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Research report

# Gender specific effects of ethanol in mice, lacking $CCK_2$ receptors

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**Abstract**

Neuropeptide cholecystokinin (CCK) has been reported to suppress ethanol intake, but there is contradictory evidence about the role of $CCK_2$ receptors. In the present study anxiolytic, hypolocomotor and sedative effects of acute ethanol administration, but also voluntary ethanol consumption were studied in male and female mice, lacking $CCK_2$ receptors (−/−). Ethanol (1.0 and 2.0 g/kg) induced a significant reduction of anxiety-related behaviours in the elevated plus-maze, but this effect was statistically significant only in female homozygous mice (−/−). In male mice, lacking $CCK_2$ receptors (−/−), but not in their wild-type littermates (+/+), the suppression of vertical locomotor activity was caused by ethanol at a dose 0.5 g/kg. The highest dose of ethanol (2.0 g/kg) produced statistically significant reduction of horizontal locomotor activity only in female wild-type (+/+) mice, but this effect was related to increased basal activity when compared to female mutant (−/−) mice. Duration of the loss of righting reflex was not significantly affected by genotype or gender, but blood ethanol levels at regain of righting reflex were significantly lower in female homozygous mice (−/−) compared to their wild-type (+/+) littermates, indicating increased sensitivity to the sedative effect of ethanol. Ethanol intake, but not preference, at concentration 10% was significantly increased in female mice, lacking $CCK_2$ receptors (−/−). The present study revealed an altered response to the acute effects of ethanol in $CCK_2$ receptor deficient mice (−/−). These changes are gender-specific and could be attributed to the altered activity of dopaminergic system in male mice and increased activity of GABA-ergic system in female mice as established in our previous studies.


*Keywords:* Ethanol; $CCK_2$ receptor-deficient mice; Anxiety; Locomotor activity; Sensitivity; Ethanol intake

## 1. Introduction

Cholecystokinin (CCK) is a gastrointestinal peptide widely distributed in the central nervous system [35]. CCK is involved in the regulation of various physiological functions in the brain, such as modulation of anxiety and stress-related behaviours, regulation of feeding, nociception, memory and reward-related behaviours [7,13,36]. There is substantial evidence that CCK acts as a neurotransmitter and that it exerts a modulatory influence through several classic neurotransmitters including dopamine, GABA and opioid peptides [6]. By now two subtypes of CCK receptors have been identified. $CCK_1$ receptors are located in the pancreas, gallbladder and distinct brain regions. $CCK_2$ receptors represent the vast majority of CCK receptors found in the central nervous system [30].

CCK has been reported to suppress ethanol drinking and preference in rodents [15,16,20,39] and the role of $CCK_1$ receptor has been underlined [8,9,27]. Studies concerning the role of $CCK_2$ receptors in ethanol preference have yielded contradictory results. Crespi [8] described that pre-treatment with $CCK_2$ antagonists did not affect ethanol drinking in rats. However, Little et al. [23], and Croft et al. [11] reported that $CCK_2$ antagonists decreased stress-induced ethanol preference in mice. Ethanol consumption has recently been studied in male mice lacking $CCK_2$ receptors, but no differences compared to wild-type (+/+) mice have been established [27]. On the other hand, chronic ethanol consumption has been shown to alter the brain CCK-ergic system [18,41] and $CCK_2$ receptors have been reported to have relevance in ethanol withdrawal-induced anxiety and convulsions [5,42,43].

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In the present study, female and male homozygous (−/−) $CCK_2$ receptor deficient mice were used to reveal a possible role of CCK in ethanol-related behaviours. Previous studies have established that male $CCK_2$ receptor deficient mice display an increased sensitivity of dopamine $D_2$ receptors in the striatum and an altered response to dopaminergic drugs such as amphetamine and apomorphine [12,19]. Altered responses to opioid- and GABA-ergic drugs along with distinct changes in opioid- and GABA-ergic systems have also been reported [12,32–34,38,40]. Considering that as a result of significant neurochemical alterations mice lacking $CCK_2$ receptors display altered responses to such commonly abused drugs as amphetamine, morphine and diazepam [12,19,34], it was assumed that ethanol-related behaviours together with ethanol consumption would also be modified due to the changes in opioid-, dopamine- or GABA-ergic systems. The study was carried out employing both male and female mice, because the phenotype of mice, lacking $CCK_2$ receptors, is significantly affected by gender [1]. Behavioural effects of ethanol were studied in the elevated plus-maze, locomotor activity and loss of righting reflex tests. Also, ethanol consumption and preference, and preference for non-alcoholic tastants were determined.

## 2. Materials and methods

### 2.1. Animals

$CCK_2$ receptor deficient mice were provided from the original background 129sv/C57BL6 mice [26]. $CCK_2$ receptor deficient mice were generated by homologous recombination by replacing a part of exon 2 and exons 3–5 [29]. Breeding and genotype analysis were performed in the Department of Physiology, University of Tartu [19]. Male and female homozygous (−/−) $CCK_2$ receptor-deficient and wild-type (+/+) mice (90 days old) were used in the behavioural experiments. Mutant mice were crossed back six times to the C57/BL6 background to minimise the possible genetic effects from the 129Sv strain. Mice were kept in the animal house at $20 \pm 2^{\circ}$C under a 12:12 h light/dark cycle (lights off at 19:00 h). Tap water and food pellets were available *ad libitum*. In all experiments ethanol-naïve mice were used. All animal procedures were approved by the University of Tartu Animal Care Committee in accordance with the European Communities Directive of 24 November 1986 (85/609/EEC).

### 2.2. Elevated plus-maze

The number of animals per group was $N = 8$–10. The mouse elevated plus-maze is a reduced copy of the rat plus-maze [17]. The plus-maze consists of two opposite open (17.5 cm × 5 cm) arms without sidewalls and two enclosed arms of the same size with 14 cm high sidewalls and an end wall. To determine the exploratory activity, the open arms were divided into three equal parts by lines. The entire plus-maze apparatus was elevated to a height of 30 cm and placed in a brightly lit room (illumination level: ∼500 lx in open arms). Standard 5 min test duration was employed [22], and the maze was wiped with damp and dry towels between the subjects. Test sessions were video-recorded and the videotapes were subsequently scored by a trained observer unaware of testing conditions. The following parameters were observed: (1) number of entries on the open arms; (2) time spent on the open arms of the plus-maze; (3) total number of closed and open arm entries; (4) ratio between the open and total arm entries; (5) number of unprotected head-dips; (6) number of lines crossed. Time spent on the open arms, number of open arm entries, and ratio between the open and total arm entries are the conventional measures of anxiety in the elevated plus-maze [22]. Three doses of ethanol (0.5, 1.0 and 2.0 g/kg) were studied. Ethanol (diluted in physiological saline, 10% (v/v) for 0.5 and 1.0 g/kg or 20% (v/v) for 2.0 g/kg) was injected intraperitoneally 20 min prior to testing.

### 2.3. Locomotor activity

The number of mice was $N = 10$–12 per treatment group. For the study of locomotor activity the animals were placed singly into soundproof photoelectric motility boxes (448 mm × 448 mm × 450 mm) connected to a computer (TSE; Technical & Scientific Equipment GmbH, Germany) for 30 min. The illumination level of the transparent test boxes was ∼400 lx. After removing the mouse from the box, the floor was cleaned with damp towels and dried thoroughly. Time in locomotion (s), distance travelled (m) and number of rearing were registered. Three doses of ethanol (0.5, 1.0 and 2.0 g/kg) were used. Ethanol was injected intraperitoneally 20 min prior to testing.

### 2.4. Loss of righting reflex

Mice ($N = 14$–15 per group) were given an intraperitoneal injection of 4.0 g/kg of ethanol (20%, v/v). At the onset of ethanol-induced sedation (the loss of righting reflex), each mouse was placed on its back in a V-shaped paper-trough. Time (s) between the injection and the loss of righting reflex and time (min) between the loss of righting reflex and the regain of righting reflex defined as the ability to right itself on all four paws three times within a 30 s interval were taken. Tail blood samples were collected at the regain of righting reflex to determine blood ethanol concentration.

### 2.5. Ethanol intake test

Fifty millilitres plastic tubes with tips cut off were used for ethanol intake and taste preference tests. Tubes were controlled for leakage for 7 days and subsequent intake measurements were adjusted for leakage. Throughout the experiment, total fluid and food intake, and body weight were measured every 7 days. The number of mice was $N = 15$ per group. Prior to testing mice were housed individually and were habituated to drinking from two tubes containing plain water for 7 days. Mice were then given 24 h access to two tubes, one containing plain water and the other containing ethanol in water. The concentration of ethanol (v/v) was increased every 7 days. Initially, mice received 3%, followed by 6% and finally 10% ethanol solution. The positions of the tubes were counterbalanced between groups and changed every 2 days to control for position preference. Average ethanol consumption (calculated in g/kg of body weight per day) was obtained for each ethanol concentration by weighing tubes at the beginning and end of the exposure. As a measure of relative ethanol preference, an ethanol preference ratio was calculated for each ethanol concentration by dividing total ethanol solution consumed by total fluid (ethanol plus water) consumption. Food intake was calculated weekly at every ethanol concentration (expressed as g/kg of body weight per day) by weighing food granules at the beginning and end of the exposure to ethanol. All spillage was collected and included in calculations.

### 2.6. Taste preference

Ten days after the end of ethanol consumption testing, the same mice used in alcohol intake test were given *ad libitum* access to two tubes, one containing plain water and the other a solution of sucrose or quinine. The compounds were presented in the following order: sucrose solutions (1.70% and 4.3%) followed by quinine solutions (0.03 and 0.10 mM). Mice had 48 h access to each solution, the position of solutions was counterbalanced between groups and switched 24 h after presentation. Millilitres of solution consumed per kilogram of body weight per day and preference for either compound were measured and calculated as described in the previous section.

### 2.7. Blood ethanol concentrations

Five microlitres of blood was taken from the tail vein 30, 60, 120 and 240 min after intraperitoneal injection of ethanol (2.0 or 4.0 g/kg 20% (v/v), diluted in physiological saline). Each group consisted of six animals. Blood samples were analysed immediately by enzymatic colour test using LKM 139 and miniphotometer LP 20 (Dr. Bruno Lange GmbH, Germany) according to manufacturer's instructions.