PHARMACOLOGY AND CELL METABOLISM

Effect of an Acute Consumption of a Moderate Amount of Ethanol on Plasma Endocannabinoid Levels in Humans

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Abstract — Aims: Animal experiments have shown that the endocannabinoid system (ECS) plays an important role in the regulation of ethanol intake. We investigated these effects in healthy volunteers who consumed a moderate amount of ethanol (red wine) and measured plasma levels of the endocannabinoids (ECs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) to test whether alcohol consumption influences the ECS in humans. Grape juice or plain non-sparkling water served as non-alcoholic control liquids. Methods: In total, 55 adults were enrolled in this study and assigned to one of three groups drinking either 250 ml of red wine (28.0 g of ethanol, <0.8 g of sugar and 187.5 kcal), grape juice (41.0 g of sugar, 187.5 kcal) or plain water within 10 min. Twenty minutes and 45 min thereafter, AEA, 2-AG, ethanol and glucose levels were determined from venous plasma samples.

Results: AEA, 2-AG and plasma glucose levels were significantly reduced after red wine consumption. AEA had its maximal decline at 20 min (from 0.23 ± 0.12 to 0.18 ± 0.07 ng/ml, P < 0.01), whereas the nadir of 2-AG was seen after 45 min and dropped from 6.68 ± 4.13 to 5.49 ± 3.22 ng/ml (P < 0.05). Grape juice highly affected blood glucose level after 20 min, with a return to baseline after 45 min. ECs remained almost unchanged by this intervention. Water intake had no significant effect on AEA (0.21 ± 0.08 at baseline and 0.19 ± 0.06 after 45 min) but resulted in a gradual reduction in 2-AG concentrations which became significant at 45 min when compared with baseline.

Conclusions: The consumption of a moderate amount of red wine reduces plasma AEA and 2-AG concentrations, whereas the volume and caloric equivalent of the sugar containing, non-alcoholic liquid grape juice does not affect plasma ECs. Plain water has a differential effect on the ECS by reducing 2-AG concentrations without affecting AEA.

INTRODUCTION

In the late 1980s, the human endocannabinoid system (ECS) was discovered (De Petrocellis and Di Marzo, 2009). It consists of cannabinoid receptors, endogenous ligands, the so-called endocannabinoids (ECs) and specific proteins involved in EC biosynthesis and degradation. At least two G-protein-coupled receptors, called cannabinoid receptor type 1 or 2 (CB1, CB2) mediate EC signaling. CB1 receptors are mainly present in the central nervous system, while CB2 receptors were described on other cells and tissues as their ligands are present in the digestive tract (Sanger, 1997). The ECS is assumed to be a major component in the interaction of physical responses and gastrointestinal function. It plays an important role in the brain and in the periphery in the regulation of energy balance, food intake and appetite control (Di Marzo et al., 1998, 2001; Matias and Di Marzo, 2007). There is evidence that CB1 and CB2 receptors as well as their ligands are present in the digestive tract (Sanger, 2007). CB1 receptors are located in neurons of the enteric nervous system, whereas CB2 receptors were described on inflammatory cells (Di Marzo and Izzo, 2006). Nevertheless, little is known about the role of the ECS in the control of gastric function and emptying in man. The ECS could represent an essential regulator of the gut-brain interaction.

In contrast to the considerable experimental evidence pointing to an important role of the ECS in mediating ethanol effects and despite the extremely high incidence of moderate ethanol consumption in many populations, only very few studies have investigated the effect of acutely
administered low doses of ethanol on the ECS in healthy humans (Joosten et al., 2010). We therefore performed a study in volunteers who consumed a moderate amount of ethanol as red wine and determined the effect of this one-time intervention on the plasma EC concentrations. Grape juice and plain water served as control liquids.

METHODS

Objectives
We performed an open label study to investigate the acute effects of a moderate amount of red wine on the ECS in adults, using the consumption of an equal caloric and volume load of grape juice or an identical volume of plain non-sparkling water as control intervention. We wanted to differentiate ethanol effects from those of a related compound with high sugar and very low ethanol content (grape juice) and from that of a pure volume effect in the absence of glucose or ethanol administration (plain water).

Ethical considerations
This investigation was approved by the Ethical Committee of the University of Munich (Protocol Nr: 088-08). Prior to study enrollment, every participant gave written informed consent.

Participants
Fifty-five healthy female and male individuals (see also Table 1; gender m/f: 32/23; age: 35.7 ± 8.1 years; height: 175.2 ± 8.9 cm; weight: 69.3 ± 10.3 kg; body mass index: 22.5 ± 2.4) participated in this experiment which was performed at the Department of Anaesthesiology of the University of Munich. All participants were on no particular medication, not addicted and normal alcohol-drinking individuals. In the alcohol-drinking group, 11 of 20 participants were drinking alcohol on a daily basis (alcohol amount: 22.5 ± 2.4) participated in this experiment which was performed at the Department of Anaesthesiology of the University of Munich. All participants were on no particular medication, not addicted and normal alcohol-drinking individuals. In the alcohol-drinking group, 11 of 20 participants were drinking alcohol on a daily basis (alcohol amount: 8.65 ± 9.72 g/day). The other two groups did not differ in this regard (grape juice group: 7 out of 17; 9.12 ± 12.4 g/day; water group: 8 out of 18; 11.4 ± 13.6 g/day). All three different study groups were not significantly different regarding any important metabolic condition.

Description of the investigation and specific procedures
Study protocol
All participants refrained from drinking and eating 4 h prior to protocol administration and were hence fasting (Fig. 1). Fluid consumption occurred in the early afternoon and was comparable in all study groups. After arrival in the study center, blood was drawn into an ethylenediaminetetraacetic acid (EDTA) prefill tube (S-Monovette®; Sarstedt, Nümbrecht, Germany) in an upright-sitting position for baseline data collection. The ‘red wine’ group (Group 1) then consumed 250 ml of a moderate amount of ethanol (red wine) within 10 min (Fig. 1). Twenty minutes after the end of drinking, a second blood drawing was performed, whereas the third blood collection was done 45 min after fluid intake. The other two study groups followed an identical protocol but consumed 250 ml of grape juice (Group 2) or plain water (Group 3).

Table 1. Demographic data of the different groups; data are mean ± SD; no significant differences were found between the groups

<table>
<thead>
<tr>
<th></th>
<th>Red wine (Group 1, n = 20)</th>
<th>Grape juice (Group 2, n = 17)</th>
<th>Water (Group 3, n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/f)</td>
<td>11/9</td>
<td>10/7</td>
<td>11/7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36.3 ± 7.9</td>
<td>35.3 ± 8.3</td>
<td>35.4 ± 8.5</td>
</tr>
<tr>
<td>Size (m)</td>
<td>1.75 ± 0.09</td>
<td>1.75 ± 0.07</td>
<td>1.76 ± 0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.6 ± 11.2</td>
<td>70.2 ± 10.7</td>
<td>70.4 ± 9.2</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.0 ± 2.5</td>
<td>22.8 ± 2.2</td>
<td>22.7 ± 2.5</td>
</tr>
</tbody>
</table>

Measurement of EC concentrations
For EC measurements, venous blood samples were drawn before fluid intake (baseline), after 20 min and after 45 min. Blood samples were collected into EDTA containing tubes (S-Monovette®, Sarstedt), immediately centrifuged in a cooled centrifuge (4°C), plasma transferred in Eppendorf tubes and then kept at −80°C. Under those storage conditions, ECs remain stable for up to 6 months (Di Marzo et al., 2009). Previous experiments have shown that EC generation in native, not cooled blood samples continues ex vivo (Vogeser et al., 2006). For this reason, immediate sample processing under cool conditions was performed until final storage.

To determine the plasma concentrations of the ECs AEA and 2-AG, high performance liquid chromatography-tandem mass spectrometry was applied, which has previously been described (Vogeser et al., 2006). This method is linear within a range of 0.1–2 ng/ml for AEA and 0.5–10 ng/ml for 2-AG. The lower detection limit of this method (defined as a signal/noise ration >4:1) is 0.025 ng/ml for AEA and 0.33 ng/ml for 2-AG. In biological matrices, 2-AG (including its deuterated analog) rapidly isomerizes to 1-AG (Vogeser and Schelling, 2007). For this reason, we quantified 2-AG as the sum of 1- and 2-esters of arachidonic acid (see also Feuerrecker et al., 2011).

As a control, we determined EC concentrations in the study fluids red wine, grape juice and water. EC concentrations were below detection limits in all liquids.

Measurement of blood ethanol and glucose levels
Blood ethanol levels were measured with the enzymatic UV test using an ethanol-dehydrogenase technique (Olympus AU2700, Beckman Coulter, Krefeld, Germany). For determination of blood glucose the hexokinase method was applied (Olympus AU2700, Beckman Coulter).

Statistical analyses
Deviation from normal distribution of sample data was tested using the Kolmogorov–Smirnov test. Changes in parameters (e.g. plasma EC or glucose concentrations) across the three time points of measurement were analyzed with a repeated measurement general linear model (RM-GLM) with time point as a within-subject variable and group assignment as a between-subject variable. Fisher’s Least Significant Difference test was used to define which groups differed significantly from each other. Sex was used as a covariate in some of these analyses.

Additional within group analyses across the three time points were performed using one-way RM analyses of
variance (RM-ANOVA) followed by a Holm–Sidak post hoc test.

The relationship between continuous data (e.g. plasma glucose and EC concentrations) was quantified by calculating Pearson’s $r$ in normally distributed data and Spearman’s $\rho$ in case of non-parametric data.

Demographic variables between groups were compared by ANOVA. A $P<0.05$ was regarded as statistically significant. Data are presented as mean ± SD with exception of figures, in which mean ± SEM are shown to increase clarity. Statistical calculations were performed using PASW Statistics 17.0 and Sigma Plot 11.0, Chicago, IL, USA.

RESULTS

General data

Fifty-five healthy volunteers participated in this study and were included in the final analysis. All participants were within the normal range of the body mass index. There were no significant differences with regard to sex distribution, degree of regular physical activity, alcohol consumption or other demographic variables between groups (Table 1). Detailed information of the administered fluids in this study is given in Table 2.

Plasma EC levels

The RM-GLM demonstrated a significant within-subject change in EC plasma concentrations over time (type III sum of square = 0.02, $F = 3.6, P = 0.03$ for AEA and type III sum of square = 27.9, $F = 17.9, P < 0.01$ for 2-AG) with a significant time point by group interaction (type III sum of square = 0.04, $F = 4.0, P < 0.01$).

Effect of ethanol (Group 1)

Both AEA and 2-AG decreased significantly at 20 min after wine intake (AEA from $0.23 \pm 0.12$ ng/ml at baseline to $0.18 \pm 0.07$ ng/ml, $P < 0.01$ and 2-AG from $6.68 \pm 4.13$ to $5.49 \pm 3.22$ ng/ml, $P = 0.01$). Forty-five minutes after wine intake, AEA remained nearly unchanged compared with the 20 min value, whereas 2-AG further and significantly declined (to $4.98 \pm 3.39$ ng/ml, $P < 0.01$ compared with the 20 min concentration) (Fig. 2).

Effect of grape juice (Group 2)

Plasma levels of AEA and 2-AG remained nearly unchanged when compared with baseline concentrations across the observation period (AEA = $0.22 \pm 0.13$ ng/ml at baseline vs. $0.21 \pm 0.11$ ng/ml after 45 min; 2-AG $3.15 \pm 1.83$ ng/ml before grape juice consumption vs. $2.78 \pm 1.21$ ng/ml 45 min thereafter) (Figs. 3 and 4).

Effect of plain water (Group 3)

Water consumption had no significant effects on AEA plasma levels, AEA concentrations were $0.21 \pm 0.08$ ng/ml at baseline, $0.25 \pm 0.10$ ng/ml after 20 min and $0.19 \pm 0.06$ after 45 min, $P = 0.13$). Likewise, no significant changes at 20 min after drinking of water were seen for 2-AG and 2-AG concentrations at baseline were $3.1 \pm 1.9$ and $2.7 \pm 1.4$ ng/ml at 20 min. At 45 min, a further drop in 2-AG concentrations was seen to $1.91 \pm 1.16$ ng/ml and, when compared with baseline values, the level of statistical significance was reached ($P<0.01$) (Figs. 3 and 4).

Blood glucose, ethanol concentrations and ECs

Red wine consumption resulted in a significant increase in plasma alcohol concentration (to $0.49 \pm 0.14$ g/l at 20 min, $P < 0.01$ and $0.55 \pm 0.12$ g/l at 45 min, $P < 0.01$, Table 3 and Fig. 2). Increased plasma alcohol concentrations were associated with a significant reduction in blood glucose levels at 20 min (from $93.3 \pm 13.8$ to $83.9 \pm 12.9$ mg/dl, $P < 0.01$), which remained at low levels during the observation period. After drinking 250 ml grape juice, blood glucose levels significantly increased (from $88.2 \pm 10.0$ to $123.1 \pm 17.3$ mg/dl, $P < 0.01$), before returning to baseline values at the 45 min time point. In contrast, blood glucose concentrations did not change in the water drinking Group 3 (Table 3).

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**Table 2. Characteristics of administered liquids**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Red wine (Group 1)</th>
<th>Grape juice, Naturell (Group 2)</th>
<th>Water, Adelholzener, non-sparkling (Group 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sugar content (g/dl)</td>
<td>&lt;0.8</td>
<td>18.0</td>
<td>—</td>
</tr>
<tr>
<td>Glucose (g/dl)</td>
<td>8.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fructose (g/dl)</td>
<td>8.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (Vol%)</td>
<td>14.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (g/dl)</td>
<td>11.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ethanol (g/250 ml)</td>
<td>28.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calories (kcal/dl)</td>
<td>75.0</td>
<td>75.0</td>
<td>—</td>
</tr>
<tr>
<td>Calories (kcal/250 ml)</td>
<td>187.5</td>
<td>187.5</td>
<td>—</td>
</tr>
</tbody>
</table>

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*a* Commendatore G.B. Burlotto, Via Umberto I, 9, 12060 Verdone, Italy.

*b* Haus Rabenhorst, Scheurener Str. 4, 53572 Unkel, Germany.

*c* Adelholzener Alpenquellen GmbH, St.-Primus-Straße 1-5, 83313 Siegsdorf, Germany.

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**Fig. 1. Study protocol; NPO, nil per os (4 h for drinking and eating), BDC, baseline data collection.**
2-AG concentrations at baseline before ingestion of the trial compounds across the whole study sample correlated positively with plasma glucose levels \((r = 0.43, P < 0.01, n = 55)\). This is in agreement with previous findings showing a positive correlation between impaired glucose clearance and 2-AG, but not AEA, plasma levels in obese humans (Cote et al., 2007). There was no significant relationship between blood glucose concentrations and AEA at baseline \((r = 0.17, P = 0.22, n = 55)\). The only consistent relationship between blood glucose concentrations and AEA at baseline \((r \geq 0.42, P < 0.05)\) was seen in the alcohol group. In this group, both AEA and 2-AG concentrations were weakly but positively related to blood glucose levels \((r \geq 0.42, P < 0.05)\) at the two time points. In contrast, after water ingestion blood glucose and AEA were negatively related at 20 min \((r = -0.51, P = 0.02)\) with no significant relationship at 45 min. At this time point, 2-AG levels correlated positively with blood glucose concentrations \((r = 0.56, P = 0.02)\) with no relationship at 20 min. In the grape juice group, no significant correlation between blood glucose and EC levels was seen.

We found no significant relationship between alcohol concentrations and plasma EC levels at any time point.

**Effect of sex**

When sex was included as a covariate into the RM-GLM, the model showed a significant between-subject effect for 2-AG, group assignment and sex (type III sum of square = 87.5, \(F = 5.7, P = 0.02\)). Female individuals showed a stronger trend toward lower 2-AG levels after red wine consumption than male participants \((4.14 \pm 2.77 \text{ ng/ml}, P = 0.09\) at 20 min and \(3.53 \pm 2.11 \text{ ng/ml}, P = 0.08\) at 45 min). No relationship between sex and AEA levels was observed (data not shown). Females also had significantly higher plasma alcohol concentrations than male...
et al. known to have reduced plasma EC concentrations (Hill et al., 2009). Thus, the ECS could play a role in the complex relationship between alcohol use, CAD and the risk for depression. This line of reasoning is, however, very preliminary and definitively requires additional studies but our findings could suggest directions for further research.

To the best of our knowledge, there are no investigations done in humans dealing with a fast one-time intake of moderate amounts of ethanol and their influence on plasma ECs. Joosten et al. (2010) recently reported AEA levels and moderate ethanol consumption combined with lunch during an observation period of 3 weeks. In their study they included only female participants who had to drink 60 min prior to a meal a first can of beer followed by a second one during lunch. The control group followed the same protocol drinking non-alcoholic beer (<0.1 g ethanol). The total intake of ethanol was ~13 g in the ethanol containing beer group. At the last day of the study, AEA plasma levels were determined. AEA levels decreased over time but there was no significant difference between the non-alcohol and the alcohol-drinking groups. The problem with this study are the many confounding variables such as the fasting regime, the relative slow intake of ethanol, as well as the food intake and the low amount of ethanol which could all have influenced the study results (Jesudason and Wittert, 2008).

There are no other studies which have evaluated the isolated effect of ethanol in humans on plasma EC levels. Therefore, only investigations in rodents or in vitro experiments can be compared with our findings. In rats, acute ethanol intake caused a reduction of AEA levels in several brain regions and peripheral tissues (Ferrer et al., 2007). The same AEA kinetic was reported by Basavarajappa and Hungund (1999) investigating SK-H-SH cells exposed to ethanol. These previously published experimental data are in good accordance with our findings.

In addition to a decline in AEA levels, we found a significant decrease in plasma 2-AG concentrations after ethanol consumption in our volunteers. This confirms findings of Rubio et al. (2007) who found a central reduction of 2-AG in the hypothalamus, the amygdala and significantly in the prefrontal cortex.

A limitation of our study results from the fact that we only investigated peripheral EC activity which may not necessarily reflect central EC signaling. There is, however, evidence that EC activity in peripheral nucleated blood cells is related to changes in central EC levels which could theoretically allow the use of peripheral EC measurements as a diagnostic tool for a number of neuropsychiatric disorders (Centonze et al., 2008).

In addition to ethanol-related changes in EC signaling, we determined the acute effects of sugar ingestion via grape juice on AEA and 2-AG levels. In contrast to red wine, grape juice resulted in a significant increase in blood glucose levels after 20 min, whereas plasma EC concentrations remained nearly unaffected. Various studies investigated the effects of the CB1 receptor antagonist rimonabant on glucose tolerance assessed by an oral glucose tolerance test (Scheen et al., 2006) but little is known about the acute effects of sugar intake on the ECS. Recently, it has been reported from experiments in rats that different types of sugar affect mRNA expression of enzymes involved in the degradation and synthesis of AEA and 2-AG in the hypothalamus. As only mRNA expression was measured, no conclusions regarding the activity of the enzymes involved in EC synthesis or degradation can be made. Despite these findings, it was speculated that the circulating levels of AEA and 2-AG are probably unaffected or slightly altered from the energy intake perspective (Erlanson-Albertsson and Lindqvist, 2010). The ECS is assumed to be a rapid processing system and might not be adequately displayed by the mRNA expression, as the authors state.

### Table 3. Plasma ethanol and glucose levels; data are presented as mean ± SD; Comparison of data in each group with baseline data

<table>
<thead>
<tr>
<th>Group</th>
<th>Time point</th>
<th>Blood glucose (mg/dl)</th>
<th>Ethanol plasma concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red wine (n = 20)</td>
<td>Baseline 93.25 ± 13.84</td>
<td>0.00 ± 0.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>83.90 ± 12.93*</td>
<td>0.49 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td>45 min</td>
<td>83.32 ± 10.74*</td>
<td>0.55 ± 0.12*</td>
</tr>
<tr>
<td>Grape juice (n = 17)</td>
<td>Baseline 88.24 ± 10.00</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>123.12 ± 17.28*</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>45 min</td>
<td>89.00 ± 12.81</td>
<td>—</td>
</tr>
<tr>
<td>Water (n = 18)</td>
<td>Baseline 91.61 ± 12.74</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 min</td>
<td>90.00 ± 9.40</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>45 min</td>
<td>89.72 ± 8.02</td>
<td>—</td>
</tr>
</tbody>
</table>

*P < 0.05, one-way RM-ANOVA with Holm–Sidak post hoc test.

study participants (0.59 ± 0.081 vs. 0.41 ± 0.14 g/l, P < 0.01 at 20 min and 0.61 ± 0.087 vs. 0.49 ± 0.12 g/l, P = 0.04, at 45 min).
Taking the findings of this study together, some other neuroendocrine aspects have to be addressed. ECs were thoroughly investigated in obese people and a number of studies have shown the relation between full meals and blood EC levels. Food intake leads to a reduction in peripheral ECs (Matias et al., 2006; Gatta-Cherifi et al., 2011). Similar results were shown for both individuals—normal weight and obese—following euglycemic hyperinsulinemic clamp (Di Marzo et al., 2009). It was also shown that moderate amounts of alcohol increased insulin sensitivity (Lazarus et al., 1997) and this could have resulted in the moderate decline in blood glucose levels observed in our study after the consumption of red wine. Therefore, the fact that insulin decreases AEA levels in lean subjects (Di Marzo et al., 2009), and/or our observation that AEA plasma concentrations positively correlate with blood glucose levels prior to the drinking test, could both explain why red wine consumption was accompanied by lower AEA levels. Interestingly, in our study, the grape juice drinking group showed no significant reduction of plasma ECs after consumption although the caloric intake of 187.5 kcal should have been sufficient to provoke an insulin response. As no insulin levels were measured one might only speculate that the insulin levels were too low to result in a decrease in EC levels or that the time interval of 4 h between the meal and the experiment was not sufficient to increase EC levels to an extent that would be then significantly reduced by the raise in insulin levels determined by grape juice consumption.

Other neuroendocrine interactions have been described for the ECS whereby cortisol and also ghrelin exert an important stimulatory action on EC biosynthesis. In fact, in a recent study, it could be shown that alcohol administration also leads to a decrease in ghrelin levels (Zimmermann et al., 2007). How these hormones interact and might contribute to an alcohol addiction need to be investigated in further studies.

In summary, this study demonstrated differential effects of fluid intake on the ECS. Moderate amounts of ethanol appear to reduce AEA and 2-AG, the caloric equivalent of a related, sugar containing fluid did not influence the ECS and plain water seems to affect only plasma 2-AG concentrations but not AEA. Further studies need to address the relationship between peripheral EC signaling and behavioral changes in humans induced by alcoholic and non-alcoholic beverages in more detail.

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