**Intracerebroventricular Ethanol Injection:**

**Water and Food Intake Alterations**

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**Abstract**

Currently, the pharmacodynamic study allows the use of multiple experimental models for detection and quantification of the pharmacologic and toxicological effects of different substances. Some studies suggest that the ethanol possess properties orexigenics, therefore in small doses stimulate the appetite. But, suggestions exist that it can modify the hunger mechanism, for the suppress appetite, by anorexigenics properties. The present study was carried in the attempt of if establishing the acute effect promoted by low doses of ethanol (EtOH) (control NaCl vs. dose-response curve 1,4 µmol, 2,8 µmol and 5,6 µmol) water and food intake for the administration i.c.v (intracerebroventricular) in male Wistar Hannover rats (220-300g). Analysis showed that 1,4 µmol dose that ethanol significantly increased the water intake to the 60 and 120 minutes; 2,8 µmol dose the increased to the 60 and 180 minutes when compared with the control. But 5,6 µmol dose decreased the water intake to the 60,120, and 180 minutes when compared with the control NaCl-injected animals. Analysis showed that 1,4 µmol dose that ethanol significantly increased the pellets ingested to the
60 and 120 minutes; 2.8 \textmu mol dose the increased to the 60 and 120 minutes and decreased to the 180 minutes when compared with the control. But 5.6 \textmu mol dose significantly decreased the pellets earned to the 60, 120, and 180 minutes when compared with the control NaCl-injected animals. The acute administration i.c.v of ethanol can promote transitory water and food intake alterations. The effects are dose-dependent.

**Keywords:** central nervous system, ethanol, intracerebroventricular, pharmacodynamic

**INTRODUCTION**

Currently, the pharmacodynamic study allows the use of multiple experimental models for detection and quantification of the pharmacologic and toxicological effects of different substances. The experiences "in vivo" make possible to show signals and specific symptoms, quantitative parameters, probable mechanisms of action, secondary effects, tolerance and interaction with other substances. The intracerebroventricular injection of a substance allows the direct administration in the central nervous system in a minimum concentration (\textmu mol, pmol or nmol) necessary for the promotion of the biological effect when compared with the peripheral administration. Studies using the injections i.c.v. (intracerebroventricular) has elucidated more clearly the neuropharmacology and neurophysiology of some drugs [15]. The gradual accumulation of knowledge makes possible a more specific boarding on its consume and abuse. Ethanol (EtOH) is classified as a sedative or depressive drug of the central nervous system. However, it can have action stimulant [8]. Its effect is considered biphasic and dose-dependent [23]. Many people use the ethanol (EtOH) during their meals. The relationship between ethanol consumption and body weight regulation is complex. Some studies suggest that the ethanol (EtOH) possess properties orexigenics, therefore in small doses stimulate the appetite, and produce a positive energy balance in humans. However, this primary disorder of the appetite could contribute for the obesity [6] and hypertension, collaborating for the concretion of the projections of the Worldwide Organization of the Health for the decade of 2020 [17]. But, suggestions exist that it can modify the hunger mechanism, for the suppress appetite, by anorexigenics properties [1]. In moderate doses, alcohol has a mild stimulatory effect on food intake [2]. In other experiment, the ethanol is tested as an aperitif [27], leaving period of around 20-30 min before presenting the meal or the alcoholic beverage is administered along with the meal, but the aperitif administration, particularly when food deprived, could enhance the psychoactive effects of alcohol [22] by facilitative effect of immediate alcohol in central nervous system. Clearly evidence from epidemiological investigation suggests that the relationship between alcohol and weight gain depends on the weight status of the individual, frequency and quantity of alcohol consumed, the habitual diet as well as other factors such as gender and age [14]. It is important to
understand the effects of ethanol on water and food intake and potential consequences. EtOH can activate the hypothalamic-pituitary-adrenal (HPA) axis through primarily central mechanisms, i.e. those that involve the paraventricular nucleus (PVN) and/or its afferents, as also for other brain areas potentially involved in a variety of neuronal responses and behaviors. The peak of increase of pro-opiomelanocortin (POMC) and adrenocorticotropin (ACTH) to the 15 minutes and to the 30 minutes for corticotrophin-releasing factor (CRF) after the injection i.c.v-injected ethanol [21]. In this way, the present study was carried in the attempt of if establishing the acute effect promoted by low doses of ethanol (EtOH) (dose-response curve) water and food intake alterations, for the administration i.c.v. in rats. Similarly the citations of others publications, the concentration of ethanol adopted in injection i.c.v. did not cause neuronal damage [26].

MATERIAL AND METHODS

The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the study. Male Wistar-Hannover rats (220-300g) were instrumented with an i.c.v. guide cannula and kept in individual cages under controlled temperature (18 - 22°C) and lighting conditions (0700h-1900h), with free access to tap water and standard laboratory rodent ration. Briefly, the animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg.kg -1 body weight) and a stainless steel cannula was stereotaxically implanted into the lateral cerebral ventricle (LCV), using previously reported techniques and pre-established coordinates: anteroposterior (0.2 mm from bregma), lateral (1.5 mm from bregma) and vertical (4.0 mm from bregma) [24]. The position of the cannula was visually confirmed by 2% blue Evans infusion through the i.c.v. cannula at the end of the experiment. Seven days after the stereotaxic surgery rats were submitted to i.c.v. acute microinjection of 4 µl of NaCl (Co, n= 10) or ethanol (EtOH) 1.4 µmol, 2.8 µmol e 5.6 µmol [1] (EtOH, n = 10). Water intake and pellets earned was recorded for 60, 120, 180 minutes. The parameters between basal and experimental periods were calculated and expressed as. %. Statistical analysis of the data was performed using analysis of variance of one-way (ANOVA) for repeated measures and t-test Bonferroni for measurements different enters the groups of animals. A p value = 0.05 was considered to indicate significance.

RESULTS

All rats survived and were clinically healthy up to the 7th day after a cannula was positioned in the LCV. The figure and table 1 and 2 shows the effects of i.c.v. microinjections of vehicle (NaCl) or various doses of ethanol (EtOH) performance. These results are from the study the low dose progression (i.e. 1.4, 2.8 and 5.6µmol). The figure and table 1 show water intake (per 100-g body
weight) and mean ±SD (ANOVA and t-test \( p \leq 0.05 \)) = *. The control = (2.55 ± 0.13 - 60 minutes), (1.16 ± 0.11-120 minutes), (0.27 ± 0.08-180 minutes). 1.4 \( \mu \text{mol} = (3.89 ± 0.31* - 60 \text{ minutes}) \), (2.05 ± 0.08*-120 minutes) (1.32 ± 0.50 - 180 minutes). 2.8 \( \mu \text{mol} = (2.97 ± 0.18* - 60 \text{ minutes}) \), (1.12 ± 0.21-120 minutes) (0.89 ± 0.16*-180 minutes). 5.6 \( \mu \text{mol} = (1.65 ± 0.17* - 60 \text{ minutes}) \), (0.46 ± 0.20*-120 minutes) (0.02 ± 0.01*-180 minutes). Analysis show that 1.4 \( \mu \text{mol} \) dose that ethanol significantly increased the water intake to the 60 and 120 minutes and 2.8\( \mu \text{mol} \) dose the increased water intake to the 60 and 180 minutes when compared with the control. But 5.6\( \mu \text{mol} \) dose decreased the water intake to the 60,120, and 180 minutes when compared with the control NaCl-injected animals. The figure and table 2 show pellets ingested (per 100-g body weight), mean ±SD (ANOVA and t-test Bonferroni \( p \leq 0.05 \)) = *. The control (2.21 ± 0.06- 60 minutes), (1.14 ± 0.21- 120 minutes), (0.34 ± 0.09-180 minutes) 1.4 \( \mu \text{mol} = (3.01 ± 0.06*-60 \text{ minutes}) \), (0.84 ± 0.12*-120 minutes), (0.36 ± 0.10 - 180 minutes). 2.8 \( \mu \text{mol} = (2.97 ± 0.14* - 60 \text{ minutes}) \), (0.83 ± 0.09*- 120 minutes), (0.22 ± 0.04*-180 minutes) 5.6 \( \mu \text{mol} = (1.28 ± 0.10*-60 \text{ minutes}) \), (0.75 ± 0.15* - 120 minutes), (0.00 ± 0.00*-180 minutes) Analysis show that 1.4 \( \mu \text{mol} \) dose that ethanol significantly increased the pellets ingested to the 60 and 120 minutes; 2.8 \( \mu \text{mol} \) dose the increased to the 60 and 120 minutes and decreased to the 180 minutes when compared with the control. But 5.6 \( \mu \text{mol} \) dose significantly decreased the pellets ingested to the 60, 120, and 180 minutes when compared with the control NaCl-injected animals.

**DISCUSSION**

According to the results, the injection technique to intracerebroventricular of ethanol presented to be efficient for the evaluation of water and food intake alterations. Tables 1 and 2 demonstrate that the responses are accentuated in first the 60 minutes and the stimulation with the solution of ethanol (EtOH) in concentrations of 1.4 \( \mu \text{mol} \) and 2.8 \( \mu \text{mol} \) promotes the increase and in the 5.6 \( \mu \text{mol} \) concentration promotes decrease of the water and food intake. The response is transitory with tendency to decrease to the 120 and 180 minutes, some that sensitivity to ethanol (EtOH) either considered changeable between the rats [28]. The ingestion of water and salt is controlled for the volemia and osmolality through of sensorial receptors [10]. The injection i.c.v. of ethanol (EtOH) stimulates the central nervous system including hypothalamus where originates thirsty sensation, and the cortical region that becomes it conscientious, confirming the increase of the water ingestion in the 1.4 \( \mu \text{mol} \) and 2.8 \( \mu \text{mol} \) concentration but, the decrease in the 5.6 \( \mu \text{mol} \) concentration. The increase or the decrease of the water ingestion (figure 1) can suggest that the interaction of ethanol (EtOH) with the osmoreceptors is dose-dependent. The paraventricular and the supraoptic nucleus are considered osmoregulatory centers [7] and the VP (vasopressin) sends signals (V1 receptors) to hypothalamus directly or indirectly to stimulate the production of CRH (corticotropin-releasing hormone) for the
hypothalamic neurons. The stimulation of CRH/AVP is related to the release of ACTH (adrenocorticotrophic hormone) and corticosterone after acute administration of ethanol (EtOH) [19]. The water ingestion is increased in the 1,4 μmol and this could be related to the inhibition of the AVP promoted for ethanol (EtOH) in this concentration. Inquiries demonstrate [19.25] that the i.p. or intragastric injection of the alcohol rapidly and significantly increase plasma ACTH levels as well neuronal activity of paraventricular nucleus (PVN) of the hypothalamus, particularly in cell bodies that express corticotropin-releasing factor (CRF) and vasopressin (VP). The problem in the characterization of ethanol in to precise sites of action is had the ability of this substance to readily diffuse throughout the brain [9]. The CRF (corticotrophin-releasing factor) and POMC (proopiomelanocortin) can be involved in the reply of the hypothalamic-hypophyseal system axis after the stimulation. In the brain, cellular bodies contend POMC are located in the arcuate nucleus and the periaarcuate region of hypothalamus. The administration i.c.v. of ethanol (EtOH) demonstrated that can influence the food intake (Figure 2) but the effect also is considered dose-dependent. This confirms previous investigations indicating that alcohol may delay or disrupt satiety signals, thus contributing to over consumption [5.3.29]. The lateral hypothalamic area (LHA) possesses osmossensitive and glucosensitive cells. The neurons of the LHA possess glucoreceptors and response the alterations of the glucose participating of the control of the food ingestion [13]. The acute administration of ethanol (EtOH) increases the beta-endorphins release known by stimulating the appetite [12]. The stimulation of food intake by ethanol may be a learned response and could operate in conjunction with psychoactive effects of ethanol [4]. However, the biological effect promoted by the acute administration i.c.v. of ethanol (EtOH) can involve the leptin that reduces the ingestion for the reduction of orexins peptides (NPY and orexin) and increase of anorexins peptides (CRH and alpha-MSH) [16.18], which could be associated the ingestion after the stimulation with ethanol in the concentration of 5,6 μmol. A study affirms that ethanol induces the acute resistance the leptin for the mechanism of phosphorilation of the STAT3 and the reduction of receptor in hypothalamus [11].

CONCLUSION

The present work confirms and extends results previously reported by other showing. The ethanol can stimulate water and food intake response was induced by i.c.v. injections of peptide in μmol amounts and can promote transitory water and food intake alterations. The effects are dose-dependents. The exposition modifies the activity of neurons that regulate the activity of the hypothalamic-hypophyseal system axis. However, due to existence of an association between the central pathways is possibility that the agents injected in a specific area can act in the other cerebral areas for diffusion. The experimentation with ethanol (EtOH) demonstrates that exist differences between the experimental protocols, the stimulation pathways, the periods of evaluation, and the animal species. Currently,
we are investigating the molecular mechanism and via of cellular signaling related to the alterations promoted for the acute administration i.c.v of ethanol and water and food intake, because they had not been established.

REFERENCES


Figure and Table 1: Water intake (ml/per 100-g body weight)

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<th>60 minutes</th>
<th>120 minutes</th>
<th>180 minutes</th>
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<tr>
<td>control</td>
<td>2.55 ± 0.13</td>
<td>1.16 ± 0.11</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>1.4 μmol</td>
<td>3.89 ± 0.31*</td>
<td>2.05 ± 0.08*</td>
<td>1.32 ± 0.50</td>
</tr>
<tr>
<td>2.8 μmol</td>
<td>2.97 ± 0.18*</td>
<td>1.12 ± 0.21</td>
<td>0.89 ± 0.16*</td>
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<tr>
<td>5.6 μmol</td>
<td>1.65 ± 0.17*</td>
<td>0.46 ± 0.20*</td>
<td>0.02 ± 0.01*</td>
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</tbody>
</table>

Mean ±SD (p ≤ 0.05) = *
Figure and Table 2: Pellets earned (g/per 100-g body weight)

Table 2: Pellets Earned (g/per 100-g body weight)

<table>
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<th>Pellets Earned (g/per 100-g body weight)</th>
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<td>60 minutes</td>
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<tr>
<td>(n=10)</td>
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<tr>
<td>control</td>
<td>2.21 ± 0.06</td>
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</tr>
<tr>
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<td>2.97 ± 0.14*</td>
</tr>
<tr>
<td>5.6 μmol</td>
<td>1.28 ± 0.10*</td>
</tr>
</tbody>
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Mean ±SD  
(p ≤ 0.05) = *  
Control NaCl vs EtOH dose progression

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