

EVALUATION OF THE IMPACT OF ETHANOL ON THE NERVOUS CONTROL OF MICTURITION - THE ANALYSIS OF THE LINK BETWEEN THE CONCENTRATION OF ALCOHOL IN THE BLOOD AND THE VOLUME OF URINE IN THE BLADDER

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EVALUACIJA UTICAJA ETANOLA NA NERVNU KONTROLI MOKRENJA – ANALIZA VEZE IZMEĐU KONCENTRACIJE ALKOHOLA U KRVI I ZAPREMINE MOKRAĆE U MOKRAĆNOJ BEŠICI

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ABSTRACT

Numerous biochemical and electrophysiological studies have determined that the functions of certain receptors and ion channels for which they are in some way affected by ethanol. Some of these are involved in the neural control of micturition, and it is therefore possible that ethanol induces urinary retention. The aim of this study was to analyse the relationship between the concentration of alcohol in the blood and the volume of urine in the bladder measured during autopsy. The study was based on autopsy protocol data from 702 people. The inclusion criteria were age not exceeding 50 years and a blood alcohol level of at least 0.5 parts per thousand. A correlation between the blood alcohol concentration and a bladder urine volume over 200 ml can be portrayed as part of a range limited by curves asymptotically converging at a value of 3 per mil. This phenomenon may be explained by the effect that alcohol has on receptors involved in the micturition reflex, which should typically be engaged at approximately 200 ml of urine in the bladder. The lower limit of this range can be explained by GABA-A stimulation as well as the inhibition of NMDA receptors or by decreasing influx through calcium channels. Both of these effects inhibit the micturition reflex. However, the upper limit, which indicates reduced urine retention, may be caused by the action of high concentrations of ethanol at the ends of the adrenergic fibres, causing their dysfunction, or by changes in the intracellular calcium ion concentration.

Key words: Alcohols, Bladder, Ethanol, Urine

SAŽETAK

Brojne biokemijske i elektrofiziološke studije su utvrdile da su funkcije određenih receptora i jonskih kanala, na neki način, pogođene dejstvom etanola. Neki od njih su uključeni u nervnu kontrolu mokrenja, i zato je moguće da etanol izaziva retenciju urina. Cilj ove studije bio je da analizira odnos između koncentracije alkohola u krvi i zapremine urina u mokraćnoj bešici, merenih prilikom obdukcije. Studija je zasnovana na podacima iz obduccionih protokola 702 ljudi. Kriterijumi za uključivanje su bili starost ispod 50 godina i količina alkohola u krvi najmanje 0,5 promila. Korelacija između koncentracije alkohola u krvi i zapremine urina u mokraćnoj bešici preko 200 ml može biti predstavljena kao deo opsega ograničenog krivama koje asimptotski konvergiraju ka vrednosti 3 promila. Ovaj fenomen se može objasniti efektom koji alkohol ima na receptore koji su uključeni u refleks mokrenja, koji se obično aktiviraju pri zapremini od oko 200 ml mokraće u mokraćnoj bešici. Donja granica tog opsega se može objasniti stimulacijom GABA-A receptora kao i inhibicijom NMDA receptora, ili smanjenjem influksa kroz kanale za kalcijum. Oba ova efekta inhibišu refleks mokrenja. Međutim, gornja granica, koja ukazuje na smanjenu retenciju urina, nastaje dejstvom visokih koncentracija etanola na završetke adrenergičkih vlakana, izazivajući njihovu disfunkciju, ili promene u intracelularnoj koncentraciji jona kalcijuma.

Ključne reči: alkoholi, mokraćna bešika, etanol, urin



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INTRODUCTION

Several case reports linking ethanol and urinary bladder dysfunction with resulting urinary retention have been published. [1,2] Animal studies using the urinary bladders of rabbits have demonstrated that ethanol increases bladder capacity and decreases detrusor muscle contraction activity. However, the mechanism regulating this phenomenon is unclear. [16] Opinions differ as to whether this is controlled by the effect of an increased intracellular calcium level, nitric oxide synthesis inhibition or the regulation of receptor function. [3, 4, 6]

Ethanol influences the functioning of receptors involved in the regulation of reflex micturition. GABA-A receptors inhibit the voiding reflex, whereas the NMDA receptors activate it. The influence of the autonomic nervous system is also important. Parasympathetic postganglionic axons in the pelvic nerve release acetylcholine (Ach), which results in bladder contraction by stimulating M3 muscarinic receptors in the bladder smooth muscle. However, sympathetic postganglionic neurons release noradrenaline (NA), which activates beta-3 adrenergic receptors to relax the bladder smooth muscle and alfa-1 adrenergic receptors to contract the urethral smooth muscle. [3]

MATERIALS AND METHODS

The study was performed via a retrospective analysis of autopsy protocols archived at the Forensic Medicine Department of Jagiellonian University Medical College from 2006 to 2010. The study group comprised 702 people younger than 50 years of age with a blood ethanol concentration at or above 0.5 per mill. We limited the age of the decease to a maximum of 50 years to exclude the influence of benign prostatic hyperplasia, which can cause urinary retention. The exclusion criteria also included the presence of drugs and toxic substances such as diazepam, carbamazepine, non-steroidal anti-inflammatory drugs, chlorpromazine, morphine and bencyclane in the blood sample. This action eliminated their influence on the results by causing urinary retention. [1] The statistical analysis was performed using Statistica (Statsoft, v8.0) software.

The statistical analysis was performed for all cases and a group without a history of chronic ethanol abuse. Urine volume was measured in millilitres (mL), and the ethanol blood concentration was per mills (ppm). Descriptive statistical analysis was performed for all cases. Ninety-five per cent confidence intervals (95% CI) were also calculated on the basis of the Poisson distribution. The distributions of the blood alcohol concentration and bladder urine volume were checked for normality with the use of the Shapiro-Wilk test. If P values were less than 0.01, there was no normality. However, when a data set is larger than 100 cases, parametric methods are the most appropriate. Simple linear correlations and linear multiple regression were used for the assessments.

RESULTS

Table 1. The results of the linear correlation analysis of urine volume in the urinary bladder and blood ethanol concentration in the two groups.

Data analysis was performed in the following groups:	Pearson's correlation coefficient	The coefficient of determination (R ²)	Level of significance (p-value)
All cases (n=702)	0.20	0.04	0.00001
Cases without alcoholics (n=473)	0.18	0.03	0.00007

The coefficient of determination showed the degree of fit of our model to a model with linear correlations between variables. According to the low level of correlation, which was 3-4%, there was no statistically significant linear correlation.

Analysed relationship to the variable: volume of urine in the bladder	All cases (n=702)	Cases without alcoholics (n=473)
blood alcohol level ²	Beta = 0.19 R ² = 0.03 p = 0.00002	Beta = 0.16 R ² = 0.02 p = 0.0001
blood alcohol level ³	Beta = 0.17 R ² = 0.03 p = 0.00007	Beta = 0.12 R ² = 0.01 p = 0.004
sqrt(blood alcohol level)	Beta = 0.2 R ² = 0.04 p = 0.00001	Beta = 0.18 R ² = 0.03 p = 0.00005
log(blood alcohol level)	Beta = 0.19 R ² = 0.04 p = 0.00001	Beta = 0.2 R ² = 0.04 p = 0.00002
e ^{blood alcohol level}	Beta = -0.01 R ² = 0.006 p = 0.9	Beta = 0.002 R ² = 0.0006 p = 0.8

Table 2. The results of a linearised linear regression of urine volume in the urinary bladder and blood ethanol concentration with the analysed factors.

When we considered the coefficients of determination, no nonlinear model could be described by any function that could explain this correlation.

A scatterplot of correlations between blood ethanol concentration and urine volume in the urinary bladder was made to investigate more advanced correlations.

In cases with a urine volume above 200 ml, we observed that dispersion of the blood ethanol concentration decreased. The scatterplot of correlations between blood ethanol concentration and urine volume in the urinary bladder could be limited by asymptotic curves convergent at a blood ethanol concentration of 3 per mills. This correlation was more noticeable in cases without a history of chronic alcohol abuse.

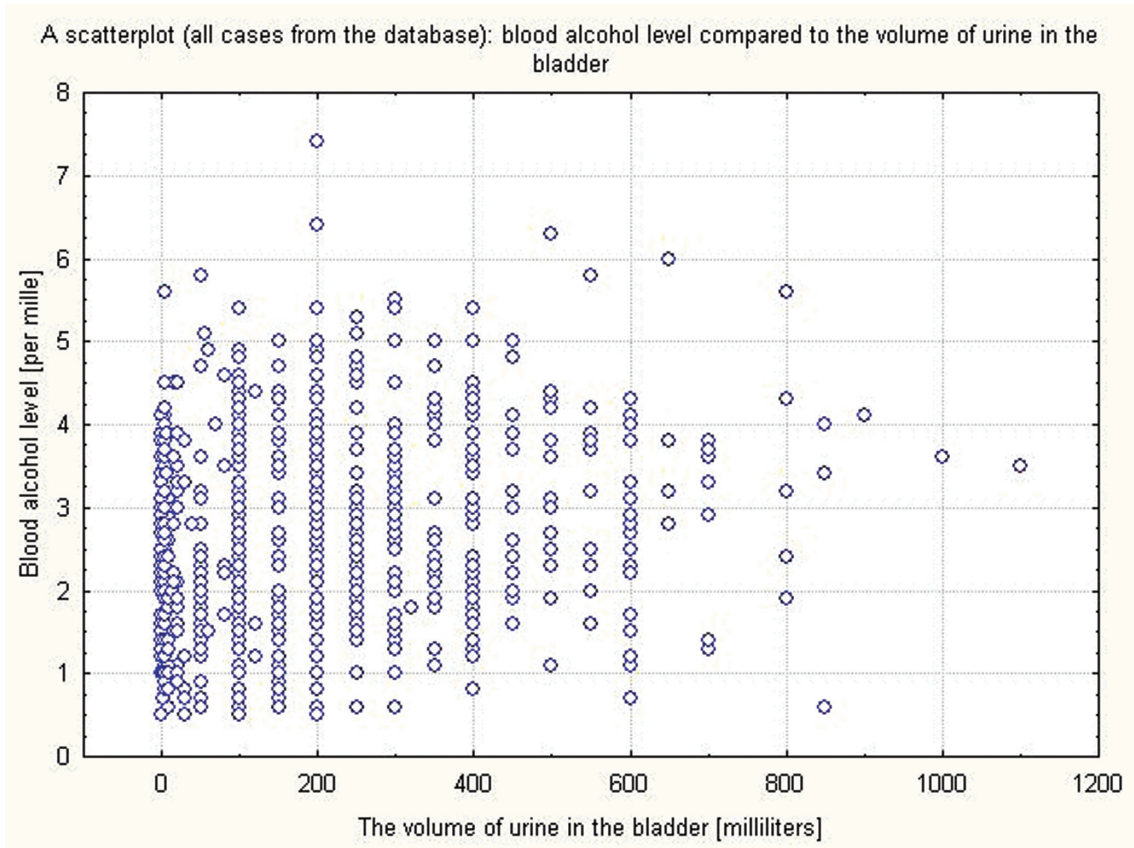


Figure 1. Figure showing the relation between urine volume in the urinary bladder and blood ethanol

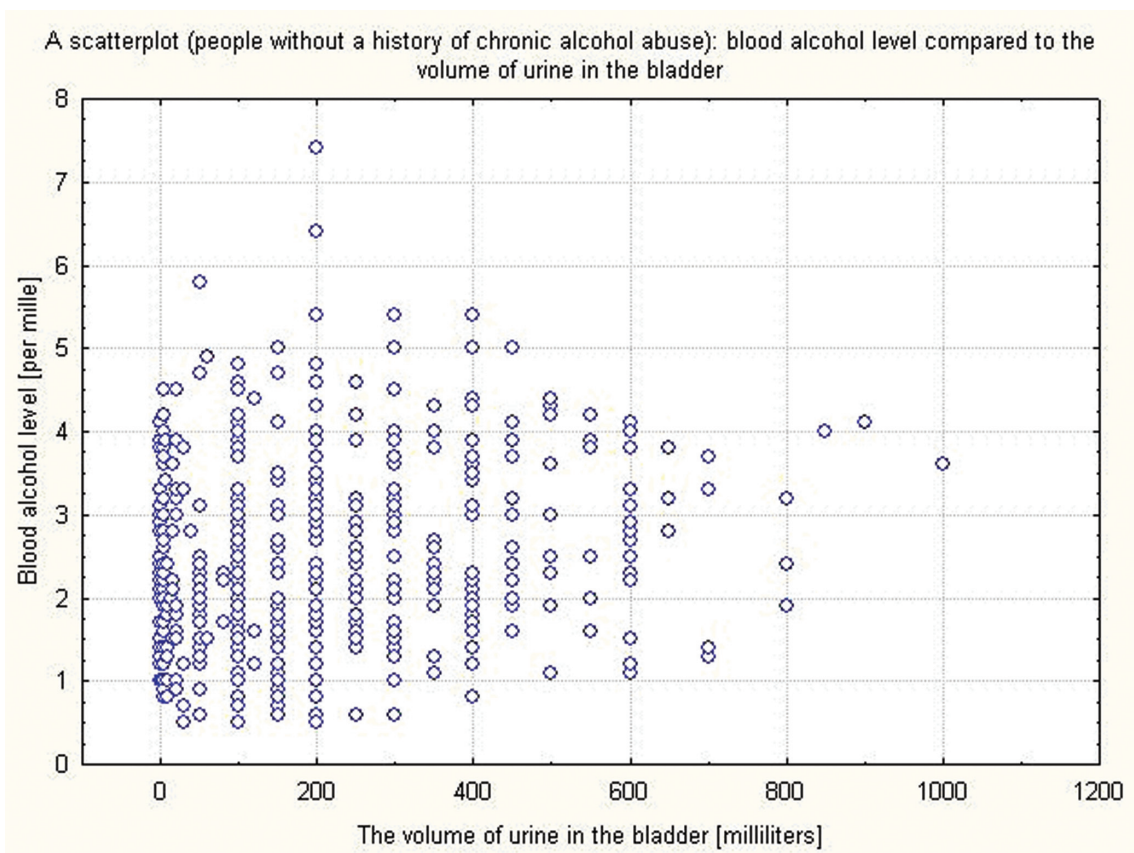


Figure 2. Relation of urine volume in the urinary bladder and blood ethanol concentration in the group without a history of chronic ethanol abuse.

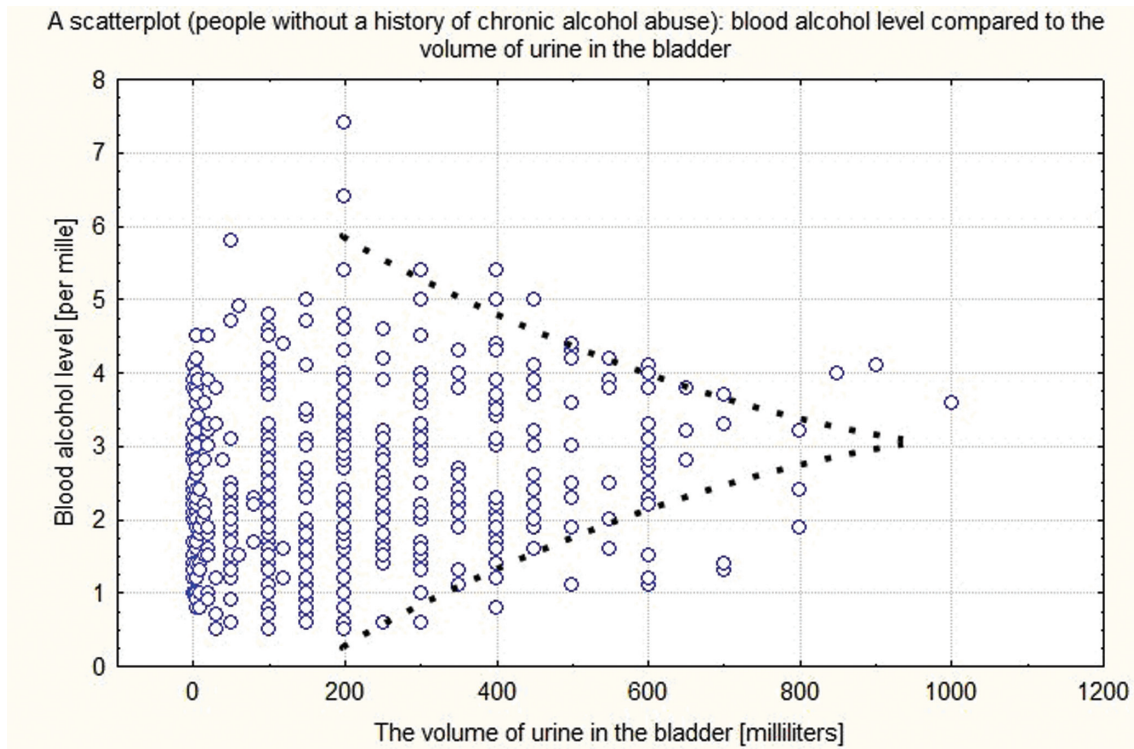


Figure 3. A scatterplot of urine volume in the urinary bladder and blood ethanol concentration with asymptotic limiting curves (cases without a history of chronic ethanol abuse).

As shown in figure 3, limitation curves were created to gain a better visual understanding of our argument.

DISCUSSION

Molecular pharmacology studies have disclosed a few primary targets for ethanol such as N-methyl-D-aspartate (NMDA), γ -aminobutyric acid A (GABA-A), glycine, 5-hydroxytryptamine-3 (5-HT₃), neuronal nicotinic acetylcholine (nACh) receptors and L-type Ca^{2+} channels. [5,7,8,9] Some of these neurotransmitter systems are responsible for the effects of ethanol on urine retention described in our study. Experiments performed on rats by Matsumoto have shown that the intravenous administration of an NMDA receptor antagonist blocks the bladder contractions evoked by electrostimulation of the PMC (pontine micturition centre), supporting the role of NMDA receptors in mediating excitatory transmission in the descending limb of the spinobulbospinal micturition reflex pathway. [18] In the next section, we describe the results of Lovinger's experiment, which confirmed the inhibitory effect of ethanol on NMDA receptors and may explain our findings. In Pehrson's experiment, the blockade of GABA-A and GABA-B receptors in the spinal cord and brain stimulated rat micturition, which confirmed the assumption that activation of GABA-A and GABA-B receptors may be responsible for the continuous inhibition of the micturition reflex within the CNS. [19] The results of studies confirming the stimulating effect of ethanol on GABA receptors and the implications of these findings with respect

to our results are described in the next section. The action of ethanol on serotonin receptors cannot be considered because it affects subtype 5-HT₃, whereas subtypes 5-HT₁ and 5-HT₇ are involved in controlling the micturition reflex. Additionally, the impact of ethanol on acetylcholine receptors cannot be considered because although several studies have confirmed its action on nicotinic receptors, its effect on muscarinic receptors remains unclear, and research has focused primarily on chronic abuse. [20,21] Göthert's experiment suggested a relation between the ethanol concentration and noradrenaline output. The noradrenaline output from peripheral sympathetic nerves was inhibited only by lethal concentrations of alcohol. Many studies have shown that noradrenaline causes detrusor muscle relaxation and inhibits adrenergic fibres, resulting in detrusor contraction. [22] The influence of ethanol on ion channels, particularly calcium channels, which subsequently increases the intracellular calcium concentration and may facilitate detrusor muscle constriction, might also be important. [16]

We separately analysed a group with a history of chronic alcohol abuse to investigate neuroadaptation of receptor systems caused by the effects of long-term ethanol use on neurotransmitters. Ethanol tolerance was caused by a decreased number of GABA-A receptors (down-regulation); changes in receptor structure and a decreased response by neurotransmitters; or an increased number of NMDA receptors (up-regulation). [5, 7-12] This adaptation of the receptor system to chronic activation/inhibition could have interfered with our outcomes if the receptor response participates in the effect of ethanol on micturition.



In our analysis, the described correlation in the group with a urine volume above 200 ml could be explained by the receptor response because micturition is activated by this amount of urine. [4] Moreover, after the exclusion of alcoholics, the scatterplot was more restricted, which could be explained by adaptation of the receptor system.

As shown in Figure 3, the lower limiting curve demonstrated increased urine retention. This could be the effect of micturition reflex inhibition or an inability to contract the detrusor muscle. The research of Lovinger on NMDA receptors in rat ganglions showed that ethanol inhibition was dependent on the ethanol concentration. Increasing inhibition potential was observed from a blood ethanol concentration of 0.12 per mills (2.5 mM) to 2.3 per mills (50 mM); a further concentration increase had no influence on the strength of receptor inhibition. The IC₅₀ (the concentration that inhibits 50% of receptors) was 0.46 per mills (10 mM) [8-10]. Stimulated NMDA receptors activate the micturition reflex. Inhibition of NMDA receptors explains the increasing urinary retention observed at a low blood ethanol concentration. We hypothesise that the lower limitation curve at a blood ethanol concentration of 0.5 per mills to 3 per mills was caused by this mechanism. Our research indicates that ethanol activates GABA-A receptors at a blood concentration above 2.76 per mills (60 mM), but receptors containing the delta (δ) subunit were activated by a blood ethanol concentration of at least 0.14 per mills (3 mM). [5, 12] The GABA-A receptors that participated in the micturition reflex consisted of $\alpha_2\delta$ subunits, and their activation inhibited the micturition reflex and influenced the lower limitation curve. [11] Another explanation of the lower limitation curve could be the influence of ethanol on smooth muscle tension, which is regulated by calcium channels. This leads to decreased intracellular calcium inflow and limits detrusor muscle contraction. A study using PC12 cells showed this effect at ethanol concentrations of 1.15-1.38 per mills (25-30 mM). However, an ethanol concentration above 4.6 per mills (100 mM) increased the free intracellular calcium level. This effect could explain the upper limiting curve, when decreased urinary retention was caused by detrusor muscle contraction. [10, 15] The mechanism through which the calcium level was increased is unclear, but calcium can be released from intracellular depots. [6, 16] Ethanol may also cause adrenergic nerve fibres to malfunction, which could influence the upper limiting curve. Laboratory experiments on isolated rat detrusor muscle have shown that ethanol can weaken the beta-adrenergic response upon stimulation. [13] Research on noradrenaline nerve excretion in the presence of ethanol revealed 50% decreased excretion (IC₅₀) in response to chemical stimulation at an ethanol concentration of 5.6 per mills (129 mM). [14] According to cited research, the inhibition of urine retention as an effect of a high ethanol concentration could be explained by adrenergic system inhibition and dominance of the cholinergic system. [17]

CONCLUSION

It has been shown that ethyl alcohol affects the micturition reflex and can cause urine retention. Ethanol inhibited the micturition reflex at a blood concentration below 3.0 per mills. Higher ethanol concentrations inhibited urinary retention via inhibition of the adrenergic system and activated the micturition reflex.

A low ethanol concentration affected NMDA receptors, GABA-A receptors and the intracellular calcium level, inhibiting the micturition reflex and leading to urinary retention. A higher ethanol concentration inhibited the adrenergic system and affected the intracellular calcium level, inhibiting urinary retention and facilitating detrusor muscle contraction.

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