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ORIGINAL ARTICLE

Plasticity and impact of the central renin–angiotensin system during development of ethanol dependence

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Abstract Pharmacological and genetic interference with the renin–angiotensin system (RAS) seems to alter voluntary ethanol consumption. However, understanding the influence of the RAS on ethanol dependence and its treatment requires modeling the neuroadaptations that occur with prolonged exposure to ethanol. Increased ethanol consumption was induced in rats through repeated cycles of intoxication and withdrawal. Expression of angiotensinogen, angiotensin-converting enzyme, and the angiotensin II receptor, AT1a, was examined by quantitative reverse transcription polymerase chain reaction. Increased ethanol consumption after a history of dependence was associated with increased angiotensinogen expression in medial prefrontal cortex but not in nucleus accumbens or amygdala. Increased angiotensinogen expression also demonstrates that the astroglia is an integral part of the plasticity underlying the development of dependence. The effects of low central RAS activity on increased ethanol consumption were investigated using either spirapril, a blood–brain barrier-penetrating inhibitor of angiotensin-converting enzyme, or transgenic rats (TGR(ASrAOGEN)680) with reduced central angiotensinogen expression. Spirapril reduced ethanol intake in dependent rats compared to controls. After induction of dependence, TGR(ASrAOGEN)

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680 rats had increased ethanol consumption but to a lesser degree than Wistar rats with the same history of dependence. These data suggest that the central RAS is sensitized in its modulatory control of ethanol consumption in the dependent state, but pharmacological or genetic blockade of the system appears to be insufficient to halt the progression of dependence.

Keywords Alcoholism · Angiotensin converting enzyme inhibitor · Animal model · Transgenic rats · Gene expression

Introduction

Angiotensinogen (AOGEN), the precursor for active angiotensin peptides, is produced by astroglia and widely distributed in the brain. It is processed through cleavage by renin and angiotensin converting enzyme (ACE) into the octapeptide angiotensin II (AngII), which binds to G-protein coupled receptors of the AT1 type in the brain stem, thalamus, and limbic areas to control blood pressure and body fluid homeostasis. Centrally administered AngII increases the release of dopamine, serotonin, and stress hormones [1–5]. Thus, AngII is believed to be involved in reward and coping with stress. A role for the brain renin–angiotensin system (RAS) in regulation of ethanol consumption has been debated [6–9] and was recently clarified by Maul et al. [10, 11] who demonstrated that central AngII augments ethanol consumption in rodents via AT1a receptors. Furthermore, studies in rodent lines that exhibit high ethanol consumption point to the RAS as a genetic factor for susceptibility to alcoholism [12–14]. Taken together, these findings suggest that reducing central RAS activity may be a strategy for treating alcoholism. However, the effects of lowering central RAS activity have not been studied in ethanol-dependent animals.

Alcoholism is a chronic disorder characterized by recurring relapses into compulsive ethanol drinking and a loss of control over intake. The transition into dependence requires prolonged exposure to and repeated intoxication with ethanol (for review, see [15, 16]). Understanding the impact of the RAS on ethanol dependence and its development requires modeling the neuroadaptations that occur with prolonged ethanol exposure. We and others recently demonstrated that, in rodents, repeated cycles of ethanol vapor intoxication and withdrawal for a prolonged period of time induces a phenotype of long-lasting increased ethanol drinking that models several facets of human alcoholism [17–19]. Similar to the human condition, a minimum duration of dependence is required for lasting up-regulation of ethanol preference [20]. Elevated ethanol consumption is sensitive to the clinically effective anticraving compound, acamprosate, while ethanol intake of nondependent rats is unaffected by the same treatment [17, 21–23]. Furthermore, these animals present increased behavioral sensitivity to stress and alterations in gene expression profiles within medial prefrontal cortex and amygdala [17, 24, 25]. This experimentally induced syndrome has recently been labeled *the postdependent state* [26]. Together, these findings provide pharmacological validation that the neuroadaptive processes induced by prolonged exposure to cycles of intoxication and withdrawal parallel those in human alcoholics and can be used shed light on underlying neural mechanisms.

In the present study, our hypothesis was that development of ethanol dependence involves the central RAS and that such changes are functionally related to dependence-induced ethanol drinking. We first studied the expression of transcripts encoding AOGEN, ACE, and AT1 in dependence-related forebrain regions. As a temporal threshold has been reported for development of persistent increases in voluntary alcohol drinking [20], we included animals with either 7 or 4 weeks history of daily intermittent alcohol exposure and nonexposed controls. The ability of the longer but not the shorter of these treatments to induce increased alcohol drinking was confirmed. Subsequent behavioral analysis therefore focused on effects of the 7-week exposure. We targeted the central RAS either pharmacologically using spirapril, an ACE inhibitor that penetrates the blood–brain barrier and reduces ethanol consumption via AngII signaling [10, 11, 27], or genetically using transgenic rats that express AOGEN-specific antisense RNA in the brain [28].

Materials and methods

Animals

All animal experiments were approved by the institutional review board and conformed to NIH and established European Community guidelines for the care and use of animals (ethics permit S84/98, Stockholm South). Sixty-eight normal Wistar rats (Mollegard, Denmark) and 17 transgenic rats (TGR(ASrAOGEN)680, [28]) obtained from the breeding colony of M.B. (Max-Delbrück-Center Berlin, Germany) were used in the experiments. Rats weighed 220–250 g at the beginning of the experiment and were housed 4 per cage under a reversed light/dark cycle (light of 11 A.M., light on 11 P.M.) with free access to food and water.

Ethanol vapor exposure

Experimental procedures were performed as described previously [17]. Briefly, four rat cages were placed in 1-m^3 glass and steel chambers and exposed to air or ethanol vapor. Vapor concentrations were controlled by pumping ethanol

into electrically heated stainless steel coils connected to airflow regulators with high-performance liquid chromatography (HPLC) pumps. Ethanol concentrations were continuously monitored via a spectrometer. Exposure was according to the following sequence: 1 week of habituation to the chambers (no ethanol), 1 week of continuous exposure to 22 ml/L ethanol to adapt to the novel odor, 7 weeks of exposure to ethanol vapor adjusted to produce blood alcohol concentrations (BAC) of 150–250 mg/dl for 17 h (Wistar, 4 P.M.–9 A.M.) or 14 h (TGR(ASrAOGEN)680, 4 P.M.–6 A.M.) each day. Once a week during exposure, rats were weighed and blood was collected from their tail veins. Serum was analyzed with NAD/NADH enzyme spectrophotometry (Sigma Diagnostics, St. Luis, MO). Symptoms of ethanol withdrawal were monitored as previously described [17]. Ethanol vapor exposure was followed by a 3-week period of abstinence to eliminate potential effects of acute withdrawal symptoms on subsequent voluntary drinking behaviour. After 2 weeks of abstinence, rats were moved to individual cages, to habituate to the new housing environment and reduce potential stress effects on drinking. A group of control rats that were not exposed to ethanol vapor was maintained under similar conditions.

Gene expression analysis

A separate group of animals went through the exposure paradigm as described above for either 4 or 7 weeks followed by a 21-day period of abstinence without ethanol. Rats were decapitated between 1 and 3 P.M. Brains were removed and frozen in −40°C isopentane and stored at −70°C. Bilateral samples from medial prefrontal cortex (mPFC), nucleus accumbens, and amygdala were obtained from 2-mm-thick coronal slices (amygdala, 2-mm punch diameter, bregma −1.8 to −3.8; mPFC and nucleus accumbens, bregma +2.7 to +0.7; [29]. Total RNA was extracted with TRIzol (Gibco, Baltimore, MD) followed by RNeasy clean-up (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Amygdala and mPFC RNA samples had 260/280 ratios of 1.9–2.1. RNA integrity was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany), and only material without signs of degradation was used for reverse transcription polymerase chain reaction (RT-PCR). Real time RT-PCR was performed as a two-step assay using TaqMan reverse transcription reagents and Universal PCR Master Mix with an ABI Prism 7900 HT, according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA). Reverse transcription was performed using 100 ng total RNA from each sample. ABI TaqMan assays Rn00593114_m1, Rn00561094_m1, and Rn00578456_m1 were used as probes for Agt, ACE, and the At1a receptor. Expression values were normalized to the mean of three endogenous controls (Actx, B2m, Cyca) as previously described by [30] and expressed as ratios of the control group using the ddCt method as described by Applied Biosystems (Foster City, CA). The endogenous control assays for Actx and Cyca have been described [31]. For B2m we used ABI TaqMan assay Rn00560865_m1. There were no group differences in expression of the endogenous control genes

Two-bottle free choice

After the abstinence period, increasing concentrations of ethanol in a 0.2% saccharin solution were made available to rats for 19 days in a continuous, two-bottle free choice between ethanol–saccharin and saccharin alone solutions as described previously [17]. Briefly, ethanol concentration was increased as follows: days 1–5, 2% ethanol; days 6–10, 4% ethanol; and from day 11, 6% ethanol (*v*/*v* solutions). Consumption of ethanol–saccharin and saccharin alone were measured between 9 and 10 A.M. each Monday, Wednesday, and Friday. Bottle positions were alternated daily to avoid development of place preference.

Drug treatment

Exposed and control rats were treated with the blood–brain barrier-penetrating ACE inhibitor, spirapril (AWD, Dresden, Germany). After stabilization of 6% ethanol consumption, on day 18 of the two-bottle free choice, ethanol consumption (g kg^{-1} day^{-1}) of exposed and nonexposed rats was measured, and the rats were assigned to one of two groups (n=8/group) with equivalent ethanol consumption. For the spirapril treatment groups, the drug was added to the drinking water in increasing doses as follows: days 19–28, 0.5 mg/kg; days 29–38, 2.5 mg/kg; days 39–45, 5 mg/kg. Drinking solutions were freshly prepared each time consumption was measured, and doses were calculated from the mean total liquid consumption of the two prior readings. Oral dosing was preferred to injection to avoid adverse reactions to long-term injection treatment. The oral spirapril dosage regime was reported to reduce central ACE activity by ~40% at the 5 mg/kg dose [10, 11, 27].

Statistical analysis

Data met assumptions of normality and homogeneity of variance and were analysed using standard parametric tests. Gene expression data were analyzed by one-way analysis of variance (ANOVA) and Dunnett's post hoc test for comparison with a control group. Drinking data are expressed as gram per kilogram per day or milliliter per kilogram per day and were analyzed by two-way or repeated measures ANOVA. Correction for multiple testing

was done by adjusting the p value for family-wise error rate according to Holm's sequentially rejective multiple test procedure.

Results

Gene expression of Agt, Ace, and At1a in the forebrain of postdependent rats

Based on our previous observation that animals with a 7-week but not a 4-week history of cyclic exposure to ethanol intoxication and withdrawal showed long-term changes in ethanol consumption [20], we used these time points to study neuroadaptation of the central RAS, particularly forebrain expression of the precursor molecule AOGEN (Gene symbol: Agt), the precursor peptidase ACE (Ace), and the main central Ang II receptor, At1a. Tissue samples from rats that completed a 4- or 7-week exposure paradigm followed by a 3-week resting period, which eliminated acute withdrawal and allowed gene expression to stabilize, and non-exposed control rats were subjected to quantitative real-time RT-PCR analysis. We focused on forebrain regions with known relevance for addictive behavior (i.e., mPFC, nucleus accumbens, and amygdala complex). Corresponding to the acquisition of the high-drinking phenotype, we found a significant increase in mPFC Agt mRNA levels in animals that were exposed for 7 but not 4 weeks compared to controls (Fig. 1; $F(2,30)=4.3$, $p<0.05$, Dunnett's post hoc test $p<0.01$, 7-week exposed vs controls). A trend towards an exposure effect was found for At1a mRNA ($F(2,30)=3.19$, $p=0.055$), while Ace transcript levels appeared to be unaffected ($F(2,29)=0.7$). There were no differences in expression of any of these genes in nucleus accumbens and amygdala of exposed and control rats (data not shown).

Effect of spirapril on postdependent rats

We compared the effect of spirapril, an ACE inhibitor with high brain accessibility, on ethanol consumption in ethanol-dependent and nondependent rats. As in our previous experiments, 17-h exposure to ethanol vapor induced BACs in the range of 150–250 mg/dl in Wistar rats [17]. BACs decreased to undetectable levels within 5 h of each daily exposure. Towards the end of the 7-week exposure period, mild symptoms of withdrawal in the form of tail stiffness and piloerection were observed during the last hours of the ethanol-off periods of each daily cycle. Withdrawal intensity did not reach a level at which seizures were seen. After the 3-week resting period, rats were introduced to the two-bottle free choice drinking paradigm and once stable drinking behavior from the 6% ethanol bottle was reached,

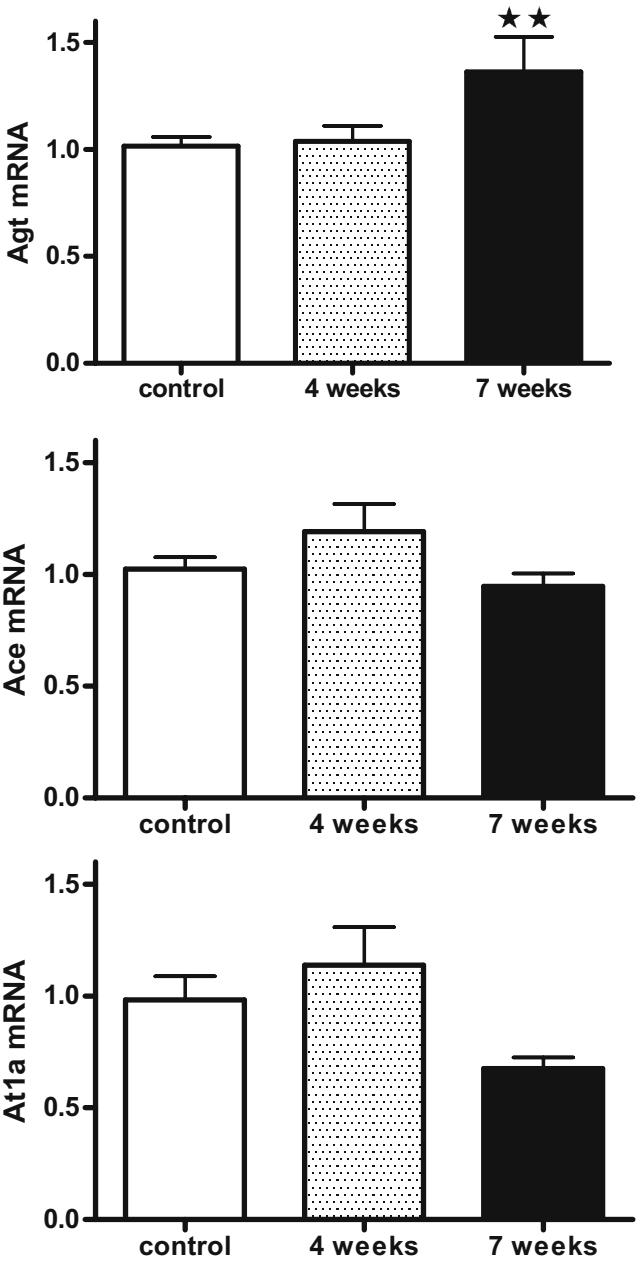

Fig. 1 Expression of RAS components in medial prefrontal cortex. Animals went through 4 or 7 weeks of cyclic ethanol intoxication and withdrawal followed by a 3-week resting period without access to ethanol. Agt, Ace, and At1a mRNA levels were determined by real-time RT-PCR as described in "Materials and methods". Values are expressed as fold change ± SEM compared to the control group. $^{\star}p<0.05$, $^{\star\star}p<0.01$, Dunnent's post hoc test 7 weeks vs control. For detailed statistics, see "Results"

they were assigned to the experimental groups. Individual mean daily consumption levels during each spirapril treatment period were analyzed by two-way ANOVA with ethanol exposure and spirapril treatment as main factors (Table 1; Fig. 2). Similar to our previous experiments a greater than twofold increase in voluntary ethanol drinking during the two-bottle free choice was observed in the

Table 1 Statistical analysis for effects of history of dependence and spirapril treatment on drinking behavior

	0 mg/kg spirapril		0.5 mg/kg spirapril		2.5 mg/kg spirapril		5 mg/kg spirapril	
	$F_{[1,19]}$	p	$F_{[1,19]}$	p	$F_{[1,19]}$	p	$F_{[1,19]}$	p
Ethanol								
HD	19.68	0.00028**	19.90	0.00027**	24.47	0.00009***	25.53	0.00007***
Spi	0.01	0.93079	0.21	0.64866	6.63	0.01859	13.79	0.00147*
HD×Spi	0.18	0.67318	0.33	0.57158	0.01	0.96818	1.16	0.29437
Saccharine								
HD	1.76	0.20043	2.03	0.17080	0.54	0.47290	0.02	0.88333
Spi	0.13	0.72366	0.48	0.49650	0.04	0.84971	0.09	0.76797
HD×Spi	0.39	0.53807	0.73	0.40289	1.56	0.22699	1.32	0.26555
Total fluid								
HD	11.49	0.00308*	14.66	0.00113*	6.86	0.01691	12.04	0.00274*
Spi	1.11	0.30461	0.01	0.91446	2.02	0.17157	0.74	0.40048
HD×Spi	0.00	0.98985	0.10	0.75480	0.56	0.46497	0.05	0.82587

Ethanol, saccharine, and total fluid consumption over 1 week of treatment for each dose of spirapril were compared by two-way ANOVA for effects of the history of dependence (HD) and the spriapril treatment (Spi) and their interactions. Raw p values are shown. Values passing correction for multiplicity of testing are marked by stars: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

exposed group vs controls (mean 6% ethanol consumption days 11–18 3.52±0.27 vs 1.32±0.14 g kg^{-1} day^{-1}). The exposure paradigm caused a significant increase in consumption from the ethanol bottle over the entire length of the experiment. This effect was reflected in a moderate increase in total fluid consumption. Drinking from the saccharine-alone bottle was not affected, demonstrating behavioral specificity. Together, these data demonstrate the robustness of the exposure paradigm in inducing long-term, high ethanol drinking behavior. At higher doses, spirapril treatment was effective in reducing ethanol consumption in animals with and without a history of ethanol dependence. The only significant difference between the treatment groups was observed at the 5 mg/kg dose of spirapril and was between the two groups with a history of ethanol dependence (Tukey's HSD post hoc test, $p<0.05$). There was no significant interaction between dependence history and spirapril action. Spirapril had no effect on drinking from the non-ethanol bottle or on total consumption.

Effect of cyclic ethanol intoxication and withdrawal in TGR (ASrAOGEN)680 rats

To determine whether reduced central RAS activity has a protective effect on developing ethanol dependence, TGR (ASrAOGEN)680 rats were exposed to the cyclic ethanol vapor intoxication paradigm. Mean BACs at the end of the 12-h ethanol-on phase were 150–470 mg/dl over the 8 weeks of exposure (Fig. 3a). During the ethanol-off phase, BACs dropped to undetectable levels within 8 h. Because ethanol metabolism in TGR(ASrAOGEN)680 rats appears to be slower than in Wistar rats, the cycling times were adjusted to allow complete withdrawal and recovery.

During the cyclic ethanol exposure paradigm pronounced behavioral signs of withdrawal, including seizures, were observed in TGR(ASrAOGEN)680 rats. At the end of the exposure period, there were no differences in body weight between exposed and non-exposed animals (443± 10 vs 441±7 g). After the 3-week resting period, ethanol was made available in a two-bottle free choice paradigm. We observed a significant increase in drinking from the 6% ethanol bottle in exposed vs non-exposed transgenic rats (repeated measures ANOVA, $F(1,12)=13.28$, $p<0.01$; Fig. 3b). There were no effects on total volume consumed. The group differences in ethanol consumption were stable over an observation period of 45 days (data not shown).

Discussion

Here, we studied the role of the central RAS during development of dependence. We demonstrate that post-dependent rats increase their ethanol intake more than twice to the levels seen in controls replicating several prior reports from our own as well as other laboratories [17, 18, 20, 32]. The increased intake was seen after a 3-week recovery period after completion of the vapor exposure and persisted for more than 2 months. It is therefore related to long-term neuroadaptations rather than acute withdrawal.

We found that the induction of increased ethanol consumption was associated with a significant up-regulation of AOGEN transcript levels and a potential down-regulation of local AT1 mRNA. No changes in gene expression levels were observed after a period of intermittent ethanol intoxication that was insufficient to produce changes in drinking behavior. Interestingly, the up-regulation of

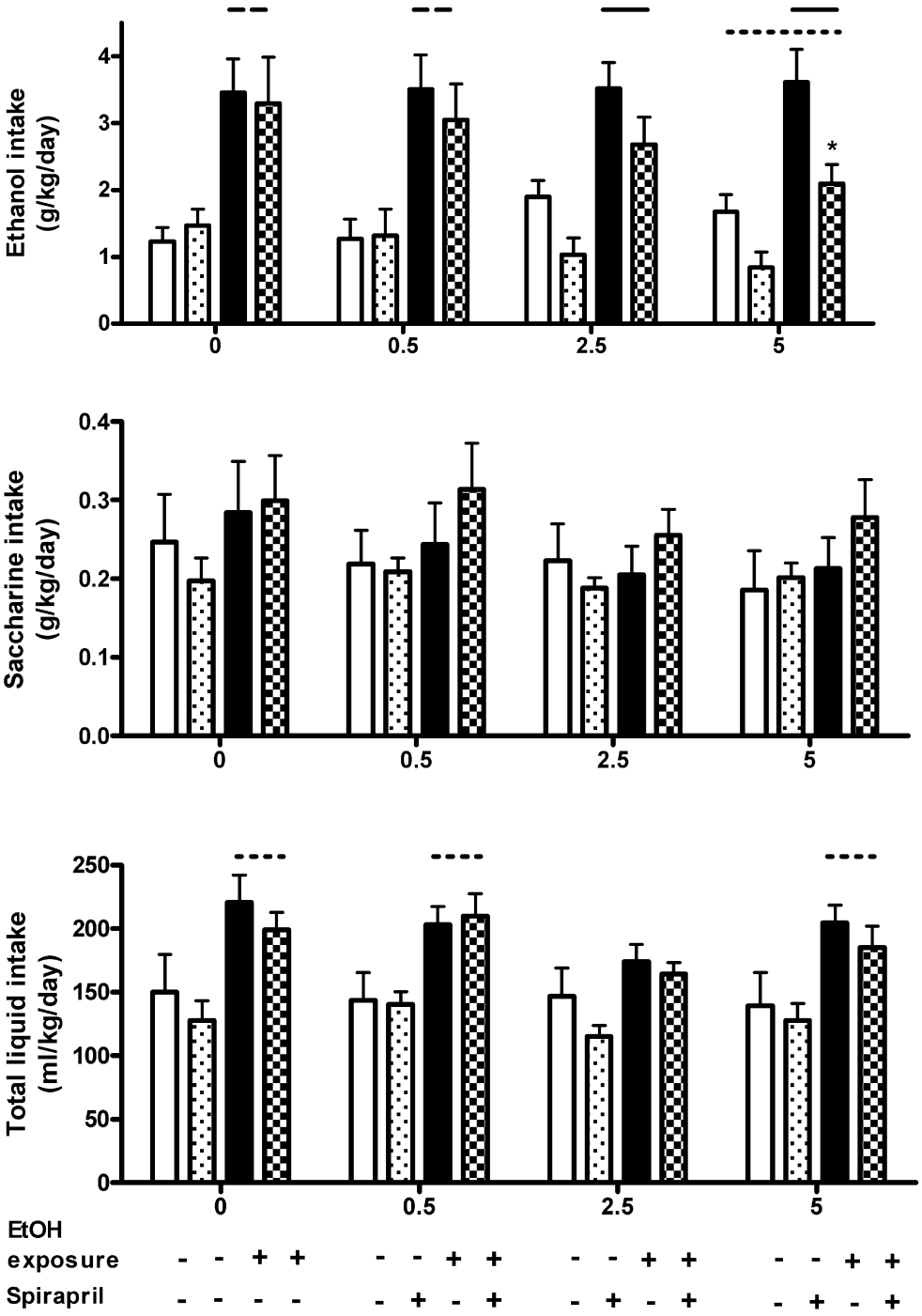

Fig. 2 Effect of orally administered spirapril on self-administration of ethanol in rats with and without a history of dependence. Animals were exposed to the intermittent ethanol intoxication and withdrawal paradigm (*dark boxes*) or matched controls (*light boxes*). After establishing ethanol consumption in a two-bottle free choice paradigm (6% ethanol/0.1 % saccharine or 0.2% saccharine only in tap water) rats were treated with increasing doses of spirapril (*hatched boxes*). Consumption is shown as daily mean consumption per kilogram body weight over 1 week of treatment for each dose of spirapril (0.5–5.0 mg/kg). Two-way ANOVA for exposure and treatment showed a significant effect on ethanol consumption (*short lines*) which also resulted in a significant increase in total liquid consumption. There was a significant overall effect of spirapril treatment (*long lines*) at the 2.5 and 5 mg/kg doses. *Dotted lines*, $p<0.05$; *broken lines*, $p<0.01$; *straight line*, $p<0.001$. There was not an exposure × treatment interaction. However, post hoc tests showed a significant spirapril (5 mg/kg) effect for ethanol-exposed rats, $*p<0.0$. See "Materials and methods" and "Results" for further details

AOGEN is seen only in the mPFC, a region known to integrate reinforced behaviors. The precise nature of mPFC control on ethanol consumption is unclear. Notably, this region seems also to be crucially involved in regulating ethanol drinking in the selected high alcohol accepting AA line, a widely used genetic model of alcoholism [33]. At the same time, the amygdala, a major site of RAS action in the brain and an important structure for fear and anxiety behaviors, as well as the nucleus accumbens, which is typically associated with positive reinforcing properties of drugs, were not affected. Furthermore, increased AOGEN expression in postdependent animals emphasizes that astroglia is an integral part of the plasticity underlying the development of dependence.

Establishing the involvement of RAS components in dependence-induced neuroadaptation on the level of gene expression prompted us to search for correlates of altered RAS function. We did not attempt to measure altered peptide content and receptor density directly in the brain for several reasons: first, levels of RAS components in most

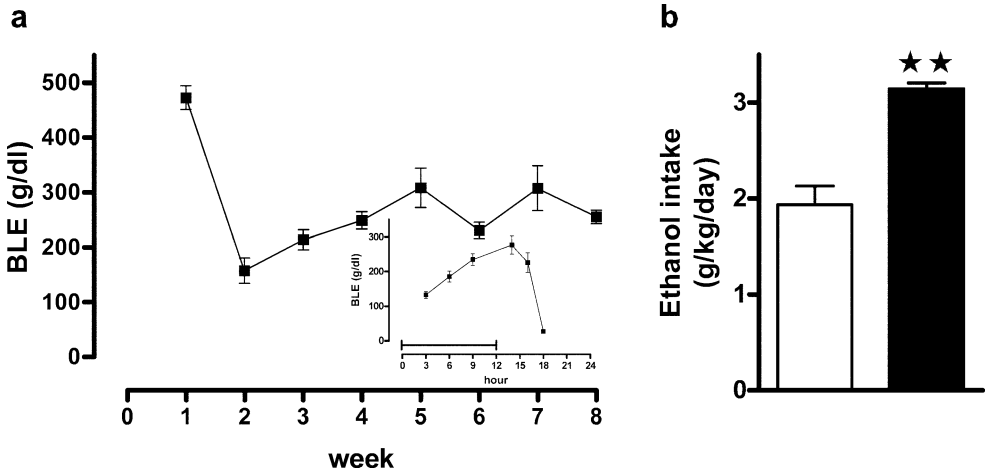

Fig. 3 Long-lasting increases in voluntary ethanol consumption in TGR(ASrAOGEN)680 rats after cyclic ethanol exposure. **a** Blood alcohol concentration (BAC; mean ± SEM) obtained weekly at the end of a 12-h exposure period. *Insert*: during each 24-h cycle, BAC dropped to undetectable levels, precipitating withdrawal. *Bar*: ethanol-on period. **b** Two-bottle free choice, continuous access drinking was assessed after a 3-week resting period that followed the last exposure cycle and stabilization of 6% (*v*/*v*) ethanol consumption vs 0.2% saccharine solution. The increase in ethanol consumption in the ethanol-exposed group (*black bar*) compared to controls (*white bar*) was significant, $^{**}p<0.01$). Values are expressed as mean daily consumption ± SEM over a 1-week period of 6% ethanol consumption. For detailed statistics, see "Results"

forebrain regions are low and the observed alteration in gene expression levels are modest, making it technically demanding to detect respective changes on the protein level. In our experience, existing tools, in particular antibodies, have produced inconsistent results in brain tissue. Second, the existence of multiple pathways leading to formation of active angiotensin peptides and altered signaling at angiotensin receptors in the brain makes the interpretation of changes in RAS components notoriously difficult [34]. Instead, we studied the functional consequences of established brain RAS manipulations in postdependent rats, i.e., using pharmacological or genetic tools [10, 27, 28]. Of the two commonly used pharmacological treatment approaches to lower RAS activity, i.e., antagonism at the AT1 receptor or reduction in AngII through ACE inhibition, the latter has been explored with respect to its effect on ethanol consumption [8, 12, 35, 36]. Furthermore, to our knowledge, currently used AT1 antagonists do not efficiently cross the blood–brain barrier. Previous observation points to higher efficacy of the ACE inhibitor abutapril for reducing ethanol intake in rats with elevated RAS activity [35]. However, these and similar experiments using earlier generations of ACE inhibitors are difficult to interpret because of the low brain availability of these drugs [37, 38]. In this study, we use spirapril, which based on its structure should cross the blood–brain barrier more efficiently, has a demonstrated effect on central ACE activity [27, 39] and which according to experiments in mice lacking AOGEN reduces ethanol consumption via central AngII [11]. We find increased efficacy of spirapril in postdependent animals, presumably reflecting increased RAS activity. In the absence of an effect of spirapril on general fluid intake, which could potentially confound ethanol consumption, we conclude that spirapril acts on both components of ethanol consumption: basal consumption related to thirst or caloric intake and consumption for ethanol-specific reinforcement. Spirapril shares this sensitized action in ethanol-dependent rats with several other drugs, including the neuropeptide Y antagonist, BIIE0246 [40]; the endocannabinoid receptor 1 antagonist, SR141716A [41]; and the CRH receptor 1 antagonists, antalarmin [42] and MTIP [43]. A common feature of these drugs is that their neurochemical targets mediate anxiety and consummatory behaviors. Hence, these drugs seem qualitatively different from an anticraving compound such as acamprosate, which acts exclusively on increased consumption in the dependent state, but does not affect basal ethanol consumption.

We were particularly interested in knowing if low RAS activity might provide a protective mechanism for development of ethanol dependence. We addressed this question by exposing transgenic rats that express an AOGEN-specific antisense RNA in the brain to the cyclic intoxication paradigm. TGR(ASrAOGEN)680 rats exhibit >90% reduced AOGEN levels in the brain, resulting in a reduction in central AngII formation, increased AT1 expression, and lower ethanol consumption compared to wild-type controls [11, 28, 44]. During the cyclic intoxication procedure, TGR (ASrAOGEN)680 rats had higher BACs and showed somewhat slower ethanol metabolism than Wistar rats. This could be attributed to the observation that TGR(ASrAOGEN) 680 rats are leaner then Wistar rats both in terms of age-matched weight and body fat [45, 46], or it may reflect an altered distribution volume of ethanol in the genetically

modified rats. TGR(ASrAOGEN)680 appear to have higher, although nonsignificant, baseline ethanol consumption compared to Wistar rats. In contrast, in the previous report by Maul et al. [11], the transgenic line was drinking less ethanol compared to the parental strain. However, this dissociation was strongly dependent on the ethanol concentration in the drinking solution and under conditions similar to the present experiment, i.e., 6% ethanol, TGR(ASrAOGEN) 680 rats were not different from controls. TGR(ASrAOGEN) 680 rats showed a stable, ~50% increase in ethanol consumption. Although this is a smaller increase than typically seen in our experiments with genetically unmodified animals, a direct quantitative comparison is not possible because of issues of genetic background etc. The important conclusion is that, similar to what we and others have repeatedly reported [17, 18, 43, 47], a history of dependence gives rise to elevated voluntary ethanol intake also in the TGR(ASrAOGEN)680 rats. The role of the central RAS therefore seems to be one of modulating the expression but not the acquisition of excessive postdependent drinking.

In summary, using an animal model that reflects aspects of the natural progression of clinical ethanol dependence, we found evidence (1) for an involvement of the central RAS in neuroadaptations underlying dependence, (2) that a likely functional consequence of these alterations is a sensitized action of the RAS on ethanol consumption in the dependent state, and (3) that a blockade of this system is insufficient to halt the process. In contrast to the anticraving drug acamprosate which only affects the pharmacological reinforcement of ethanol but not the thirst or caloric component that could also promote high ethanol intake, spirapril seems to reduce both the reinforcing and caloric/thirst factors contributing to high ethanol intake. Thus, ACE inhibitors with high bioavailability in the brain, like spirapril, could in fact add an alternative treatment approach to alcoholism. The ability of ACE inhibitors to reduce ethanol consumption may be beneficial for the large population of hypertensive, nondependent patients who commonly consume amounts of ethanol that exceed the recommended upper limits. To our knowledge, this has not been evaluated. There is one report of a genetic association of the ACE D allele, which causes increased ACE activity and may effect higher AngII levels, and excessive ethanol consumption in humans [9]. Only one clinical study has directly addressed a potential link between ACE inhibitor treatment and ethanol consumption [36]. In that study, 10–20 mg/day of elanapril had no effect on daily ethanol consumption in normotensive alcoholics. However, first generation ACE inhibitors, like elenapril or captopril, are highly hydrophilic and thus unlikely to cross the blood–brain barrier [27]. Notably, ACE inhibition in the brain by spirapril was found to be well within the dose range (0.3–30 mg/kg p.o.) for antihypertensive effects in hypertensive SHR rats [11, 39]. It may be worthwhile to evaluate the newer generation of centrally acting ACE inhibitors for effects on ethanol consumption in long-term, follow-up studies in nondependent, hypertensive clinical populations as well as in hypertensive alcoholics.

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