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Research report

Effects of neuropeptide Y and corticotropin-releasing factor on ethanol intake in Wistar rats: interaction with chronic ethanol exposure

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Abstract

Neuropeptide Y (NPY) and corticotropin-releasing factor (CRF) have opposing effects on stress-associated and consummatory behaviors in rodents. Recent studies also suggest that both peptides influence ethanol intake. In the present study, the effects of administration of CRF and NPY into the lateral ventricle on ethanol intake in naive and ethanol-vapor-exposed Wistar rats were examined. A limited access paradigm was used to measure intake of a 10% (v/v) ethanol solution in Wistar rats trained to drink using a sucrose fading procedure. Ethanol vapor exposure for 8 weeks significantly elevated ethanol intake in this limited access paradigm relative to pre-exposure levels. The effects of icv administration of CRF (1 μg), NPY (10 μg) or NPY/CRF combined (10 and 1 μg, respectively) on ethanol intake were then assessed. In non-vapor-exposed subjects, icv infusion of NPY had no effect on ethanol intake, while a significant suppression of drinking was seen following icv administration of CRF. Administration of NPY in combination with CRF had no effect on ethanol intake in non-ethanol-vapor-exposed rats. In vapor-exposed subjects, both NPY and CRF reduced ethanol intake, but when given in combination, no difference from vehicle was detected. Locomotor activity was measured during drinking sessions and was unaffected by peptide administration. These studies underscore the importance of a history of exposure to chronic ethanol vapor in the regulation of ethanol intake by NPY. Furthermore, the results presented here suggest that a balance between the stress-related peptides NPY and CRF may be involved in the regulation of ethanol intake.

Keywords: Ethanol; Dependence; NPY; CRF; Self-administration; Rat

1. Introduction

One theory, which has been advanced to explain ethanol use and abuse, is the "tension-reduction hypothesis" [9]. This theory suggests that ethanol-mediated relief from "stress" may be one factor motivating ethanol consumption, particularly in alcohol-dependent individuals [31,32,40,41]. Stress and anxiety have been reported to increase ethanol craving and consumption [5,31,33,51]. The anxiolytic properties of ethanol have been demonstrated in animal models [55], and a positive correlation between anxiety levels and ethanol consumption has also been shown [6,34,35]. These studies provide potential evidence for links between stress, anxiety and alcohol consumption.

Corticotropin-releasing factor (CRF [64]) and neuropeptide Y (NPY [53,54]) are two neuropeptides that have been implicated in modulation of anxiety-related behaviors and stress responses. CRF is a potent anxiogenic agent [12,36,52,56] and regulates neuroendocrine and behavioral responses to stress through actions in the hypothalamus, amygdala and other brain regions [20,29]. NPY is an anxiolytic peptide [21,22,39,66] whose expression is regulated by stress [59,60]. The interplay and/or balance between these two systems have been suggested to be of importance in regulating stress, anxiety, sleep and depression [16,20,67].

There are several studies linking disturbances in central NPY systems to the regulation of ethanol intake [23]. For instance, genetic linkage studies of rats selectively bred for ethanol preference (the P-line from Indiana University) have identified a chromosomal region including the NPY gene that may account for two-thirds of the genetic vari-

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ance between the preferring line and their non-preferring counterpart [10]. Furthermore, ethanol-preferring rats have lower levels of NPY in the amygdala compared to non-preferring rats [24]. A direct link between NPY signaling and regulation of ethanol consumption was first suggested by the demonstration that NPY overexpression in mice reduced ethanol self-administration, but the knockout of NPY expression increased ethanol self-administration [57]. In the rat, evidence for an involvement of NPY in regulation of ethanol intake is supported by the reductions in ethanol consumption resulting from icv administration of NPY in the genetically selected alcohol preferring P and HAD (high alcohol drinking) lines from Indiana University [2,3,19]. However, in outbred Wistar rats, icv or localized administration of NPY affects neither ethanol intake [25,26,49] nor operant ethanol self-administration [8]. In addition, studies have reported increased ethanol intake in hamsters following icv administration of NPY [30] and in rats following administration of NPY into the paraventricular nucleus [28]. We hypothesize that these inconsistencies may be due to a differential regulation of ethanol intake by NPY when intake is low/moderate (as in outbred rats) relative to when intake is high (as in selectively bred alcohol preferring rats). Therefore, the use of alternative animal models of elevated ethanol self-administration could enhance our understanding as to whether the results of NPY on ethanol intake in genetic models of alcoholism are applicable to other paradigms.

Another brain peptide which may potentially mediate the stress–ethanol interaction and alcohol dependence is CRF [48]. Acute administration of ethanol (intraperitoneal injection) increases plasma adrenocorticotropin hormone (ACTH) and corticosterone [43]. Administration of an anti-CRF antibody blocks this effect suggesting a CRF-dependent mechanism for the effect of ethanol on ACTH release. Central administration of CRF into the third ventricle has been demonstrated to dose-dependently suppress ethanol intake in a limited access paradigm in rats [4]. Rats bred for high ethanol preference also have lower brain CRF content in the hypothalamus, amygdala and cortex relative to their non-preferring counterparts [15]. Furthermore, CRF-deficient mice have been shown to consume twice as much ethanol in both continuous and limited access paradigms [37]. These data suggest that high central CRF activity decreases ethanol intake, an idea which is seemingly inconsistent with the "tension-reduction hypothesis". Further, it has been reported that icv administration of a CRF receptor antagonist reduces ethanol intake in rats with a history of ethanol vapor exposure [63]. These data seem to indicate that activation of central CRF systems contributes to the enhanced alcohol consumption in rats with a history of ethanol exposure. To date the circumstances under which ethanol intake is enhanced or inhibited by central CRF systems remain unclear. Furthermore, whether the effects of CRF on ethanol intake are due to regulation of consummatory behavior, changes in sensitivity to the rewarding properties of ethanol, increased levels of anxiety or a combination of these factors remains to be determined.

When administered alone NPY or CRF often elicit opposing neurobehavioral effects, but there is also ample evidence indicating that central NPY and CRF administration can modulate each other's effects. NPY fibers present in the paraventricular nucleus of the hypothalamus are closely associated with CRF cell bodies [65]. Furthermore, corticosterone replacement in adrenalectomized rats strongly potentiates NPY gene expression, peptide content and radioligand binding in the hypothalamus [1]. NPY and CRF systems have also been demonstrated to reverse each other's effects on arousal [16,67] and anxiety-like behavior [7,27]. Despite the known interactions of NPY and CRF systems, there are no published studies assessing the potential interactive effects of the combined administration of NPY and CRF on ethanol consumption. The demonstration of such an interaction would further support the concept that a balance between NPY and CRF activity, which is critical for maintaining proper stress responses and anxiety levels, is important in the regulation of ethanol consumption.

Chronic ethanol administration through ethanol vapor exposure can mimic many aspects of heavy drinking and the withdrawal syndrome seen in alcohol-dependent individuals [13,14,44–46,50]. Using a continuous exposure paradigm resulting in blood alcohol concentrations of >150 mg/dl, elevated ethanol intake can be induced in experimental animals [45]. However, this effect is transient. A paradigm using intermittent exposure has recently been found to elicit long-term behavioral effects which included increased voluntary ethanol consumption [42,61]. This exposure-induced increase in ethanol intake is also blocked by acamprosate suggesting that the underlying events leading to increased intake in exposed animals are of relevance to the human condition [42].

The primary goal of this study was to examine the combined effects of CRF and NPY on ethanol intake in a limited access model. In light of previous findings of suppressed ethanol drinking following icv administration of CRF [4], it was hypothesized that CRF would suppress ethanol intake in rats not exposed to ethanol vapor. We further hypothesized that NPY alone would produce no effect on ethanol consumption, but that an interaction effect might be seen with NPY counteracting CRF-induced suppression of ethanol intake. Based on the finding that NPY reduces ethanol intake rats selectively bred for high ethanol consumption [2,3,19], we posited that in rats exposed to ethanol vapor a decrease in intake would be seen following administration of NPY, as well as, after administration of CRF and the combination of NPY and CRF.

2. Methods

2.1. Subjects

Male Wistar rats ($n = 26$; Charles River, USA) were used in this study. Upon receipt, body weight averaged 196 ± 3 g