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The effect of short-term lyophilized beer consumption on established hypertension in rats

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Abstract

Effects of short-term lyophilized beer (LB) consumption on normotensive (WKY) and hypertensive (SHR) rats are reported. It was found that LB contains high quantities of bioactive compounds and has a high antioxidant potential. The WKY and SHR rats were divided into four groups of 8, two experimental and two controls, which were named LBWKY and LBSHR and ControlWKY and ControlSHR, respectively. LB was given to the rats of the LBWKY and LBSHR groups intragastrically at a dose of 2.72 g/kg in a volume of 10 ml/kg for 10 days. The rats of the control groups received saline solution. The following indices were determined: body weight gain, heart rate, systolic blood pressure, using a tail cuff method and GABA accumulation in the hypothalamus and the pons-medulla as measured by GABA-T inhibition. It was found that the treatment of rats with LB had no effect on the blood pressure and heart rate values. In both rat strains, LB decreased GABA accumulation in the hypothalamus and the pons-medulla.

A significant reduction of body weight gain was observed in both LB-treated groups when compared with the corresponding controls.

In conclusion, LB contains high quantities of bioactive compounds and possesses a high antioxidant potential. Diet supplemented with LB causes significant reduction of the central GABAergic activity in WKY and SHR rats without any effect on cardiovascular function. In addition, in both animal strains there was an apparent inverse association between LB intake and body weight gains.

Keywords: lyophilized beer; Antioxidant potential; Rats; Body weight gain; GABA accumulation

1. Introduction

In most Western countries alcohol beverages are an integral part of diets (Renaud and Lorgeril, 1992). Epidemiological, experimental and clinical investigations show that such diets, supplemented with various kinds of alcoholic beverages have a cardioprotective effect (Thun et al., 1997; Gronbaek, 2006). Moderate consumption of alcoholic beverages leads to cardioprotective effects: decrease in the plasma lipids levels and increase in the antioxidant and anticoagulant activities both in laboratory animals and humans (Renaud and Lorgeril, 1992; Gorinstein et al., 1997a; Gorinstein et al., 1997b; Thun et al., 1997; Klatsky, 2004; Mann and Folts, 2004; Gronbaek, 2006). Contrary, some authors have shown that consumption of alcoholic beverages increases blood pressure (Vandongen and Puddey, 1994).

Until now, red wine has been the cardioprotective beverage of choice (Klatsky and Armstrong, 1993). But Innes,

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1998, suggests that beer may also have beneficial effects on risk factors of the heart diseases. According to Innes, effectiveness, low cost, acceptable adverse-event profile and single-dose dispensers of beer are the basis for replacing red wine as the cardioprotective beverage of coronary atherosclerosis in hypertensive patients. It was shown that moderate wine drinkers have lower hypertension-related mortality (Renaud et al., 2004). However, consumption of alcoholic beverages has also adverse effects (Marway et al., 1993; Preedy et al., 1994). Therefore, lyophilized beverages are successful used in experiments on laboratory animals and investigations of humans: the level of the plasma lipids was decreased and the plasma antioxidant activity increased (Gorinstein et al., 1998a; Gorinstein et al., 1998b; Carbonneau et al., 1998; Serafini et al., 1998). This positive influence of lyophilized beverages is a results of their biologically active compounds mainly phenolic compounds (Carbonneau et al., 1998; Serafini et al., 1998). Statistical data show that coronary heart disease patients are often suffering also from high blood pressure (Vandongen and Puddey, 1994). Could moderate lyophilized beer consumption influence the blood pressure as the mentioned cardioprotective indices? In order to answer this question we decided to assess the effect of moderate dose of lyophilized beer in experiment on normotensive and hypertensive rats.

In a convincing experiment, Uchida et al., 1989, have found that tannin, a high molecular weight phenolic compound, prolongs the lifespan of stroke-prone spontaneously hypertensive rats. However, there has been no investigation done to assess whether the dry matter of alcoholic beverages could positively influence the cardiovascular system function of normotensive and hypertensive rats. Therefore, it was decided to perform such experiment.

The importance of the GABAergic mechanisms for the regulation of cardiovascular system function has been well established. Some investigators imply that the central GABAergic system in SHR is impaired (Sasaki et al., 1990). We attempted to clear up whether lyophilized beer as a cardioprotective beverage of choice might improve the disturbed GABAergic function in hypertension and normalize blood circulation. In our experiments we decided to use the lyophilized beer with a high content of phenolic compounds with high antioxidant potential.

2. Materials and methods

2.1. Reagents

Trolox (6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid) and 2,2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt (ABTS) were purchased from Sigma Chemical Co (St. Louis, MO, USA). All reagents were of analytical grade.

2.2. Beer samples

The Maccabee beer samples were produced by Tempo Beer Industries, Natania, Israel. To receive alcohol free examples they were freeze-dried and then analysed as previously described (Gorinstein et al., 1998a; Gorinstein et al., 1998b).

2.3. Chemical analysis of beer samples

Proteins, albumins, glucose, maltose, maltotriose, and dextrins were determined by conventional analyses. The determination of total polyphenols and beer antioxidant potential was done as follows:

(a) Total polyphenols were measured at 765 nm using Folin–Ciocalteu reagent with gallic acid as a standard and were expressed as mg/g of gallic acid equivalent (Singleton and Rossi, 1965).
(b) Total flavonoids were determined by a colorimetric method. A 0.25 mL of beer was diluted with 1.25 mL of distilled water; then 75 μL of a 5% $NaNO_2$ solution was added to the mixture. After 6 min, 150 μL of a 10% $AlCl_3 \times 6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min. A 0.5 mL quantity of 1 mol/L NaOH was added, and the total was made up to 2.5 mL with distilled water. The solution was well mixed, and the absorbance was measured immediately against the prepared blank at 510 nm using a spectrophotometer in comparison with the standards prepared similarly with known (+)-catechin concentrations. The results were expressed as mg/g of catechin equivalent.
(c) The antioxidant capacities were determined by $ABTS^{\cdot+}$ radical using $K_2S_2O_8$ and MnO_2:
(1) 2, 2′-azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) diamonium salt ($ABTS^{\cdot+}$) radical cation was generated by the interaction of ABTS (250 μM) and $K_2S_2O_8$ (40 μM). After addition of 990 μL of $ABTS^{\cdot+}$ solution to 10 μL of beer or plasma or Trolox (final concentration 0–20 μM) in phosphate buffered saline (PBS), the absorbance was monitored exactly 1 and 6 min after the initial mixing (Miller et al., 1996).
(2) $ABTS^{+}$ was prepared as well by passing a 5 mM aqueous stock solution of ABTS through manganese dioxide on a Whatman no. 5 filter paper. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μM Whatman PVDF syringe filter. This solution was then diluted in a 5 mM phosphate buffered saline, pH 7.4 to an absorbance of 0.70. The percentage decrease of the absorbance at 734 nm in (1) and (2) was calculated and plotted as a function of the concentration of the samples and of Trolox for the standard reference data and expressed as nM(TE) Trolox equivalent/g (Miller et al., 1996).

2.4. Animals

All procedures used in the present research were in compliance with KBN Animal Care Committee guidelines.

Thirty two adult male, 14-week old spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto (WKY) rats were used. The rats were maintained in an air-conditioned room at 21–22 °C, with a 12 h light–dark cycle. Standard pellet food (Labofeed-H) and tap water were available ad libitum throughout the experiment.

2.5. Experimental procedures

The animals were divided into four groups of 8, two experimental and two controls, which were named LBWKY and LBSHR and ControlWKY and ControlSHR, respectively. Lyophilized beer was given to the rats of the LBWKY and LBSHR groups intragastrically at a dose of 2.72 g/kg in a volume of 10 ml/kg for 10 days. The rats of the control groups received saline solution.

In both strains of rats, blood pressure and heart rate were recorded 24 h before the completion of the experiment. Systolic blood pressure (BP) was measured by tail cuff plethysmography with a photoelectric system (IITC/Life Sci. Instr. Mod.179, Woodland Hills, CA, USA). A mean value from at least four consecutive readings was used for calculations. Heart rate (HR) was counted from the blood pressure pulse wave. On the last day of study, the animals were sacrificed by decapitation and discrete brain structures taken for the GABA content determination.

2.6. Biochemical studies

The brain GABA accumulation was determined using the GABA-T inhibition method of Löscher et al., 1996. To prevent a post-mortem increase in GABA content, the rats used for the determination of GABA were injected with 3-mercaptopropionic acid (100 mg/kg, 2.5 min before killing) according to Carmona et al., 1980. Then, the rats received aminooxyacetic acid intraperitoneally at a dose of 50 mg/kg, 60 min before decapitation according to Löscher et al., 1991. The brains were quickly removed and dissected on ice-cold Petri dishes. The hypothalamus and the pons-medulla were isolated according to Balcom et al., 1975. Tissues were homogenized in a Potter–Elvehjem homogenizer and centrifuged (20 min, 8000×*g*, 4 °C). The clear supernatants were stored at −80 °C until assayed. The GABA concentration was measured spectrofluorimetrically (Uchida and O'Brien, 1964). Fluorescence was determined using a Shimatzu spectrofluorometer at 380/450 nm. GABA content was expressed in μM/g tissue. The detection limit of GABA was 0.1 nM.

2.7. Statistical analysis

The results of the investigation in vitro are given as the means ± SD of five measurements When appropriate, differences were tested by ANOVA. In the assessment of the antioxidant potential, Spearman correlation coefficient (R) was used. P-values of <0.05 were considered significant.

3. Results

3.1. In vitro

The used beer samples were rich in all bioactive compounds: proteins (Fig. 1A), amino acids (Fig. 1B), and carbohydrates (Fig. 1C).

The contents of polyphenols, flavonoids (epicatechin and quercetin) and some hydroxycinnamic phenolic acids (*p*-coumaric, gallic and ferulic) in the beer samples are shown in the Table 1. As can be observed from the table, these beer samples were also rich in the mentioned bioactive compounds.

Table 2 reflects the changes in the antioxidant potential of the beer samples after lyophilization as determined by with $K_2S_2O_8$ and MnO_2 tests. The decrease in the antioxidant potential of the lyophilized beer samples was not significant ($P > 0.05$). Therefore, the contribution of ethanol to the antioxidant potential of beer was minimal (R^2 from 0.35 to 0.39).

3.2. In vivo

Weight gains, food consumption and feed efficiency ratio in all 4 groups are summarized in Table 3. The data show that body weight gain slowed down significantly in rats receiving lyophilized beer (LBSHR and LBWKY).

The dynamics of the blood pressure and heart rate changes are presented in Fig. 2. A progressive increase in the blood pressure of SHRs was observed. The treatment with lyophilized beer did not affect this phenomenon. There were also no significant alterations in the heart rate. Likewise, the treatment of normotensive rats (LBWKY) with lyophilized beer had no effect on their BP and HR.

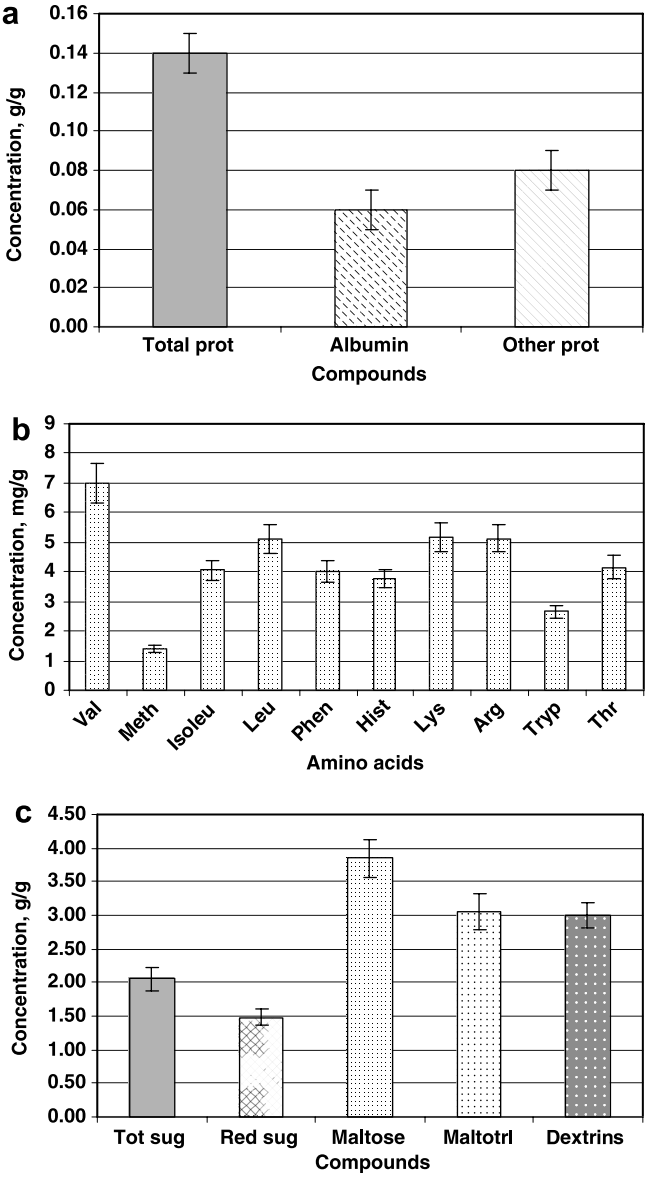

Fig. 1. Composition of the used beer samples: (A) Proteins. Abbreviations: Tot prot: total proteins; other prot: other proteins; concentration in g/g lyophilized sample. (B) Essential amino acids. Abbreviations: Val: valine; Meth: methionine; Isoleu: isoleucine; Leu: leucine; Phen: phenylalanine; Hist: histidine; Lys: lysine; Arg: arginine; Tryp: tryptophan; Thr: threonine; concentration in mg/g lyophilized sample. (C) Carbohydrates and sugars. Abbreviations: Tot sug: total sugars; red sug: reducing sugars; maltotri: maltotriose; concentration in g/g lyophilized sample.

The influence of the different diet treatments on the GABA accumulation induced by aminooxyacetic acid (AOAA) in the hypothalamus and the pons-medulla is depicted in Fig. 3. The administration of lyophilized beer decreased the GABA accumulation in the brain of SHR as well as WKY rats. The results suggest significant reductions in the GABA turnover rate in these two brain areas following lyophilized beer treatment. The GABA content in the hypothalamus and the pons-medulla was diminished in comparison with WKY rats (Fig. 3).

Table 1
Total polyphenols, flavonoids and some phenolic acids in used beer samples

Components	Concentration (mg/g)
Total polyphenols	9.33 ± 0.33
Flavonoids	1.40 ± 0.11
Epicatechin	1.77 ± 0.09
Quercetin	0.03 ± 0.01
p-Coumaric acid	0.06 ± 0.02
Gallic acid	0.08 ± 0.03
Ferulic acid	0.18 ± 0.12
Procyanidins	1.68 ± 0.11

Values are means ± SD of 5 measurements.

Table 2
Changes in the antioxidant potential of the used beer samples after lyophilization as determined by $K_2S_2O_8$ and MnO_2 tests (nM TE/g)

Samples	$K_2S_2O_8$	MnO_2
Before lyophilization	49.46 ± 3.1^a	56.49 ± 4.6^a
After lyophilization	48.11 ± 3.4^a	53.51 ± 4.2^a

Values are means ± SD of 5 measurements. Values in columns with different superscript letters differ significantly ($P < 0.05$).

Table 3
Body weight gains (g) in all 4 groups.

Groups	Weight gains (on the third day)	Weight gains (on the sixth day)	Weight gains (on the ninth day)
LBSHR	6.0 ± 0.3^b	6.0 ± 0.3^b	6.0 ± 0.3^a
ControlSHR	11.0 ± 0.7^c	13.0 ± 0.8^c	15.0 ± 0.9^b
LBWKY	0.1 ± 0.01^a	1.0 ± 0.1^a	4.0 ± 0.2^a
ControlWKY	0.2 ± 0.02^a	10.0 ± 0.6^c	12.0 ± 0.7^b

Values are means ± SD ($n = 8$).
Means in columns with different superscript letters are significantly different ($P < 0.05$).
Abbreviations: ControlWKY, control group of normotensive rats; ControlSHR, control group of hypertensive rats; LBWKY, experimental group of normotensive rats; LBSHR, experimental group of hypertensive rats.

4. Discussion

The circumstantial evidence, which links alcohol consumption with blood pressure is well documented (Klatsky et al., 1977). A large cross-sectional population study involving 86 000 males and females demonstrated no differences in blood pressure in males drinking one or two alcoholic drinks per day. However, in females both systolic and diastolic blood pressures were lower in light drinkers compared with no drinkers (Klatsky et al., 1977). Some other studies also found such J- or U-shaped associations (Harburg et al., 1980). In addition, clinical studies gave supportive evidence for a pressor effect of alcohol. So, in one of the investigations hypertensive males who regularly drank up to eight drinks daily were admitted to hospital (Potter and Beevers, 1984). After admission, alcohol was withdrawn for 3–4 days and the blood pressure fell, but increased again after alcohol was reintroduced.

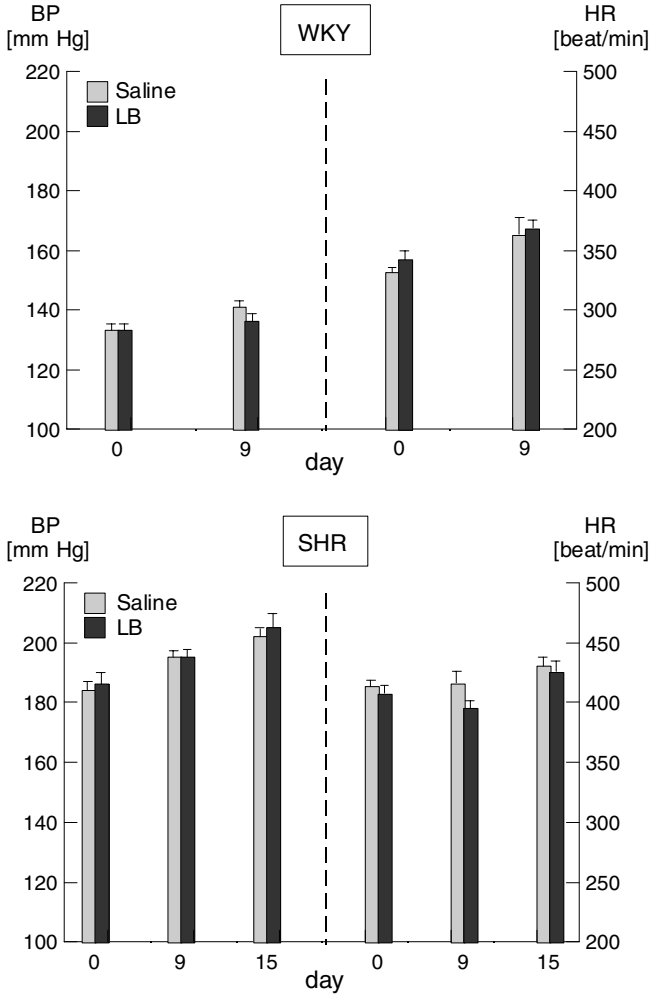

Fig. 2. Effect of lyophilized beer (LB) on blood pressure (BP) and heart rate (HR) in WKY and SHR rats ($n = 8$).

According to Vandongen and Puddey, 1994, ethanol rather than the type of beverage is the factor associated with blood pressure. However, it was shown that not only the ethanol content of alcoholic beverages is a biologically active factor, the dry matter of wine and beer may be also bioactive (Gorinstein et al., 1998a; Gorinstein et al., 1998b). Therefore, in the present study, we aimed to explore the possible association between intake of the beer dry matter and blood pressure. To the best of our knowledge, there have been no similar investigations done so far.

In our in vitro investigation we found that the lyophilized beer samples were rich in proteins, amino acids, and carbohydrates. Also the contents of polyphenols, flavonoids, and some phenolic acids in the used beer samples were high. These data are in accordance with other reports (Innes, 1998).

It should to be stressed that the antioxidant potential of the lyophilized beer samples was very high and the difference between antioxidant potential of lyophilized and not lyophilized beer samples was not significant. Thus, the contribution of ethanol to the beer antioxidant potential can be neglected. Also these data are in accordance with our

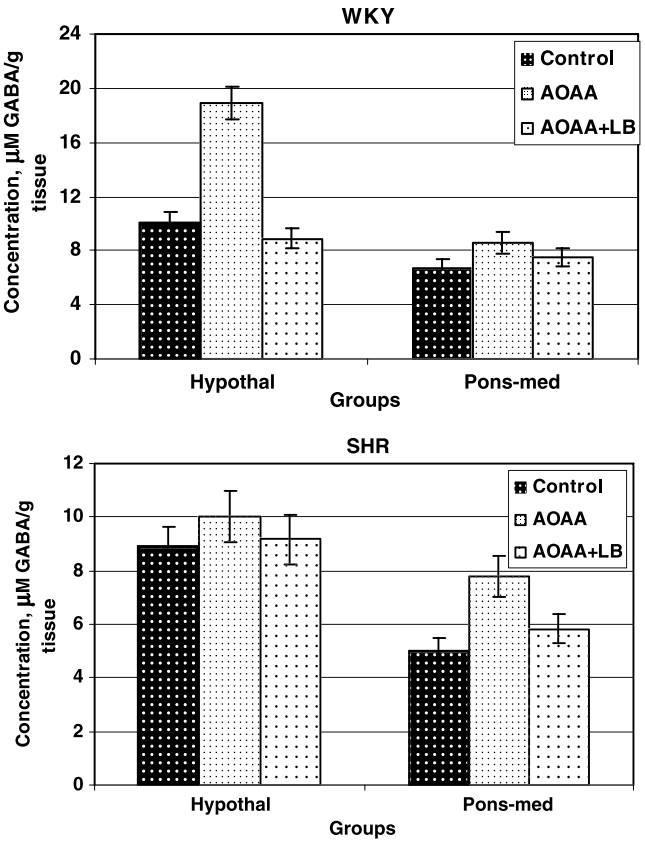

Fig. 3. Effect of lyophilized beer (LB) on GABA accumulation in the hypothalamus (*hypothal*) and the pons-medulla (*pons-med*) induced by *aminooxyacetic acid* (AOAA). ($n = 8$). $P < 0.01$, $P < 0.001$ versus AOAA group.

previous findings and the data of other authors (Gorinstein et al., 1998a; Gorinstein et al., 1998b; Carbonneau et al., 1998; Serafini et al., 1998).

The results of the investigation in vivo indicated that GABA content and consequently its turnover in the brain structures involved in blood pressure control i.e. the hypothalamus and the pons-medulla were lower in SHR than in WKY rats. It is in line with the outcomes of our previous studies and those of others who proved that the central GABAergic system in spontaneously hypertensive rats is impaired and its chronic inhibition may increase sympathetic overactivity (Czyżewska-Szafran et al., 1989; Czyżewska-Szafran et al., 1991; Singewald et al., 1992; Ichida et al., 1996).

Our present findings revealed that administration of lyophilized beer did not affect the established hypertension characteristic of the SHR strain. Similarly, LB was shown to have no influence on cardiovascular function in normotensive WKY rats. AOAA is an inhibitor of GABA-transaminase, the enzyme responsible for the GABA catabolism in the brain. AOAA significantly increases the intracellular GABA content in all brain regions. AOAA has an inherent action. The substance is commonly used in the experiments to estimate the GABA concentration and turnover rate in particular brain structures and thus to characterize the GABAergic tone of the concerned brain areas. The aim of our study was to explore the effect of LB on the GABAergic function in the brain structures involved in cardiovascular control. For that purpose it was sufficient to compare only two experimental groups of each rat strain: AOAA-injected animals with AOAA-injected and additionally LB-receiving animals.

Furthermore, this study provided evidence that the treatment of SHR and WKY rats with LB caused marked reduction in the AOAA-induced GABA accumulation in the brain structures mentioned above suggesting suppression of the central GABAergic activity.

Recently, a number of reports demonstrated that certain neurosteroids synthesized in the brain can modulate the inhibitory action of $GABA_A$ receptor (Robel and Baulieu, 1995; Barbaccia et al., 1997; Morrow et al., 1999). On the other hand, the inhibition of steroid synthesis was shown to prevent completely the inhibitory effects of alcohol associated with $GABA_A$ receptor modulation (VanDoren et al., 2000). Therefore, it seems very likely that cholesterol, as a known precursor of all neurosteroids synthesis may indirectly influence the $GABA_A$ receptor function (Papadopoulos et al., 1992).

In our previous studies on rats fed with lyophilized beer, we observed significant reduction of plasma total cholesterol level (Gorinstein et al., 1998a; Gorinstein et al., 1998b). It is possible that the lowered cholesterol concentration in the blood of the LB-treated rats contributes to the changes in the brain GABA accumulation observed in our study.

The results of the present study showed that there exists a clear association between the brain GABA accumulation and turnover, and the diet supplemented with lyophilized beer having no impact on cardiovascular function. It might be, however, that prolonged treatment with LB would intensify arterial blood pressure as a result of the permanent GABA system suppression. Therefore, further studies with a long term LB intake are needed to confirm the lack of the haemodynamic effects of LB, despite the marked attenuation of the central GABAergic activity.

At this stage of surveys, it is difficult to explain the mechanism responsible for the reduction of the body weight gains in LB-treated rats as compared with their corresponding controls. However, our results are consistent with those of other authors who report that beer intake is inversely associated with the body mass index in men and women and that alcohol beverage consumers are leaner than abstainers (Kahn et al., 1997; Männistö et al., 1997; Dallongeville et al., 1998). It is conceivable, that the impared GABAergic inhibition observed in this study produces alterations in sympathetic activity of importance for energy turnover and motor function. The regulation of body weight via regulation of sympathetic outflow is suggested in a number of reports (Eslami and Tuck, 2003; Nagai and Moritani, 2004; Hegling et al., 2005). In such case lyophilized beer owing to its influence

on the central GABAergic mechanism and consequently sympathetic regulation could be effective for body mass reduction.

At the beginning we expected that LB will positively influence BP and HR by means of the GABA content elevation in the brain areas related to cardiovascular control. It was not the case and the GABA content was lowered in those areas as compared with AOAA-alone group. Normally, the reduction of central GABA content should result in BP elevation. Surprisingly, we noted that under the experimental conditions applied LB did not exert any deleterious effect on cardiovascular function in both rat strains having reducing influence on their weight gains. Therefore, our results indicated that LB has potent biological activity and can be safe ingested by both hypertensive and normotensive objects. The weight gain reducing action may have additional beneficial effect for the treatment of obesity which is often connected with hypertension. However, to clear the significance of these observation for the putative future alimentary therapy further intensive studies with prolonged LB administration and diverse dosing will be needed.

In conclusion, lyophilized beer contains high quantities of bioactive compounds and exhibits a high antioxidant potential. A diet supplemented with lyophilized beer causes significant reduction of central GABAergic activity in normotensive and hypertensive rats without any effect on cardiovascular function. In addition, in both animal strains there exists an inverse association between lyophilized beer intake and body weight gain.

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