The effects of ethanol and vasopressin on renal function in the isolated perfused rat kidney

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Abstract

Controversial results regarding the role of vasopressin as a mediator of ethanol-induced diuresis raise the question, whether ethanol may stimulate diuresis directly on the level of the kidney, possibly by its vasodilating properties. We thus studied the effects of different ethanol-concentrations (0.5 ‰, 1 ‰, 2 ‰, and 4 ‰; n = 4 to 6, respectively) – in the presence of 10 mU/l vasopressin in the perfusion medium – and the effect of absence of vasopressin (-VP; n = 5) in isolated rat kidneys perfused for 180 minutes in a closed circuit system. A control group (con+VP; n = 6) was studied with the standard perfusion medium containing 10 mU/l vasopressin. Urine flow (UV) and free water clearance (C_{H2O}) were increased (UV: con+VP: 124 ± 78 vs. –VP: 258 ± 88 µl*min^{-1}*g kidney-1; C_{H2O}: - con+VP: 20.4 ± 7.4 vs. –VP: 51.6 ± 20.8 µl*min^{-1}*g kidney^{-1}, p < 0.05 respectively) and fractional reabsorption of sodium (FRNa) were increased in rats perfused without vasopressin (FR_{Na}: con+VP: 83.7 ± 8.3 vs. –VP: 75.0 ± 2.6 %, p < 0.05). Ethanol-treatment had no effect on urine flow, sodium and potassium excretion, glomerular filtration rate, and renal vascular resistance. These data show that ethanol-mediated diuresis cannot be explained by intrinsic effects of ethanol on the kidney, at least in an isolated and denervated perfused rat kidney model.

Key words: ethanol-induced diuresis, fluid homeostasis, renal function

Background

Historically, the diuresis accompanying ethanol ingestion has been attributed to a decrease in circulating vasopressin levels and a decrease in hypothalamic osmoreceptor sensitivity, leading to smaller increase in plasma vasopressin during a comparable osmostimulation [1]. However, several studies have questioned this classical point of view, since ethanol-induced diuresis was observed in healthy volunteers despite unchanged or even increased plasma vasopressin levels [2,3]; suggesting, that additional factors may be involved in mediating the diuretic effects of ethanol.

Carney et al. studied the effects of ethanol in anesthetized rats [4], in which vasopressin secretion was inhibited by water loading and showed that an increase in plasma ethanol was still accompanied by an increase in urine flow and natriuresis; leading to the assumption, that ethanol-diuresis may also be explained by direct effects of ethanol on the kidney.

Besides its diuretic effects, ethanol has been shown to have direct vasoactive properties and to induce vasodilatation in low and vasoconstriction in high concentrations in isolated vessels [5], raising the question, if the effects of ethanol on the kidney and ethanol-induced diuresis may be explained by variations in renal blood flow and/or glomerular filtration rate.

We hence hypothesized that ethanol-induced diuresis may be explained by intrinsic effects of this drug on the kidney. To test this hypothesis, we investigated the effect of different ethanol concentrations on renal functional parameters in an isolated perfused rat kidney model. Since the natriuretic and diuretic stability of our model is achieved by perfusion with vasopressin in a low concentration, we additionally studied the effects of vasopressin depletion in this in vitro model.

Materials and methods

All experiments were performed in accordance with the 'Guiding Principles for Research involving Animals and Human beings'. Kidneys were obtained from adult male Sprague-Dawley rats, weighing 190 - 300 g. The animals had free access to food (Altromin 1314, Lage, Germany) and water.

The preparation of the kidneys, the components of the perfusion medium and the experimental setup have been reported in detail previously [6]. In brief, kidneys were harvested from rats anesthetized with pentobarbital (60 mg/kg BW i.p.). The right kidneys were used for perfusion, the left kidneys were decapsulated and weighed for the calculation of renal functional parameters.

Kidneys were perfused with an amino acid- and substrate enriched Krebs-Henseleit buffer containing freshly drawn human erythrocytes (hematocrit, 5%) and 60 g bovine serum albumin (BSA). Of note, our standard perfusion medium contains 10 mU*l^{-1} vasopressin (Pitressin; Parke-Davis, Germany) to reduce the "wash-out of medullary hypertonicity" and to achieve greater natriuretic

stability (for a detailed discussion of this model and the complete composition of the perfusion medium please see [6]).

Experimental setup

The isolated kidneys were perfused at 37° in a closed recirculation system. From the reservoir, the perfusion medium (200 ml) was pumped through a dialyzer and regenerated against 5000 ml dialysate composed like the perfusion medium except that BSA and erythrocytes were omitted. The dialyzer also served for the equilibration ("dialung") with a prewarmed and moistened gas mixture (5% CO_2; 95% O_2). In the ethanol groups, respective concentrations were achieved by adding 99% ethanol (Merck, Germany) to the perfusion medium and the dialysate.

Kidneys were perfused with a constant perfusion pressure. The pressure signal was taken for feedback regulation of the perfusion pump. Perfusion flow and pressure were recorded continuously.

Experimental design

Six series of experiments were performed: control (n = 6), ethanol 0.5 ‰ (n=5), 1 ‰ (n=6), 2 ‰ (n=4), 4 ‰ (n=5), and a series without the standard concentration of vasopressin (n=5).

In each series kidneys were perfused for 3 hours with 100 mmHg. Measurements of renal functional parameters were performed every 30 minutes by sampling urine via an ureter catheter and perfusate from the reservoir.

Urine flow (UV) was determined gravimetrically. Urinary sodium excretion ($U_{Na}V$) and potassium excretion (U_kV) were determined by flame photometry. Perfusion flow rate (PFR) was derived from the revelations of the perfusion pump (tacho signal). Glomerular filtration rate (GFR; determined from the clearance of inulin), free water clearance (C_{H2O}), fractional reabsorption of sodium (FR_{Na}), and renal vascular resistance (RVR)

were calculated according to standard formula.

Statistical analyses

Data are given as mean ± standard deviation and are the average of six measurements during the perfusion period. Following a Kolmogorov-Smirnov test for normal distribution, differences between interventions and the control group were determined by ANOVA and posthoc Fisher's PLSD. The level of statistical significance was set to $p < 0.05$.

Results

Ethanol treatment had no significant effect on renal functional parameters (table 1). PFR tended to be lower ($p = 0.053$) and RVR to be higher ($p = 0.07$) in the 4 ‰ ethanol group in comparison with the control group.

In contrast, UV and C_{H2O} were significantly higher and FR_{Na} was lower in the vasopressin depleted group in comparison with

the control group (table 1).

Discussion

The present study was designed to investigate the effects of ethanol treatment on renal functional parameters to determine, if the alcohol-induced diuresis may be explained by intrinsic effects of ethanol on the kidney. The vasodilating properties of ethanol are well known and have been related to variations in cytosolic calcium [5]. In vivo, ethanol has been shown to induce an increase in digital arterial blood flow, a decrease in forearm pulsed-wave-velocity [7] and an increase in splanchnic blood flow [8]. Hence we hypothesized that the renal effects of ethanol may – at least in part – be mediated by vasodilatation, i.e. an increase in renal blood flow and/or variations in glomerular hemodynamics.

The results of the present study – performed in a well established in vitro model [6] with several ethanol concentrations – cleary show, that ethanol per se has no diuretic and

Table 1: Renal function in isolated rat kidneys perfused with a standard concentration of vasopressin (10 mU* l⁻¹) and different concentrations of ethanol, and in the absence of vasopressin.

	control +VP n=6	0.5 ‰ ethanol +VP n=5	1 ‰ ethanol +VP n=6	2 ‰ ethanol +VP n=4	4 ‰ ethanol +VP n=5	- VP n=5
UV, µl* min⁻¹*g⁻¹	124±78	169±76	122±49	117±73	105±46	258±88*
$U_{Na}V$, µmol* min⁻¹*g⁻¹	15.8±10.9	22.4±10.9	16.0±7.5	14.2±10.1	13.3±6.2	24.8±8.3
U_KV, µmol* min⁻¹*g⁻¹	3.0±0.6	3.8±1.1	3.1±0.7	3.22±1.3	2.5±0.8	2.9±1.0
FR_{Na}, %	83.7±8.3	78.4±3.6	78.4±7.8	85.1±7.5	80.8±5.4	75.0±2.6*
GFR, µl* min⁻¹*g⁻¹	675±161	752±246	546±135	651±168	549±133	669±227
PFR, ml* min⁻¹*g⁻¹	18.5±6.3	16.8±2.2	17.7±2.8	17.4±2.2	13.6±2.2	17.3±5.2
RVR, mmHg*min⁻¹*g⁻¹	6.1±2.2	6.0±0.7	5.8±1.0	5.9±0.7	7.9±1.9	6.3±2.1
C_{H2O}, µl*min⁻¹*g⁻¹	-20.4±7.4	-25.6±10.9	-19.4±5.8	-22.2±20.1	-14.6±4.3	51.6±20.8*

Values are mean ± SD and are the average of six measurements performed throughout the perfusion period of 180 min. g: gramm kidney weight; UV: urine flow; $U_{Na}V$: urinary excretion of sodium; U_KV: Urinary excretion of potassium; FRNa, fractional reabsorption of sodium; GFR: glomerular filtration rate ((inulin clearance); PFR: perfusion flow rate. *: significant difference in comparison with the control group ($p < 0.05$). ANOVA with posthoc Fisher's PLSD.

vasodilating properties in the isolated rat kidney. In contrast, with higher concentrations, a trend towards a reduction in renal blood flow and – consecutively – an increase in renal vascular resistance was observed; the latter being in line with observations in vitro in isolated vessels [5].

The findings in the kidneys treated with ethanol contrast with our observations made in the vasopressin – depleted group, in which a clear cut increase in urine flow and free water clearance and a reduction in fractional sodium reabsorption was noted, showing that this model is indeed reactive to the hormonal mechanisms which for long have been assumed to be the pathophysiological basis of ethanol-induced diuresis [1].

In their classical study, Eisenhofer and Johnson observed a decrease in plasma vasopressin levels following the ingestion of 75 ml ethanol and a – timely related – increase in free water clearance, suggesting, that alcohol-induced diuresis may indeed be linked to a decrease in circulating vasopressin levels [1]. These findings were confirmed by Leppaluoto and coworkers, which additionally showed, that the plasma levels of atrial natriuretic peptide are also decreased after ethanol-ingestion in healthy volunteers [9].

In contrast, a more recent study failed to detect a decrease in plasma vasopressin levels in water loaded subjects despite orally ingested alcohol inducing an increase in urine flow [2]. Comparable observations were made by Colantonio and coworkers and by our group ([3] and unpublished observations); suggesting that additional factors may be involved in mediating alcohol-induced diuresis.

Despite the fact that the data of the present study clearly show, that ethanol has no direct effects on the kidney in vitro, this does of course not rule out possible indirect effects of ethanol in vivo. Since renal sympathetic nerve activity contributes markedly to renal vascular tone in vivo [10] and ethanol ingestion has been linked to variations in sympathetic tone, which is initially decreased immediately after ethanol ingestion [11], the possibility arises, that – at least a part of the diuretic response during ethanol-diuresis – may be linked to an inhibition of efferent renal sympathetic nerve traffic. Whether this assumption is correct, can however only be clarified in denervation experiments in awake animals and awaits further study.

In conclusion the data presented in this study – investigating for the first time the effects of ethanol in an isolated perfused rat kidney model – do not support a direct role of ethanol on the kidney; suggesting that ethanol-diuresis cannot be explained by intrinsic effects of this drug on the level of the isolated and denervated kidney.

Acknowledgements

References

1. Eisenhofer G, Johnson RH. Effect of ethanol ingestion on plasma vasopressin and water balance in humans. Am J Physiol 1982; 242: R522-527
2. Taivainen H, Laitinen K, Tahtela R, Kilanmaa K, Valimaki MJ. Role of plasma vasopressin in changes of water balance accompanying acute alcohol intoxication. Alcohol Clin Exp Res 1995; 19: 759-762
3. Colantonio D, Casale R, Desiati P, De Michele G, Mammarella M, Pasqualetti P. A possible role of atrial natriuretic peptide in ethanol-induced acute diuresis. Life Sci 1991; 48: 635-642
4. Carney SL, Gillies AH, Ray CD. Acute effect of ethanol on renal electrolyte transport in the rat. Clin Exp Pharmacol Physiol 1995; 22: 629-634
5. Altura BM, Altura BT. Microvascular and vascular smooth muscle actions of ethanol, acetaldehyde, and acetate. Fed Proc 1982; 41: 2447-2451

6. Heringlake M, Wagner K, Schumacher J, Pagel H. Urinary excretion of urodilatin is increased during pressure natriuresis in the isolated perfused rat kidney. Am J Physiol 1999; 277: F347-F351

7. Sehested J, Heringlake M, Schmidt V. Neurohumoral cardiovascular responses to alcohol and their modulation by peroral fluid. Am J Cardiol 1998; 81: 761-765

8. Maule S, Chaudhuri KR, Thomaides T, Pavitt D, McCleery J, Mathias CJ. Effects of oral alcohol on superior mesenteric artery blood flow in normal man, horizontal and tilted. Clin Sci (Lond) 1993; 84: 419- 425

9. Leppaluoto J, Vuolteenaho O, Arjamaa O, Ruskoaho H. Plasma immunoreactive atrial natriuretic peptide and vasopressin after ethanol intake in man. Acta Physiol Scand 1992; 144: 121-127

10. DiBona GF, Sawin LL. Renal hemodynamic effects of activation of specific renal sympathetic nerve fiber groups. Am J Physiol 1999; 276: R539-549

11. Iwase S, Matsukawa T, Ishihara S, Tanaka A, Tanabe K, Danbara A, Matsuo M, Sugiyama Y, Mano T. Effect of oral ethanol intake on muscle sympathetic nerve activity and cardiovascular functions in humans. J Auton Nerv Syst 1995; 54: 206-214

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