence ethanol consumption and/or ethanol preference. The purpose of the present study was to preliminarily determine if intracerebroventricular administration of NPY alters ethanol drinking and/or sucrose drinking in the rat.

METHOD

Subjects

Male Wistar rats (n = 12) obtained from Charles River were used. Rats were housed two/cage in standard cages for the duration of the experiment. The vivarium was maintained on a 12 h light/dark cycle (lights on at 0600 h). Ad lib food and water were provided during the experiment, except when noted below. Animal care was in accordance with NIH and institutional guidelines.

Surgical Procedure

All rats were implanted with a 23-gauge cannula (Plastics One, Inc., Roanoke, VA) aimed at the lateral ventricle (AP + 0.6 mm, ML ± 2.0 mm, DV −3.2 mm) using the Pelligrino atlas (28) under pentobarbital anesthesia (50 mg/kg, IP). Atropine (0.06 mg, SC) was administered to minimize respiratory suppression.

Neuropeptide-Y (NPY) Administration

Rat NPY was synthesized at the Salk Institute Peptide Biology Laboratory courtesy of Dr. Jean Rivier. Solutions of NPY (0.0, 2.5, 5.0, and 15.0 μg/5 μl; 0–3 nmol) were prepared daily in sterile water prior to drinking sessions. A 5-μl volume was infused into the ventricle over a 1-min period. Injectors were kept in place for an additional 30 s following the infusion. A 10-min pretreatment time was chosen because previous studies have observed behavioral and/or EEG changes after similar pretreatment times (10,12,25).

General Procedure

Ethanol self-administration sessions were performed in plastic cages [2.5 cm (w) × 2.0 cm (h) × 4.5 cm (l)]. A 50-ml graduated cylinder with a Teflon drinking tube was mounted on each cage. Fifteen-minute self-administration sessions were run 5 consecutive days a week. Rats were trained to self-administer ethanol using a modified sucrose substitution procedure (32). The acquisition of drinking was facilitated with 23-h fluid restriction. Fluid restriction was terminated after all rats acquired drinking (>5 ml consumed/15 min) of 20% sucrose. Ad lib drinking conditions in the home cage were restored within 2–3 days of initial restriction. The following solutions were then presented consecutively across sessions to initiate ethanol drinking: 10% sucrose (10S) for three sessions, 10% sucrose/2% ethanol (10S2E) for two sessions, 10% sucrose/5% ethanol (10S5E) for two sessions, 10% sucrose/10% ethanol (10S10E) for two sessions, 5% sucrose/10% ethanol (5S10E) for two sessions, 2% sucrose/10% ethanol (2S10E) for five sessions and 1% sucrose/10% ethanol (1S10E) for five sessions. Ten percent ethanol (10E) was then presented for 5–10 sessions. All sucrose solutions were prepared weight/volume. All ethanol solutions were prepared volume/volume from 95% ethanol.

When ethanol drinking was reestablished after cannula implantation, NPY was administered twice a week Tuesday and Friday. This injection schedule minimized complications due to the potential long lasting effects of NPY by providing at least 72 h between injections. Injections were administered in a random fashion within the group. The order of infusion was: 1) 5 μg, Veh, 15 μg, 2.5 μg; or 2) 15 μg, 2.5 μg, Veh, 5 μg; or 3) Veh, 15 μg, 2.5 μg, 5 μg. All subjects received each dose. After examining NPY's effects on ethanol drinking, all rats were given access to 2% sucrose (2S) for 2 weeks. A single determination of the effects of 5.0 μg NPY on 2S intake was then examined to confirm NPY's effects on sucrose intake and feeding. Following the experiment, rats were administered a lethal dose of sodium pentobarbital (100 mg/kg, IP). Prior to decapitation, a 5μl ICV infusion of toluidene blue was administered to verify cannula placement. The brains were then frozen on dry ice and sectioned on a cryostat (60 μm). The presence of dye in the ventricles confirmed placement of the cannula in the ventricle.

Statistical Analysis

Statistical analysis was completed with the use of Systat for the Macintosh (Systat Inc). Self-administration data analyzed included the volume of ethanol and sucrose consumed and total session ethanol intake in grams/kilogram (g/kg) bodyweight. Changes in volume consumed and ethanol intake during the initiation of ethanol drinking were assessed using one-way repeated measures analysis of variance (ANOVA) with post hoc contrasts compared to the 10% ethanol condition. There were no apparent differences in the effects of NPY based on the order of infusion so data from each injection order were combined for statistical analysis. One-way repeated-measures ANOVA were used to assess changes in ethanol consumed and ethanol intake as a function of NPY dose with post hoc contrasts compared to the vehicle condition. A repeated-measures ANOVA was used to compare the volume of sucrose consumed between the baseline and NPY conditions. All repeated-measures ANOVA were adjusted using the Greenhouse-Geisser method.

RESULTS

At the start of the experiment, the weight of the rats ranged from 343–428 g (390 ± 28 g, mean ± SD). Over the course of the experiment (i.e., 15 weeks), there were significant increases in body weight, $F(1, 11) = 109.71$, $p < 0.0001$. Average weight at sacrifice was 593 ± 68 g (range = 499–716 g). The sucrose substitution procedure successfully initiated the consumption of 10% ethanol. There were significant changes in volume of each solution consumed, $F(7, 77) = 21.89$, $p < 0.0001$, and total ethanol intake, $F(6, 66) = 24.29$, $p < 0.0001$, during initiation of ethanol drinking. Ethanol intake increased as the ethanol concentration increased and then decreased as the concentration of sucrose decreased.

Average ethanol intake during the baseline session was 0.45 ± 0.06 g/kg (Table 1). Historical data from our laboratory using this self-administration paradigm in Wistar rats, and other rat strains, indicated blood ethanol levels of at least 10–15 mg/dl are achieved following consumption of approximately 0.50 g/kg. These blood levels are relatively low, but are still consistent with blood ethanol levels reported in other studies given similar ethanol intakes (31,33). The administration of NPY had no effect on the volume of ethanol consumed, $F(4, 44) = 1.56$, $p = 0.216$, as can be seen in Fig. 1. NPY also had no significant effects, $F(4, 44) = 1.48$, $p =$

TABLE 1

ETHANOL INTAKE OR SUCROSE INTAKE (GRAMS/KILOGRAM BODYWEIGHT, MEAN ± SEM) FOLLOWING THE INTRACEREBROVENTRICULAR ADMINISTRATION OF NPY (n = 12)

	Baseline	Vehicle	2.5 μg NPY	5.0 μg NPY	15.0 μg NPY
10% ethanol	0.45 ± 0.06	0.56 ± 0.10	0.40 ± 0.06	0.55 ± 0.07	0.49 ± 0.11
2% sucrose	0.42 ± 0.03	NA	NA	0.50 ± 0.05	NA

0.236, on total session ethanol intake (Table 1). In contrast, ICV infusion of 5.0 μg of NPY significantly increased, $F(1, 11) = 6.18$, $p = 0.03$, the consumption of 2% sucrose compared to baseline consumption (Fig. 1). There were no consistent effects of NPY administration on ethanol consumption or sucrose consumption on the day following NPY infusion.

DISCUSSION

The present study is one of the first to directly assess the ability of ICV NPY to alter ethanol consumption in the rat. NPY significantly increased sucrose drinking, but not 10% ethanol drinking. Increased sucrose consumption following intracerebroventricular NPY infusion is consistent with previous studies that have reported that NPY increases sucrose drinking and feeding (6,22,23). The lack of an increase ethanol drinking would appear to contrast with the existing evidence, which suggests NPY could play a role in the regulation of ethanol intake (5,8,9,11,35). Only one other published study has assessed the influence of NPY on ethanol drinking (20). In the golden hamster, low (0.04 and 0.12 μg) and high doses (3.3 to 10.0 μg) of NPY had no effect on ethanol drinking. A moderate dose (0.37 μg) increased 5% ethanol drinking, but there was no apparent dose–response relationship to NPY's effects. Although the present data do not conclusively indicate that NPY is not involved in ethanol drinking, the lack of an effect on ethanol drinking, with an increase in sucrose consumption does emphasize an important point. The central regulation of ethanol drinking and sucrose drinking by NPY systems is different, suggesting ethanol drinking is not simply a feeding behavior. It is clear that NPY has a facilitory effect in sucrose drinking and feeding, but this is clearly not the case in regard to ethanol drinking.

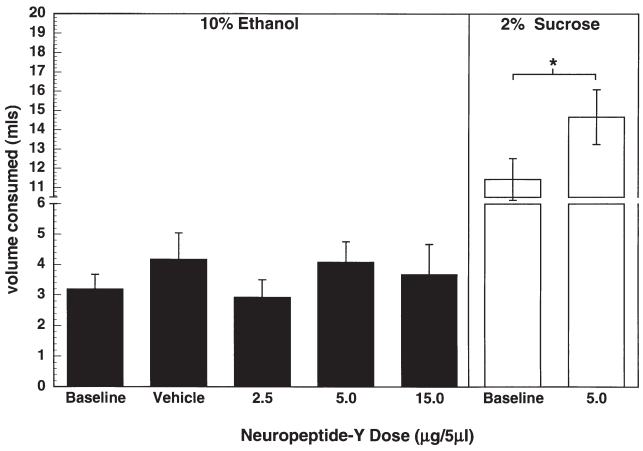

FIG 1. The effects of intracerebroventricular infusion of NPY on the consumption of 10% ethanol and 2% sucrose. Ethanol and sucrose consumption was measured in the same subjects (n = 12). Filled bars represent the volume of ethanol consumed and the open bars represent the volume of sucrose consumed. Error bars indicate the standard error of the mean. Asterisks (*) indicate a significant difference between brackets ($p < 0.05$).

The paraventricular nucleus of the hypothalamus (PVN) has been suggested to be the central locus for the orexigenic effects of NPY, as site specific injections of NPY into this nucleus increase feeding in a dose-dependent fashion (26,34). Therefore, NPY's effect on sucrose drinking in the present study are likely due to hypothalamic mechanisms. The PVN has also been shown to be involved in the regulation of ethanol intake. PVN injections of norepinephrine or serotonin, respectively, increase and decrease ethanol intake (18). Ethanol-naive P rats have higher levels of NPY in the PVN compared to NP rats (19), and chronic ethanol exposure and subsequent withdrawal significantly increases hypothalamic NPY levels (8). These data suggest that the PVN, and potentially NPY activity in the PVN, should have a facilitory role in ethanol drinking and ethanol preference.

In contrast, NPY activity in the central nucleus of the amygdala would be hypothesized to inhibit ethanol drinking. Central infusion of NPY has an anxiolytic effect (15,29,37) which is particularly potent following infusion directly into the central nucleus of the amygdala (13,14). It has been suggested that at least one factor that regulates ethanol consumption is its anxiolytic effects (30). Our electrophysiological studies have shown that NPY and ethanol have additive effects (9). In addition, it has been reported that lesions of the central nucleus of the amygdala that decrease anxiety also decrease ethanol drinking (24). These data suggest that an additive interaction of ethanol and NPY in the amygdala may result in increased anxiolysis, and consequently, decreased ethanol drinking.

In summary, NPY infusion into the lateral ventricle increased sucrose consumption but not ethanol consumption. To the best of our knowledge, this is one of the first studies assessing the direct effects of NPY on ethanol drinking in rats. The results are likely to indicate a differential regulation of ethanol and sucrose drinking by NPY systems. The contrasting effects of NPY in the PVN of the hypothalamus and the central nucleus of the amygdala are hypothesized to be the primary contributors resulting in the lack of changes in ethanol consumption following infusion of NPY into the lateral cerebral ventricle. Studies involving site specific injections into the hypothalamus or amygdala will be necessary to more definitively assess the influence of NPY on ethanol drinking. In addition, the reported long-lasting effects of NPY on feeding behaviors (22,23) and differential effects of NPY on different sucrose solutions (23) suggests that in future studies it will also be important to investigate NPY's effects on ethanol drinking with longer access periods and different concentrations of ethanol.

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