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The role of hypothalamic peptide gene expression in alcohol self-administration behavior

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ABSTRACT

Self-administration of ethanol and food share many common features and Richter hypothesized that an increase in ethanol consumption would decrease feeding to balance the excess calories contained in the ethanol. Previously, we have shown that individual alcohol consumption correlates with neurotransmitter gene expression, especially in the prefrontal cortex. To test the hypothesis of Richter, we measured hypothalamic gene expression of receptors or neuropeptides of known relevance for the regulation of food intake using qPCR and correlated this to individual ethanol consumption in Wistar rats. For validation, gene expression was first correlated with body weight. We found a correlation of dynorphin, somatostatin, melanocortin-4 receptor and serotonin 5-HT_{2C} with body weight and trends to correlation for CART, thus confirming the established role of the hypothalamus in the regulation of weight. For ethanol consumption, correlations were found for CRH receptors 1 and 2 and vasopressin while strong trends were observed for galanin receptor 1, orexin receptor 1, MCH and adrenoceptor α_{1B}. Therefore, alcohol consumption does seem to involve several hypothalamic systems which also mediate feeding responses and suggests that the hypothalamus, together with the prefrontal cortex, may determine the 'stopping point' of an individual.

1. Introduction

Consumption of alcohol shares many common features with feeding and terms like absorption, caloric content, foraging and satiety could be used interchangeably for food or alcohol self-administration. Alcoholism and obesity represent two of the most major health issues in modern society and obesity, in particular, increases the risk to develop diabetes and cardiovascular disease [28]. Alcohol-dependent patients often show increased susceptibility to obesity and eating disorders [51]

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Abbreviations: AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; CRHR, corticotrophin-releasing hormone receptor; 5-HT, serotonin; GAPDH, glyceraldehyde-3-phosphate-dehydrogenase; GAL, galanin; GR, glucocorticoid receptor; GnRF, gonadotropin-releasing factor; H3b, histone H3b; MC4, melanocortin-4 receptor; MCH, melanocortin-concentrating hormone; MR, mineralocorticoid receptor; NPY, neuropeptide Y; OR, opioid receptor; ORX, orexin; ORXR1, orexin receptor 1; POMC, pro-opiomelanocortin; qPCR, quantitative reverse-transcriptase polymerase chain reaction; RPL19, ribosomal protein L19; SDHA, succinate dehydrogenase complex A subunit A; SOM, somatostatin; VP, vasopressin.

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and an epidemiological study by Carlat et al. [6] found comorbid alcohol abuse in 38 patients (28%) identified with eating disorders. There are also interactions between food and alcohol since the intake of high-fat meals correlates with increased ethanol consumption [8].

Given the caloric content of alcohol, Richter [46] proposed that alcohol and food intake is regulated similarly such that increased alcohol consumption would lead to a decrease in eating to compensate for the excess calories. But this has been debated from both sides. If alcohol is the only fluid available to the animal, food intake decreases when concentrations of ethanol are increased [32,45] or patterns of eating are altered [52] to balance the overall amount of calories consumed, as hypothesized by Richter. However, in a more recent study, animals were given a free choice between ethanol and water and no correlation was found between alcohol and food consumption [17], indicating that the predictions of Richter depends on the availability of alcohol versus other fluids. Additional studies cast doubt on this hypothesis. While food deprivation is known to increase alcohol consumption [49], it also increases self-administration of non-caloric reinforcers like cocaine and amphetamine [9] or etonitazene [10]. Additionally, several studies report a correlation in self-administration of ethanol and the non-caloric sweetener saccharin [16,18] which suggests that mechanisms other than caloric content may be important in determining the amount of ethanol consumed by the individual.

Previously, we observed that Wistar rats quickly reach a stable ethanol consumption level [40] and we have hypothesized that this 'stopping point' is a characteristic of each individual. Tombaugh and Tombaugh [53] suggest that an animal stops drinking due to the hypnosedative or toxic effects of ethanol while others have suggested that cholecystokinin may act as a satiety signal for alcohol in a way similar to food [45]. We have previously investigated the idea of a 'stopping point' by correlating gene expression of many neurotransmitter receptors to the total alcohol consumption of an individual for the daily 30-min session [40]. Six alcohol-specific correlations in the prefrontal cortex indicated that this area, largely responsible for executive control of behavior [26], was important for alcohol self-administration and may produce the 'stop signal'. However, these results cannot exclude the role of peripheral signals or endocrine mechanisms that may be passed to cortical areas. Therefore, we cannot, for example, say whether the animal has stopped self-administering alcohol based on caloric content.

Our hypothesis was that if the stopping point for alcohol consumption is determined by caloric or feeding mechanisms, some evidence of this relationship should be apparent in the gene expression pattern of the hypothalamus. Therefore, our aim was to correlate mRNA expression of key receptors or peptides in the hypothalamus that may have relevance for alcohol self-administration. Quantitative reverse transcriptase polymerase chain reaction (qPCR) was used to measure mRNA expression and we have previously used this method to determine the effects of food restriction on gene expression of a large number of endocrine mediators [35]. We have chosen many of the same mediators or receptors for this current study but have added several that have been discussed recently with respect to alcoholism; in particular galanin [34], orexin [33] and corticotrophin-releasing hormone (CRH) [13]. The role of individual peptides and/or receptors has been reviewed extensively (for example, [38]). In Table 1 of the review by Williams et al. [59], a summary of hypothalamic neurotransmitters and their role in feeding is provided. Stimulation of feeding occurs via neuropeptide Y (NPY), melanin-concentrating hormone (MCH), orexin, galanin, agouti gene-related protein (AgRP) and dynorphin while inhibition of feeding occurs via cholecystokinin (CCK), cocaine- and amphetamine-regulated transcript (CART) or CRH [59]. In addition to feeding receptors, we have included several α adrenoceptors which were correlated with alcohol self-administration in the prefrontal cortex in our previous study [40] and also several components of the stress response axis (GR, MR and CRH receptors) since changes in HPA axis function may underlie the development of dependence [29].

This study consisted of several parts. First, we examined the relation of individual body weight to gene expression in the hypothalamus to validate and confirm the role of known mediators in food regulation. Second, we examined the correlation of alcohol consumption with hypothalamic gene expression to question the role of this structure in the 'stop signal' of the individual. Finally, consumption of saccharin, a non-caloric reinforcer, was correlated with hypothalamic gene expression as a control for specificity of the alcohol correlations [40]. An additional benefit of the saccharin analysis lies in the fact that both ethanol and saccharin are reinforcing and self-administered in animals while only ethanol contains calories. As such, we can investigate the hypothesis of Richter using qPCR and a wide range of genes, an approach which has many advantages over single-gene investigations, as discussed in [35].

2. Materials and methods

Experiments were approved by the Ethical Committee for Use of Animal Subjects at Karolinska Institutet and all animal care procedures followed the guidelines of Swedish legislation on animal experimentation (Animal Welfare Act SFS1998:56) and EU legislation (Convention ETS123 and Directive 86/609/EEC).

2.1. *Animals*

Sixteen naïve, male, Wistar rats (weighing 250 g at the start) were group housed four per cage in an animal facility with climate-controlled conditions for temperature (22 °C), humidity (50%) and a 12 h light:dark cycle with lights on at 07.00. Animals were given 1 week to acclimatize to the animal facilities after transport from Scanbur/B&K (Sollentuna, Sweden). Self-administration sessions were performed between 09.00 and 12.00 and animals received food and water *ad libitum* unless otherwise stated.

2.2. *Operant self-administration training*

All training was performed in MED-PC operant chambers (Med Associates Inc., VT, USA) and the procedure was discussed in detail in [40]. The 9-day training protocol is briefly described here. Rats were water deprived in their home cage from 17.00 the day before the start of training. On Day 1, a delivery of 0.2%