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Neurokinin-1 receptor antagonist rolapitant suppresses anxiety and alcohol intake produced by repeated withdrawal episodes

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Neurokinin-1 receptor (NK1r) antagonists have been shown to suppress operant self-administration of alcohol, voluntary alcohol consumption and stress-induced reinstatement of alcohol-seeking behaviour. Considering the long half-life and anxiolytic-like properties of NK1r antagonist rolapitant, we expected that it may be an effective option for reducing anxiety and alcohol motivation during early withdrawal. Voluntary alcohol intake (two-bottles paradigm) was recorded in male Wistar rats during the three periods: 24 days (basal level), 6-day period when rats received 5 mg·kg^{-1} rolapitant or vehicle and 12-h period after repeated withdrawal episodes (alcohol cessation for 36 h). We found that upon intraperitoneal (i.p.) administration, rolapitant rapidly penetrated into specific rat brain regions – amygdala, hypothalamus and neocortex – implicated in the control of anxiety and reward. Rolapitant did not affect basal voluntary alcohol intake, but significantly suppressed anxiety-like behaviour and alcohol consumption following withdrawal episodes. Our findings suggest that rolapitant should be further investigated as a novel treatment option for relapse prevention in alcohol-dependent patients.

Introduction

Alcohol dependence is a chronic recurrent state determined by compulsive alcohol consumption, loss of control over the consumption of alcohol and the emergence of negative emotional states, both during consumption and during the abstinence period [1]. A significant role in maintaining alcohol dependence is played by changes in the neural circuits mediating anxiety and stress disorders [2,3]. Substance P (SP) and its neurokinin-1 receptor (NK1r) are widely expressed within the brain mesolimbic areas associated with stress and addictive behaviour [4]. Stimulation of NK1r leads to the excitation of dopaminergic neurons in the ventral tegmental area – a key structure implicated in the processes involving reward and drug dependence [5]. It has been reported that the functional polymorphism of the *TACR1* locus encoding NK1r is associated with a high risk of the development of alcohol abuse in humans [6] and genetically selected alcohol-preferring rats demonstrate an increased expression of NK1r and SP-binding affinity in the central amygdala [7].

There are many disparate data on the participation of the SP/NK1r system in the modulation of drug addiction. It has been shown that morphine loses its reward properties in homozygous NK1r knockout mice [8]. Interestingly, the loss was specific to morphine, as NK1r knockout mice responded when cocaine or food was

Abbreviations
CRF, corticotropin-releasing factor; i.p., intraperitoneal; *NK1r*, neurokinin-1 receptor; sP, substance P.

used as rewards [8,9]. It was established that conditioned place preference for alcohol was reduced by NK1r deletion [10]. Importantly, the escalation of alcohol intake produced by repeated cycles of deprivation and access in wild type mice was not found in NK1r knockout mice [10]. High doses of the NK1r antagonist ezlopitant reduced operant self-administration and voluntary alcohol consumption in Long Evans rats, but not in C57BL/6 mice [11]. The NK1r antagonist L822429 prevented yohimbine-induced reinstatement of cocaine and alcohol-seeking but did not affect alcohol self-administration in Long Evans rats [12]. It is noteworthy that the clinical trials of the NK1r antagonist LY686017 showed a decrease in spontaneous and alcohol-induced cravings in recently detoxified alcoholic patients [13]. Additionally, LY686017 attenuated concomitant cortisol responses and normalized brain functional magnetic resonance imaging responses to affective stimuli after detoxification [13]. The effects of NK1r antagonists on stress-induced alcohol craving are of great interest. Indeed, the NK-1 antagonist L822429 suppressed the stress-induced reinstatement of alcohol-seeking in Wistar rats [14]. It was found that L822429 reduced anxiety-like behaviour and operant alcohol self-administration when injected into the medial amygdala of the highly sensitive to stress and stress-induced alcohol drinking Marchigian Sardinian rats [15]. At the same time, in spite of a well-established decrease in SP seen in the hypothalamus of alcohol-preferring rats [16], the SP decline in socially isolated Wistar rats was not associated with alcohol drinking levels in the two-bottle choice procedure [17].

When studying the effects of NK1r antagonists on stress-induced alcohol consumption, exogenous stress factors are commonly used; however, the influence of NK1r antagonists on the withdrawal-associated relapse has not been previously evaluated. This study is aimed to test the hypothesis that the NK1r antagonist rolapitant suppresses voluntary alcohol consumption under repeated short-term withdrawal episodes.

Results

Rolapitant concentration in brain structures and blood plasma

The concentration of rolapitant in the blood plasma and three brain structures is shown in Fig. 1. Following the first i.p. administration rolapitant distributed well to the brain tissue without significant differences in the concentration between structures. Following the second and the third injections a significant decrease in the rolapitant concentration was detected in neocortex

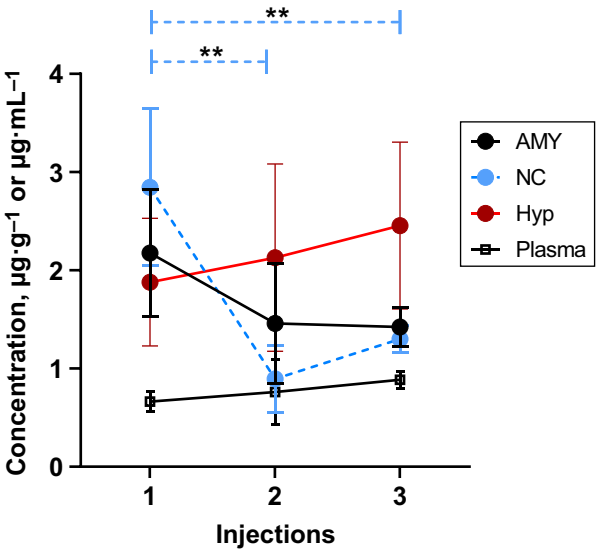

Fig. 1. Rolapitant concentration in the different rat tissues. Data present as mean ± SD; for each point $n = 4$. Concentration present at μg·mL^{-1} for plasma and at μg·g^{-1} for brain structures. AMY, amygdala; Nc, neocortex; HYP, hypothalamus; Plasma, blood plasma. Injection, the number of consecutive injections of rolapitant. Asterisks indicate statistical significance compared with the first injection (**$P < 0.01$; *post-hoc* Duncan test).

($F_{2,6} = 22$, $P = 0.0017$): the levels achieved were within 68.5% ($P = 0.001$) and 54.3% ($P = 0.003$) of those observed after the first injection. At the same time, there were no significant changes in rolapitant concentration in other brain areas and blood plasma. Thus, if administered as a single dose at 72 h intervals, the amygdala and hypothalamus exposure to rolapitant will remain relatively constant following every other injection, which suggests there is no induction or inhibition of clearance mechanisms. Thus, when rolapitant (5 mg·kg^{-1}) was administered at 72 h intervals, the amygdala and hypothalamus exposure to the drug remained relatively constant following every other injection, which suggests there is no induction or inhibition of clearance mechanisms. The reason for an observed decrease in rolapitant concentration in the neocortex is unclear.

Rolapitant does not affect voluntary alcohol consumption, but suppresses alcohol intake after repeated withdrawal episodes

Data analysis revealed a statistically significant change in the dynamics of alcohol consumption ($F_{7,105} = 18.6$, $P = 5.6 \times 10^{-16}$), as well as the effect of the interaction of the rolapitant treatment and alcohol consumption in the dynamics ($F_{7,105} = 3.1$, $P = 0.005$).

We have used the 2-bottle "alcohol vs. water" choice regimen to investigate rolapitant effect on alcohol consumption. Repeated i.p. administration of rolapitant failed to reduce alcohol intake in alcohol-experienced rats having free 24-h access to alcohol (days 24–30, Fig. 2). However, after the first withdrawal episode (day 31), we observed a tendency ($P = 0.075$) to an increase in alcohol consumption in the alcohol + vehicle (Alc + V) rats, but not in the alcohol + rolapitant (Alc + R) rats. Following the second alcohol withdrawal episode (day 34) alcohol intake in Alc + V rats was significantly higher: on 126% ($P = 0.00002$) and 74% ($P = 0.00005$) compared to days 28 and 31, respectively. Rolapitant pretreatment (Alc + R) resulted in less pronounced increase in daily alcohol intake following the second withdrawal episode: on 94% ($P = 0.00003$) and 66% ($P = 0.0005$) compared to days 28 and 31, respectively. Compared to Alc + V rats alcohol consumption of Alc + R rats was 35% lower ($P = 0.0002$) at day 34.

Rolapitant suppressed anxiety-like behaviour during the early withdrawal period

Three groups of rats Alc + R, Alc + V and control (C, $n = 11$) were used to evaluate the effect of rolapitant on alcohol withdrawal-associated anxiety-like behaviour. Control rats were individually housed and kept under the same conditions as Alc + R and Alc + V rats but were given access to water only during the entire experiment.

The light-dark box test revealed a statistically significant change in the following parameters: the number of entries to the dark compartment ($F_{2,25} = 4.4$, $P = 0.023$), the total time spent in the middle compartment ($F_{2,25} = 4.8$, $P = 0.017$), as well as the number of entries ($F_{2,25} = 9.9$, $P = 0.0007$) and total distance ($F_{2,25} = 3.7$, $P = 0.04$) in the light compartment (Fig. 3).

The Alc + V-rats were characterized by decreased number of entries to the dark compartment (44%, $P = 0.01$ compared to control group and 38%, $P = 0.037$ compared to the Alc + R group), number of entries to the light compartment (64%, $P = 0.002$ compared to control group and 68%, $P = 0.0003$ compared to the Alc + R group), time spent in the middle compartment (59%, $P = 0.007$ compared to control group) and the total distance travelled in the light compartment (53%, $P = 0.01$ compared to the Alc + R-rats). At the same time, the total locomotor activity (distance travelled in all compartments) was not significantly different between the groups.

Discussion

Rolapitant was originally developed for the prevention of emesis associated with cancer chemotherapy [18]. As an NK1r antagonist, rolapitant is potentially useful in the treatment of anxiety-related disorders and alcohol dependence [13,19]. However, many aspects of its effect on alcohol consumption are still unknown. The results from our study indicated that rolapitant reduced voluntary alcohol consumption after repeated short-term withdrawal episodes. At the same time, our data support previous findings [14,15] that rolapitant administration did not influence alcohol intake when

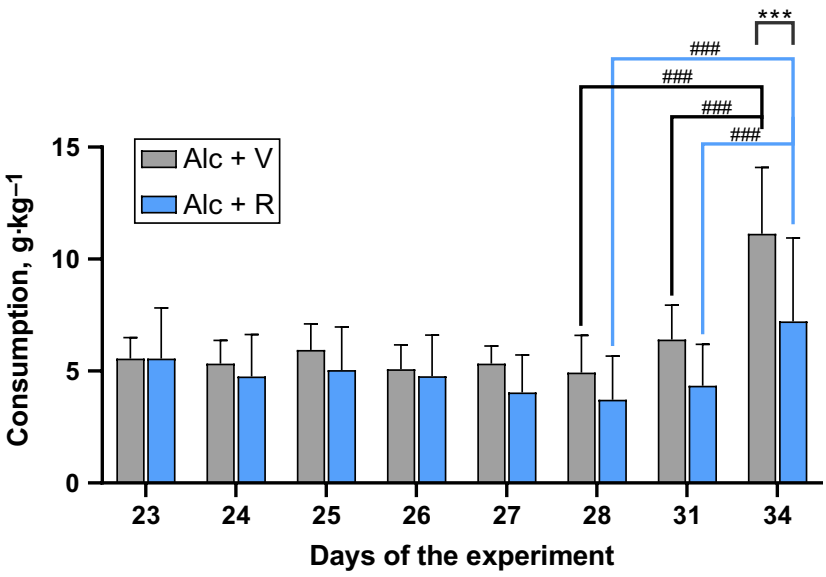

Fig. 2. Voluntary alcohol consumption. Data present as mean + SD. Alc + V, alcohol-drinking rats which received vehicle, $n = 8$; Alc + R, alcohol-drinking rats which received rolapitant, $n = 9$. DID, drinking in the dark. Asterisks indicate statistically significant differences between the groups (***$P < 0.001$; *post-hoc* Duncan test). Hash indicates statistically significant differences between different time points within one group (###$P < 0.001$; *post-hoc* Duncan test).

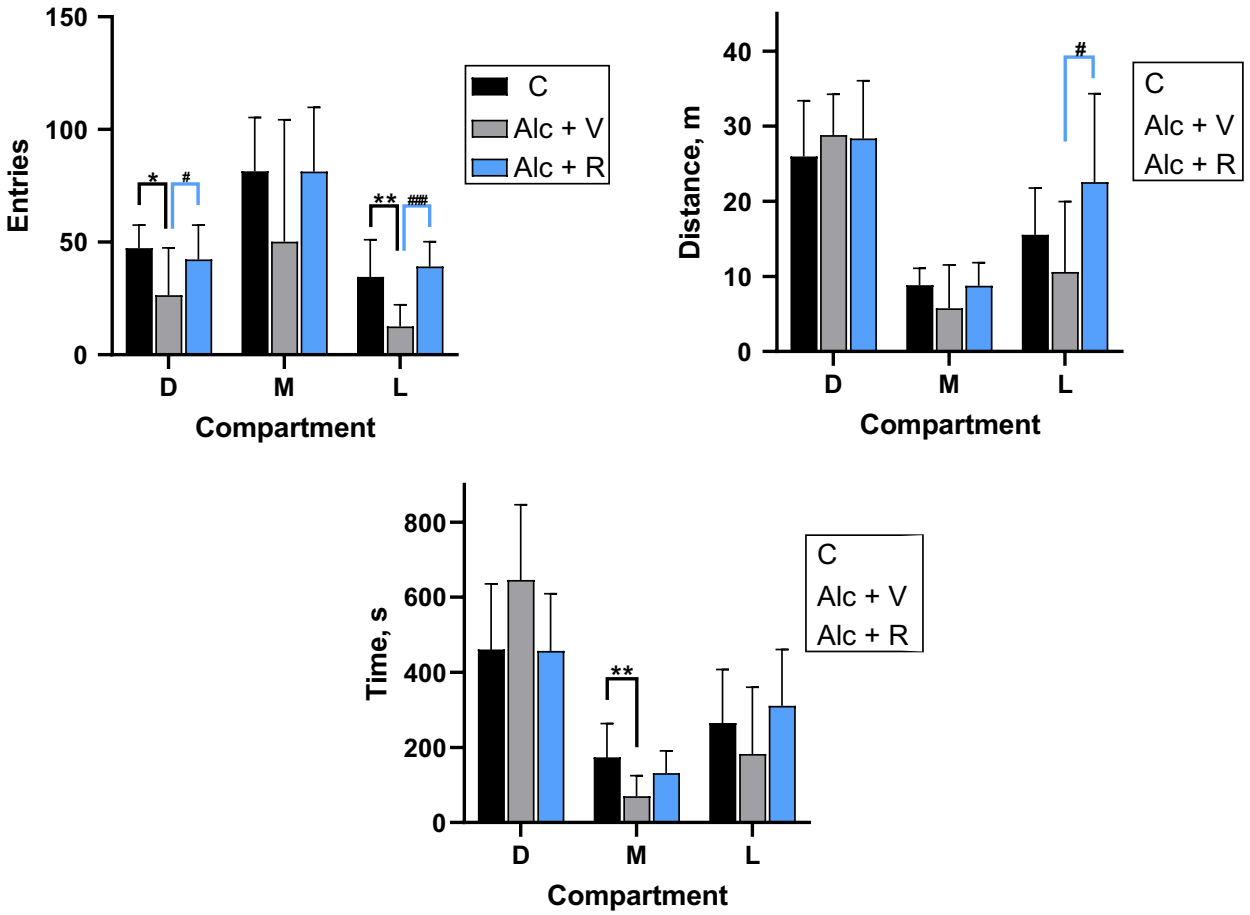

Fig. 3. Light-dark box. Data present as mean + SD. C, group of naive rats, $n = 11$; Alc + V, alcohol-drinking rats which received vehicle, $n = 8$; Alc + R, alcohol-drinking rats which received rolapitant, $n = 9$. A, number of entries to compartment. B, total distance in compartment. C, total spent time in compartment. D, dark compartment; M, middle compartment; L, light compartment. Asterisks indicate statistically significant differences between the groups compared control C group (*$P < 0.05$, **$P < 0.01$; *post-hoc* Duncan test). Hash indicates statistically significant differences between the groups except control group ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; *post-hoc* Duncan test).

continuous access to alcohol was available. Since rats exhibit increased anxiety-like behaviour after exposure to repeated withdrawal episodes we hypothesized that the effectiveness of rolapitant treatment in reducing alcohol intake following withdrawal may be due to its anxiolytic activity. Indeed, NK1r antagonists have been found to exert anxiolytic-like effects [20], although less pronounced than those reported for benzodiazepines [21]. It is well known that chronic alcohol consumption increases stress reactivity [22,23] and promotes anxiety disorders [24], which, in turn, contributes to the maintenance of and relapse to pathological alcohol use [25]. NK1r antagonists have been reported to reduce stress-associated alcohol-seeking [14]. Subsequently, here we used the light-dark box to assess the effects of rolapitant on the anxiety-related behaviour of rats in response to mild stress 24 h after withdrawal from alcohol. We found that rolapitant suppressed anxiety-related behaviour during the early withdrawal period. A number of possible mechanisms of this effect can be discussed. It has been shown that both chronic alcohol consumption and withdrawal are associated with upregulated neuronal activity in the amygdala [15] and NK1r antagonist injection into the amygdala attenuates alcohol drinking in rats with innate anxiety [15]. It is well known that the amygdala plays one of the dominant roles in the processing of emotional information: the imbalance of neuroactivity within the amygdala is responsible for the development of anxiety, dysphoria and depressive disorders [1,26,27]. It is also known that NK-1 antagonists inhibit sP-induced activation of corticotropin-releasing factor (CRF)

immunopositive neurons in the dorsal raphe nucleus which target the amygdala [28,29]. At the same time, CRF-induced hyperexcitability of amygdala plays a central role in addiction and stress response [30,31]. Thus, NK1r antagonist-induced suppression of the amygdala reactivity may be responsible for an attenuation of withdrawal-induced anxiety-like behaviour and increase of alcohol consumption. However, other mechanisms such as modulation of serotonergic and noradrenergic systems may also be involved in NK1-mediated anxiolytic effects [32]. It has been found that rolapitant reduces the monoaminergic-dependent overactivation of the hypothalamic–pituitary–adrenal axis [21]. Another explanation of rolapitant's effects on alcohol intake comes from a series of studies of NK1r antagonists as a promising medication for neuroinflammatory disease treatment [33]. It is well documented that alcohol abuse leads to the development of neuroinflammation [34]. Thus, NK1r antagonists can be considered as a potential anti-inflammatory therapy in alcohol-dependent subjects.

Our data demonstrate that rolapitant is characterized by high affinity to the rat brain tissue. This high affinity is conferred by the 3,5-ditrifluoromethylphenyl moiety leading to high lipophilicity and good brain penetration of the compound [35,36]. It has been previously reported that a single oral dose of 200 mg rolapitant was sufficient to occupy more than 90% of human cerebral NK1r receptors. Moreover, this receptor occupancy level was maintained for 120 h post-drug administration [37]. Compared to the other NK1r antagonists rolapitant has an extremely long elimination half-life estimated at approximately 160 h [38–40]. We found that when 5 $mg{\cdot}kg^{-1}$ of rolapitant was given repeatedly the suppression of alcohol intake was enhanced after the second withdrawal episode. This delayed response could be due to a possible cumulative effect arising from the accumulation of rolapitant in the brain. However, we did not see an increase in rolapitant levels following the same route of administration as we used for alcohol-treated rats. Instead, we found a slight decrease of rolapitant concentration in the neocortex following the second and third injections. A decrease in the rolapitant concentration could be explained by internalization of NK1r receptors [41], or an increase in metabolism of the compound. Further research should be conducted to investigate this phenomenon.

Taking into account that rolapitant is safe and well-tolerated across previous studies [18], with no severe adverse events [40] our data further suggest its benefit and advantage in reducing withdrawal-induced anxiety-related responses and in preventing the re-establishment of alcohol-seeking and relapse.

Materials and methods

Animals

Adult (PND 60) male Wistar rats were supplied by Stolbovaya Animal Farm (Moscow region, Russia) and group-housed 5 per cage in a 12/12-h light/dark cycle, 19–22 °C and 55% humidity with free access to water and standard lab chow until the start of experimental treatment.

All the animal procedures were conducted according to the guidelines of the Regulations for laboratory practice in the Russian Federation, 2003, the European directive (2010/63/EU) as well as to the recommendations of ARRIVE guidelines, and approved by the Institutional Ethics Committee of V.P. Serbsky National Medical Research Center on Psychiatry and Addiction (protocol code 01/2021P 04 April 2021).

Two-bottle "10% alcohol vs. water" choice drinking paradigm

At PND 70 (250–270 g) rats were housed individually in standard cages (43.5 × 28 × 16 cm). After a week of acclimatization and handling, rats were matched for body weight and randomly assigned to two groups: control rats (C, $n = 11$) were presented with two bottles containing water, and alcohol-exposed rats ($n = 25$) received one bottle containing diluted ethanol (10% v/v) and the other containing water, providing a 24-h continuous free-choice alcohol access. Alcohol and water intake were carefully measured for 24 days by weighing the bottles every 24 h at 8 a.m. (0.1 g accuracy). Rats finally consuming more than 2.5 $g{\cdot}kg^{-1}$ of pure alcohol per day were randomly assigned to Rolapitant- (Alc + R, $n = 8$) and Vehicle- (Alc + V, $n = 9$) treated groups (days 24–30). To estimate the effect of the treatment on anxiety-like behaviour during withdrawal episodes the alcohol bottle was replaced with another water bottle for 36 h followed by "drinking in the dark" (DID) regimen (two-bottle alcohol-free choice, 12 h during the dark phase; Fig. 4).

Drug administration

Animals from Alc + R group received intraperitoneal injections of rolapitant (CAS: 552292-08-7; Shandong Zhishang Chemical Co. Ltd, Jinan, China) at 5 $mg{\cdot}kg^{-1}$ in 25% ethanol (5 $mg{\cdot}mL^{-1}$) i.p. injections, every 48 h (Fig. 4). Control rats received a vehicle (25% ethanol) injection of a similar volume.

Light-dark box test

Anxiety-like behaviour was measured via light-dark box testing (TSE, Berlin, Germany) 24 h after alcohol

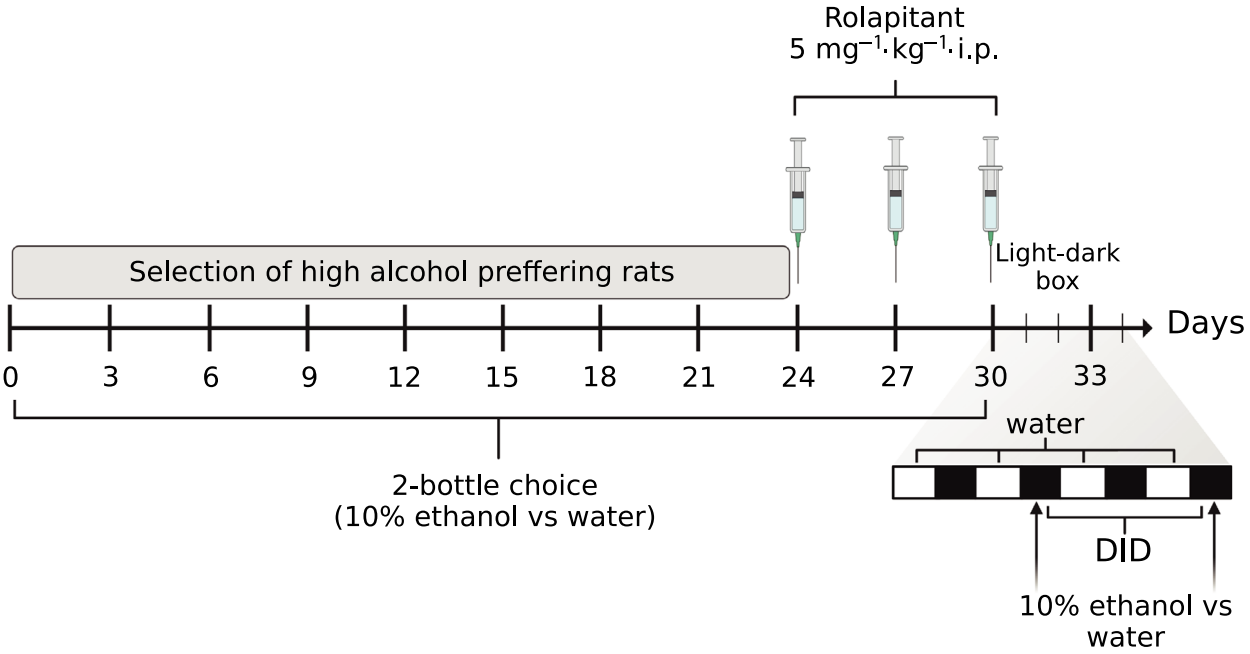

Fig. 4. Schema of the experimental protocol. Days 0–30 ("free choice" alcohol): the procedure began with a 24-day baseline period of voluntary alcohol consumption in a two-bottle paradigm (water or 10% ethanol). Rats finally consuming more than 2.5 g·kg^{-1}·day^{-1} of pure alcohol were assigned to rolapitant- (5 mg·kg^{-1} i.p., $n = 8$) or vehicle- ($n = 9$) groups treated on days 24, 27 and 30. Days 30–34: "drinking in the dark" (DID) regimen (36 h alcohol-deprivation period, after which alcohol was reintroduced for 12 h during the dark phase). The effect of the treatment on anxiety-like behaviour in the light-dark test was evaluated following 24 h period of alcohol deprivation (days 31 and 33).

withdrawal at day 31 (Fig. 4). Each rat was initially placed in the middle compartment (length × width × height, 13 × 21 × 35 cm) and was monitored for 15 min in the box with free choice to move between and within brightly illuminated (left) and dark (right) compartments of the box (both 21 × 21 × 35 cm). The following parameters were detected for each of the box compartments: number of entrances, total distance, and the total spent time.

Rolapitant distribution in the brain and plasma

A group of naive Wistar rats (PND 100, $n = 12$) has been used to determine the brain and plasma distribution of rolapitant. Rolapitant was administered according to the previous schema – 3 injections (5 mg·kg^{-1} in 25% ethanol, i.p.) with 72 h interval. Two hours after each injection 4 rats were sacrificed; brain areas – prefrontal cortex, amygdala and hypothalamus were dissected and immediately frozen in liquid nitrogen and then stored at −80 °C. Blood samples were collected in 5 mL tubes with Na_2EDTA (50 µL 0.5 M solution) and centrifuged for 10 min at 1500 ***g***, 3 °C. Plasma aliquots were stored at −40 °C until HPLC-MS analysis. The timing of brain and blood sampling was selected according to the "peak" concentration observed in the blood plasma after systemic administration of rolapitant [42]. Rolapitant was isolated from biological samples by liquid-liquid extraction: 5 mL ethyl acetate were added to 500 µL of blood plasma sample, or to 500 µL of brain tissue homogenate which was prepared in deionized water. After shaking on a vortex mixer, the samples were centrifuged at 2300 ***g***. The upper organic phase was decanted and evaporated on a vacuum centrifugal concentrator. The dry residue was recovered by 500 µL isopropyl alcohol for analysis.

Liquid chromatography–mass spectrometry

Rolapitant content was determined by high-performance liquid chromatography-mass spectrometry on a Thermo Fischer Scientific LCQ Fleet HPLC-mass spectrometer (Waltham, MA, USA) with a quadrupole ion trap detector. Prepared samples (10 µL) were injected into the loop of the chromatograph. Chromatographic separation was carried out on an XTerra MS C18 (4.6 × 150) 5 µm chromatographic column (Waters, Milford, MA, USA). Surveyor LC pump (Thermo Scientific) was used, the flow rate of the mobile phase was 0.7 mL·min^{-1}, the elution mode was isocratic, and the total analysis time was 11 min. A combination of solutions of 10 mM ammonium acetate (solution A; Clearsynth, Mumbai, Maharashtra, India) and acetonitrile (Sigma-Aldrich, Saint-Louis, MO, USA) with the addition of 10 mM ammonium acetate (90 : 10) (solution B), taken in a ratio of 75% B:25% A, was used as a mobile phase. Under these conditions, the retention time of rolapitant

was 8.40 ± 0.1 min. Rolapitant was determined by MRM-transition with m/z 501.4 → 261.21, a normalized collision energy was 35 eV. An external standard method was used for the quantitative determination of rolapitant. The calibration dependence was linear in the concentration range from 31.3 to 4000 ng·mL^{-1}. The concentration of rolapitant was determined by the formula $C = 0.016897 \times S$, where C is the concentration of rolapitant, expressed in ng·mL^{-1}, S – the area of the chromatographic peak of rolapitant. The correlation coefficient (R^2) was 0.996. Limit of quantitation – 31.3 ng·mL^{-1}.

Data analysis

The data were represented as the average ± standard deviation (SD) and analysed with the STATISTICA 12 software (StatSoft Inc., Tulsa, OK, USA). The datasets were tested for normality with the Shapiro-Wilk test (W-test) and the parametric analysis was applied if $P > 0.05$. The data obtained in the alcohol self-administration test and HPLC analysis were analysed with repeated-measures ANOVA. For the other datasets, the one-way ANOVA was applied. The *post-hoc* Duncan's multiple range test was used when appropriate.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization, VSK and AVD; methodology, IYS; validation, PKA, VOS, and DAA; formal analysis, VSK and AVD; investigation, VSK, PKA and DAA; resources, VOS; data curation, VSK; writing—original draft preparation, VSK; writing—review and editing, IYS; visualization, VSK, VOS; funding acquisition, AVD. All authors have read and agreed to the published version of the manuscript.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/febs.16400.

Data availability statement

The data presented in this study are openly available in Mendeley Data, link: Kokhan, Viktor (2021), "Neurokinin-1 receptor antagonist suppresses anxiety and alcohol intake produced by repeated withdrawal episodes", Mendeley Data, V1, doi: 10.17632/tw9xc9fjkp.1.

References

1 Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry*. 2016;**3**:760–73.
2 Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, et al. Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. *Biol Psychiat*. 2010;**67**:831–9.
3 Breese GR, Sinha R, Heilig M. Chronic alcohol neuroadaptation and stress contribute to susceptibility for alcohol craving and relapse. *Pharmacol Ther*. 2011;**129**:149–71.
4 Sandweiss AJ, Vanderah TW. The pharmacology of neurokinin receptors in addiction: prospects for therapy. *Subst Abuse Rehabil*. 2015;**6**:93–102.
5 Schank JR. Neurokinin receptors in drug and alcohol addiction. *Brain Res*. 2020;**1734**:146729.
6 Seneviratne C, Ait-Daoud N, Ma JZ, Chen G, Johnson BA, Li MD. Susceptibility locus in neurokinin-1 receptor gene associated with alcohol dependence. *Neuropsychopharmacology*. 2009;**34**:2442–9.
7 Schank JR, Tapocik JD, Barbier E, Damadzic R, Eskay RL, Sun H, et al. Tacr1 gene variation and neurokinin 1 receptor expression is associated with antagonist efficacy in genetically selected alcohol-preferring rats. *Biol Psychiat*. 2013;**73**:774–81.
8 Murtra P, Sheasby AM, Hunt SP, De Felipe C. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. *Nature*. 2000;**405**:180–3.
9 Ripley TL, Gadd CA, De Felipe C, Hunt SP, Stephens DN. Lack of self-administration and behavioural sensitisation to morphine, but not cocaine, in mice lacking NK1 receptors. *Neuropharmacology*. 2002;**43**:1258–68.
10 Thorsell A, Schank JR, Singley E, Hunt SP, Heilig M. Neurokinin-1 receptors (NK1R:s), alcohol consumption, and alcohol reward in mice. *Psychopharmacology*. 2010;**209**:103–11.
11 Steensland P, Simms JA, Nielsen CK, Holgate J, Bito-Onon JJ, Bartlett SE. The neurokinin 1 receptor antagonist, ezlopitant, reduces appetitive responding for sucrose and ethanol. *PLoS One*. 2010;**5**:e12527.
12 Schank JR, King CE, Sun H, Cheng K, Rice KC, Heilig M, et al. The role of the neurokinin-1 receptor in

stress-induced reinstatement of alcohol and cocaine seeking. *Neuropsychopharmacology*. 2014;**39**:1093–101.
13 George DT, Gilman J, Hersh J, Thorsell A, Herion D, Geyer C, et al. Neurokinin 1 receptor antagonism as a possible therapy for alcoholism. *Science*. 2008;**319**:1536–9.
14 Schank JR, Pickens CL, Rowe KE, Cheng K, Thorsell A, Rice KC, et al. Stress-induced reinstatement of alcohol-seeking in rats is selectively suppressed by the neurokinin 1 (NK1) antagonist L822429. *Psychopharmacology*. 2011;**218**:111–9.
15 Ayanwuyi LO, Stopponi S, Ubaldi M, Cippitelli A, Nasuti C, Damadzic R, et al. Neurokinin 1 receptor blockade in the medial amygdala attenuates alcohol drinking in rats with innate anxiety but not in Wistar rats. *Br J Pharmacol*. 2015;**172**:5136–46.
16 Slawecki CJ, Jimenez-Vasquez P, Mathe AA, Ehlers CL. Substance P and neurokinin levels are decreased in the cortex and hypothalamus of alcohol-preferring (P) rats. *J Stud Alcohol*. 2001;**62**:736–40.
17 Thorsell A, Slawecki CJ, Khoury A, Mathe AA, Ehlers CL. Effect of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. *Alcohol*. 2005;**36**:91–7.
18 Navari RM. The safety of rolapitant for the treatment of nausea and vomiting associated with chemotherapy. *Expert Opin Drug Saf*. 2019;**18**:1127–32.
19 Heldt SA, Davis M, Ratti E, Corsi M, Trist D, Ressler KJ. Anxiolytic-like effects of the neurokinin 1 receptor antagonist GR-205171 in the elevated plus maze and contextual fear-potentiated startle model of anxiety in gerbils. *Behav Pharmacol*. 2009;**20**:584–95.
20 Varty GB, Cohen-Williams ME, Morgan CA, Pylak U, Duffy RA, Lachowicz JE, et al. The gerbil elevated plus-maze II: anxiolytic-like effects of selective neurokinin NK1 receptor antagonists. *Neuropsychopharmacology*. 2002;**27**:371–9.
21 Kokhan V, Mariasina S, Pikalov V, Abaimov D, Somasundaram S, Kirkland C, Aliev G. Neurokinin-1 receptor antagonist reverses functional CNS alteration caused by combined γ-rays and carbon nuclei irradiation. *CNS Neurol Disord Drug Targets*. 2022;**21**:278–89.
22 Krieger H, Young CM, Anthenien AM, Neighbors C. The epidemiology of binge drinking among college-age individuals in the United States. *Alcohol Res*. 2018;**39**:23–30.
23 Lu YL, Richardson HN. Alcohol, stress hormones, and the prefrontal cortex: a proposed pathway to the dark side of addiction. *Neuroscience*. 2014;**277**:139–51.
24 Xu H, Li H, Liu D, Wen W, Xu M, Frank JA, et al. Chronic voluntary alcohol drinking causes anxiety-like behavior, thiamine deficiency, and brain damage of female crossed high alcohol preferring mice. *Front Pharmacol*. 2021;**12**:614396.
25 Becker HC. Effects of alcohol dependence and withdrawal on stress responsiveness and alcohol consumption. *Alcohol Res*. 2012;**34**:448–58.
26 Sosulina L, Strippel C, Romo-Parra H, Walter AL, Kanyshkova T, Sartori SB, et al. Substance P excites GABAergic neurons in the mouse central amygdala through neurokinin 1 receptor activation. *J Neurophysiol*. 2015;**114**:2500–8.
27 Chandra P, Hafizi S, Massey-Chase RM, Goodwin GM, Cowen PJ, Harmer CJ. NK1 receptor antagonism and emotional processing in healthy volunteers. *J Psychopharmacol*. 2010;**24**:481–7.
28 Commons KG, Connolley KR, Valentino RJ. A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. *Neuropsychopharmacology*. 2003;**28**:206–15.
29 Valentino RJ, Commons KG. Peptides that fine-tune the serotonin system. *Neuropeptides*. 2005;**39**:1–8.
30 Sharp BM. Basolateral amygdala and stress-induced hyperexcitability affect motivated behaviors and addiction. *Transl Psychiatry*. 2017;**7**:e1194.
31 Zhang X, Ge TT, Yin G, Cui R, Zhao G, Yang W. Stress-induced functional alterations in amygdala: implications for neuropsychiatric diseases. *Front Neurosci*. 2018;**12**:367.
32 Gobbi G, Cassano T, Radja F, Morgese MG, Cuomo V, Santarelli L, et al. Neurokinin 1 receptor antagonism requires norepinephrine to increase serotonin function. *Eur Neuropsychopharmacol*. 2007;**17**:328–38.
33 Martinez AN, Philipp MT. Substance P and antagonists of the neurokinin-1 receptor in neuroinflammation associated with infectious and neurodegenerative diseases of the central nervous system. *J Neurol Neuromed*. 2016;**1**:29–36.
34 Lowe PP, Morel C, Ambade A, Iracheta-Vellve A, Kwiatkowski E, Satishchandran A, et al. Chronic alcohol-induced neuroinflammation involves CCR2/5-dependent peripheral macrophage infiltration and microglia alterations. *J Neuroinflammation*. 2020;**17**:296.
35 Jiang J, Bunda JL, Doss GA, Chicchi GG, Kurtz MM, Tsao KL, et al. Potent, brain-penetrant, hydroisoindoline-based human neurokinin-1 receptor antagonists. *J Med Chem*. 2009;**52**:3039–46.
36 Fong CW. Permeability of the blood-brain barrier: molecular mechanism of transport of drugs and physiologically important compounds. *J Membr Biol*. 2015;**248**:651–69.
37 Majkowska-Pilip A, Halik PK, Gniazdowska E. The significance of NK1 receptor ligands and their application in targeted radionuclide tumour therapy. *Pharmaceutics*. 2019;**11**:443.
38 Muñoz M, Coveñas R. The neurokinin-1 receptor antagonist aprepitant: An intelligent bullet against cancer? *Cancers*. 2020;**12**:2682.

39 Singewald N, Chicchi GG, Thurner CC, Tsao KL, Spetea M, Schmidhammer H, et al. Modulation of basal and stress-induced amygdaloid substance P release by the potent and selective NK1 receptor antagonist L-822429. *J Neurochem*. 2008;**106**:2476–88.
40 Wang X, Zhang ZY, Wang J, Powers D, Arora S, Lu S, et al. Pharmacokinetics, safety, and tolerability of rolapitant administered intravenously following single ascending and multiple ascending doses in healthy subjects. *Clin Pharmacol Drug Dev*. 2019;**8**:160–71.
41 Thomas AG, Stathis M, Rojas C, Slusher BS. Netupitant and palonosetron trigger NK1 receptor internalization in NG108-15 cells. *Exp Brain Res*. 2014;**232**:2637–44.
42 Lai LML, Wu WKK, Chan MTV, Gan TJ, Habib AS, Kehlet H. Pharmacology of neurokinin antagonists and novel antiemetics. In: Kehlet H, Gan T, Habib A editors. Postoperative nausea and vomiting. Cambridge: Cambridge University Press, 2016; p. 74–89.