THE GLYCINE REUPTAKE INHIBITOR ORG 25935 DECREASES ETHANOL INTAKE AND PREFERENCE IN MALE WISTAR RATS

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Abstract — Previous findings from our group indicate that accumalble glycine receptors (GlyRs) are involved in mediating the dopamine (DA) activating effects of ethanol (EtOH), and that administration of glycine locally into the nucleus accumbens (nAc) reduces EtOH consumption in EtOH high-prefering rats. Aim: The present study examines the influence of a systemically administered glycine reuptake inhibitor, Org 25935, on EtOH preference and intake, in male Wistar rats with an EtOH preference >60% (during continuous access to a bottle of EtOH, 6% v/v, and a bottle of water), called EP>60 rats, as well as in animals with an EtOH preference <60%, called EP<60 rats. Org 25935 is an inhibitor of the glycine transporter 1 (GlyT1) protein with negligible action on the glycine transporter 2 (GlyT2) protein. Methods: Both EP>60 and EP<60 rats were limited to drink 2.5 h/day. Org 25935 or vehicle was administered intraperitoneally ~40 min before the rats were presented to a choice of drinking EtOH or water. Results: Org 25935 decreased EtOH intake and EtOH preference, as compared with vehicle, whereas water intake was unaffected. This effect was dose-dependent, developed gradually and was sustained for up to 40 days, also after introduction of an alcohol deprivation period. Conclusion: It is suggested that Org 25935, and possibly also other GlyT1 inhibitors, can represent a new pharmacological treatment principle for alcohol dependence or abuse.

INTRODUCTION

Neuroactive amino acids and their receptors are known to be involved in various behavioural effects of ethanol (EtOH). For example, we have previously reported that bilateral reversed microdialysis of glycine into the nucleus accumbens (nAc), an important part of the brain reward system, reduces EtOH intake and preference in male EtOH high-prefering Wistar rats exposed to a free choice between EtOH (6% v/v) and water (Molander et al., 2005). Based on these and other results (Molander and Soderpalm, 2005a; Molander and Soderpalm, 2005b) it was hypothesized that the reduced EtOH consumption is related to glycine’s action on strychnine-sensitive glycine receptors (GlyR) expressed in the nAc, which in turn influence extracellular dopamine (DA) levels as well as EtOH-induced DA release in the nAc. EtOH administration is known to increase DA output in the nAc of the rat (Di Chiara and Imperato, 1985; Imperato and Imperato, 1986; Blomqvist et al., 1993; Blomqvist et al., 1997), as well as in man (Boileau et al., 2003), and this increase has been hypothesized to be of importance for the development of alcohol addiction and alcoholism (Engel, 1977; Wise, 1987; Koob and Bloom, 1988; Wise and Rompre, 1989; Robinson and Berridge, 1993).

Reuptake of glycine via glycine transporter (GlyT) proteins into presynaptic nerve terminals and glial processes constitutes the mechanism by which the postsynaptic action of glycine is terminated and the extracellular levels of the amino acid are returned to basal values. There are two known types of GlyT proteins, the GlyT1 and the GlyT2, located on glia cells and glycineric neurons, respectively. The GlyT1 catalyses the removal of glycine from the synaptic cleft and the GlyT2 is required for the reuptake and reloading of glycine into synaptic vesicles (Gomeza et al., 2003). Org 25935 (cis-N-methyl-N-(6-methoxy-1-phenyl-1,2,3,4-tetrahydroonaphtha-len-2-ylmethyl)amino-methylcarboxylic acid hydrochloride) is a glycine reuptake inhibitor, which easily passes the blood–brain barrier and has its main action on the GlyT1 protein with negligible action on the GlyT2 protein. If dosed at 6 mg/kg intraperitoneally (i.p.) and administered to a rat weighing ~50 g, this compound is expected to increase striatal extracellular glycine levels by ~50–80% for ~2.5 h (Ge et al., 2001).

Glycine has been implicated in controlling neuronal excitability (Kirchner et al., 2003) and psychotic symptoms (Javitt, 2004) and consequently, GlyT inhibitors have been developed for treating epilepsy and schizophrenia (Aragon and Lopez-Corcuera, 2003; Isenhot et al., 2004). Against our background data, indicating that glycine and GlyRs are involved in the DA activating and reinforcing effects of EtOH, it was considered important to investigate whether a GlyT1 inhibitor influences voluntary EtOH consumption. The GlyT1 inhibitor Org 25935 is developed for clinical purposes and has shown to raise extracellular glycine levels after systemic administration. Therefore, the aim of the present study was to examine the effect of Org 25935 on EtOH intake and preference in a limited access two-bottle EtOH/water drinking model in male Wistar rats, before and after a period of alcohol deprivation. Rat models invoking a free choice between drinking EtOH and water and/or such a choice after a period of alcohol deprivation have been shown sensitive to the alcohol consumption reducing effect of acamprosate (Spanagel et al., 1996; Berglund et al., 1997; Olive et al., 2002) and naltrexone/naloxone (DeWitte, 1984; Stromberg et al., 1998; Holter and Spanagel, 1999), drugs recently introduced in the clinic for the treatment of alcoholism.

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MATERIALS AND METHODS

Animals
Male adult Wistar rats weighing 250–300 g (50 days old) were supplied by Beekay (Stockholm, Sweden). The animals were housed in groups of five at constant cage temperature (25°C) and humidity (65%), and were allowed to adapt for 1 week to the novel environment before screening for EtOH preference (see below) was initiated. They were kept under reversed light–dark conditions (light off at 09:00 a.m. and on at 09:00 p.m.) with free access to standard rat feed (Beekay feed). This study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

Screening for ethanol preference
Rats had continuous access to a bottle of EtOH solution and a water bottle during the screening period. The EtOH concentration was gradually increased (2% (v/v) for 3 days; 4% for 1 week and 6% for 3 days). Animals were subsequently housed individually in plastic cages. They had continuous access to two bottles containing either tap water or 6% EtOH solution. Previous observations using Wistar rats indicate that EtOH consumption is maximal approximately at this concentration (Fahlke, 1994). Water and EtOH intake were measured over a 6–7 week period. The amount (grams) of EtOH solution consumed, in percent of total fluid intake (grams), was used as an index of EtOH preference. Rats with an EtOH preference above and below 60% were denoted EP>60 and EP<60 rats, respectively. The EP>60 rats consumed 0.74 g/kg/2.5 h and the EP<60 consumed 0.46 g/kg/2.5 h during the one-week baseline period (the last 3 days are shown in the graphs). Experiment 1 examined the influence of Org 25935 on EtOH consumption in EP>60 rats and Experiment 2 that in EP<60 rats, in another set of rats.

Drugs
EtOH (AB Svensk sprit) was dissolved (2, 4, and 6% v/v) with regular tap water and presented in regular plastic 300 ml bottles. Org 25935 was kindly provided by NV Organon and dissolved in NaCl (0.9%) and administered i.p. in a volume of 2.0 ml/kg. NaCl (0.9%), 2.0 ml/kg i.p. was used as vehicle.

Experimental procedure
To achieve a baseline prior to the experiment and to let the animals adapt to the experimental procedure, all rats were allowed to drink both EtOH (6% v/v) and water for 2.5 h/day during 1 week; baseline limited access (LA) drinking. Moreover, during LA in Experiment 2, all rats were challenged with daily vehicle injections in order to minimize the influence of stress during LA in Experiment 2, all rats were challenged with daily vehicle injections in order to minimize the influence of stress on the EtOH drinking data. After the baseline LA period, daily Org 25935 or vehicle administration was started. Org 25935 or vehicle was injected i.p. and ~40 min later the rats were presented with the choice of drinking EtOH (6% v/v) or water during 2.5 h. All drinking sessions started when the light was turned off.

In Experiment 1, EP>60 rats were treated with Org 25935 (6 mg/kg, i.p.) or vehicle for 12 days and EtOH and water intake were monitored. Then all rats were exposed to 14 days of alcohol deprivation (AD), when they had free access to water, but not to EtOH. During the last 4 days of the AD period, the rats were again injected daily with either Org 25935 (6 mg/kg) or vehicle, but were still allowed to drink only water. After the AD period the rats were challenged with Org 25935 (6 mg/kg) or vehicle for 7 days and re-exposed to EtOH drinking. At the end of the second part of the experiment the dose of Org 25935 was gradually lowered (6, 3, and 1.5 mg/kg), in order to examine whether the observed effect was dose-related. The experiment was both preceded and ended by a baseline LA-drinking period, when no drug or vehicle was administered.

In Experiment 2, a new set of rats was used in order to examine how the GlyT1 inhibitor would influence EtOH preference and intake among EP<60 rats. Furthermore, this experiment was performed in order to systematically monitor rat weight and food intake. After one week of baseline LA-drinking (here including daily vehicle injections to all rats), the EP<60 rats were treated with Org 25935 (6 mg/kg) or vehicle for 12 days, in a manner identical to that in Experiment 1. This first test period was followed by an AD period, where Org 25935 (6 mg/kg) or vehicle was administered daily during the last 4 days of this period. The experiment was finished by a second test period when the rats were re-exposed to the choice of drinking EtOH (6% v/v) or water for 7 days.

Since the peak extracellular glycine levels may be expected ~50 min after administration of Org 25935 (filed data, Organon R & D), rats were presented with the water and EtOH (6% v/v) bottles ~40 min after drug administration. The choice of a 2.5 h drinking period was based on the information that if dosed i.p. at 6 mg/kg, Org 25935 is expected to increase striatal glycine levels by ~50–80% lasting ~2.5 h [filed data, Organon R & D, (Ge et al., 2001)].

Statistics
EtOH, water and food consumption data were statistically analysed using analysis of variance (ANOVA) with repeated measures followed by Fisher’s PLSD post hoc test, while rat weight was analysed by unpaired t-test. All values are expressed as means ± SEM. A probability level (P) of <0.05 was considered significant.

RESULTS

Experiment 1
As illustrated in Fig. 1a, Org 25935-treated EP>60 rats significantly reduced their EtOH intake compared with the vehicle-treated group. No difference in EtOH intake was seen during the days of baseline drinking. During the first test period, EtOH intake was significantly reduced among the Org 25935-treated animals (group effect [F(1.13) = 37.127, P < 0.0001], time effect [F(11.143) = 5.820, P < 0.0001], and interaction term [F(11.143) = 6.024, P<0.0001], Day 4–12) compared with the vehicle group, and this effect became evident after ~5 days of treatment. Following the AD period both groups significantly increased their EtOH intake (P = 0.0136, vehicle Day 15 versus vehicle Day 30 and P = 0.0074 for Org 25935 Day 15 versus Org 25935 Day 30, paired t-test). Reintroduction of Org 25935 (6 mg/kg) significantly lowered EtOH intake also after the
AD-induced increase of EtOH intake (group effect $F(1.13) = 5.530, P = 0.0351$), time effect $F(6.78) = 3.064, P = 0.096$, and interaction term $F(6.78) = 0.794, P = 0.578$, Day 30–37. Reduction of the Org 25935 dose from 6 to 3 and 1.5 mg/kg narrowed the difference between the groups to nonsignificant values. Finally, termination of Org 25935 administration completely abolished the difference in EtOH intake.

EtOH preference was also clearly affected by Org 25935 treatment in EP>60 animals, as indicated in Fig. 1b. The groups did not differ during the drug-free baseline period. In the first test period (Org 25935, 6 mg/kg) the Org 25935-treated group significantly reduced their EtOH preference (group effect $F(1.13) = 31.343, P < 0.0001$), time effect $F(11.143) = 2.747, P = 0.0030$, and interaction term $F(11.143) = 3.475, P = 0.0003$, Day 4–12) with onset of effect after ~5 days. After two weeks of AD (including 4 days of pre-treatment with Org 25935 or vehicle during the end of the AD period) there was a strong tendency for a reduced EtOH preference, compared with the vehicle group (group effect $F(1.13) = 4.353, P = 0.0572$), time effect $F(6.78) = 0.225, P = 0.9676$, and interaction term $F(6.78) = 0.219, P = 0.9695$, Day 30–37). The difference between the groups decreased when the dose was lowered to 3 mg/kg, and totally abolished after reducing the dose to 1.5 mg/kg.

As illustrated in Fig. 1c, there was no significant difference in water intake between the two EP>60 groups during the first part of the experiment. The AD period significantly increased water intake in the Org 25935-treated rats ($P = 0.0059$, Day 15 versus Day 30, paired $t$-test), but not in the control group. Total fluid intake was significantly lower in the Org 25935 group compared with the controls after 4 days of treatment (group effect $F(11.143) = 5.800, P = 0.0001$), and interaction term $F(11.143) = 3.054, P = 0.0001$, Day 4–12, Fig. 1d). Both groups significantly increased their fluid intake when comparing the time before and after the AD period ($P = 0.0001$, Org 25935 Day 15 versus Org 25935 Day 30; $P = 0.0008$, vehicle Day 15 versus vehicle Day 30, paired $t$-tests). There was no difference with respect to total fluid intake after the AD period between the Org 25935 group and controls.

Experiment 2

Fig. 2a displays the reduction in EtOH intake observed in Org 25935-compared with vehicle-treated EP<60 animals. EtOH intake did not differ between the groups during baseline drinking. However, in the first treatment period, EtOH intake was significantly reduced in the Org 25935 group (group effect
Fig. 2. Org 25935 decreases ethanol intake and preference in EP<60 male Wistar rats, n = 14–15. Data from Experiment 2, during baseline and Org 25935 administration, 6 mg/kg, i.p. Day 4–15, AD Day 16–29, reintroduction of Org 6 mg/kg Day 30–36. EtOH intake (a), EtOH preference (b), Water intake (c) and Total fluid intake (d). For statistics: see text.

[F(1.27) = 14.145, P = 0.0008], time effect [F(11.297) = 2.395, P = 0.0074], and interaction term [F(11.297) = 2.272, P = 0.0113], Day 4–15. This decrease was also pronounced after the AD period (group effect [F(1.27) = 9.927 P = 0.0040], time effect [F(6.162) = 4.562, P = 0.0003], and interaction term [F(6.162) = 3.445, P = 0.0031], Day 30–35), when Org 25935 and vehicle administration was reintroduced.

EtOH preference revealed similar results. Fig. 2b illustrates no difference during the drug-free baseline period. However, the significant interaction term indicates that Org 25935- and vehicle-treatment influenced EtOH preference differently (group effect [F(1.27) = 3.630 P = 0.0675], time effect [F(11.297) = 3.682, P < 0.0001], and interaction term [F(11.297) = 3.539, P = 0.0001]). Also here a delayed onset of effect was seen, and the group effect became statistically significant when the first 3 days of treatment were excluded (group effect [F(1.27) = 6.890, P = 0.0141], time effect [F(8.216) = 2.449, P = 0.0148], and interaction term [F(8.216) = 1.087, P = 0.3735]). A reduced EtOH preference was observed also after the AD period (group effect [F(1.27) = 6.961, P = 0.0137], time effect [F(6.162) = 2.897, P = 0.0104], and interaction term [F(6.162) = 3.825, P = 0.0041]).

The repeated measures ANOVA revealed no group effect [F(1.27) = 2.142, P = 0.1548], but a time effect [F(11.297) = 5.178, P ≪ 0.0001] and a significant interaction term [F(11.297) = 4.781, P ≪ 0.0001], with respect to water intake during the first test period, displayed in Fig. 2c. After the AD period there was no significant group effect for water intake, but a significant time effect [F(6.162) = 4.940, P = 0.0001], and interaction term [F(6.162) = 3.988, P = 0.0009]. Total fluid intake differed significantly between Org 25935- and vehicle-treated EP<60 rats during both test periods (group effect [F(1.27) = 44.786 P < 0.0001], time effect [F(11.297) = 8.349, P < 0.0001], Day 4–15, and interaction term [F(11.297) = 4.112, P < 0.0001 ]; and group effect [F(1.27) = 10.055 P = 0.0038], time effect [F(6.162) = 7.425, P < 0.0001], and interaction term [F(6.162) = 2.360, P = 0.0327], Day 30–35, Fig. 2d).

Taken together, Org 25935 treatment decreased EtOH intake and preference in both EP>60 and EP<60 rats.
Furthermore, when examining each rat’s individual EtOH intake, the Org 25935 treatment decreased EtOH intake in most animals. Only 3 of the 21 rats examined slightly increased their EtOH intake when comparing the mean of Day 1–3 (baseline LA-drinking), versus mean of Day 7–9, representing a time-point in the middle of the first test period.

Food intake in EP<60 rats was significantly reduced by Org 25935 during the first test period {group effect \( F(1.26) = 73.035, P < 0.0001 \), time effect \( F(11.286) = 3.981, P < 0.0001 \), and interaction term \( F(11.286) = 2.010, P = 0.0274 \), Day 4–15}. However, this difference disappeared over time and was no longer significant after the AD period {group effect \( F(1.27) = 0.145 P = 0.7062 \), time effect \( F(6.162) = 3.048, P = 0.0075 \), and interaction term \( F(6.162) = 2.835, P = 0.0119 \), Day 30–35, Fig. 3}.

The rat weight was monitored once a week prior to and throughout both Experiments 1 and 2. There was no significant difference in mean rat weight between the EP>60 Org 25935 treated rats compared with the controls during the whole experiment (Fig. 4a). However, mean rat weight of the Org 25935-treated EP<60 rats was significantly reduced compared with controls during the first test period {group effect \( F(1.27) = 15.663, P = 0.0005 \), time effect \( F(1.27) = 11.846, P = 0.0019 \), and interaction term \( F(1.27) = 11.213, P = 0.0274 \)}, during the AD period {group effect \( F(1.27) = 13.582 P = 0.0010 \), time effect \( F(1.27) = 66.613, P = 0.0001 \), and interaction term \( F(1.27) = 2.214, P = 0.1483 \)}, as well as during the second test period \( P = 0.0003 \), Unpaired \( t \)-test (1 week, one measurement), Fig. 4b}. Rat weight in the Org 25935-treated groups (both experiments) was not significantly reduced when comparing baseline values with the values obtained at the end of drug treatment (6 mg/kg). The mean rat weight among the Org 25935-treated EP>60 rats was instead significantly increased during this period [baseline versus second test period (Org 25935 6 mg/kg), \( P = 0.0001 \), paired \( t \)-test].

**DISCUSSION**

This is the first study demonstrating that a selective glycine reuptake inhibitor (Org 25935) dose-dependently decreases voluntary EtOH intake and preference in the rat. The reduced EtOH intake was observed both in high-preferring (EP>60) and low- and medium-preferring (EP<60) male Wistar rats. The onset of the Org 25935-induced effect appeared to be delayed by 3–5 days in this model. Therefore the rats were treated with Org 25935 for 4 days at the end of the AD period before reintroducing EtOH, which most likely explains the earlier onset of effect in the second test period.

The Org 25935 effect was maintained for the entire treatment period (40 days, Experiment 1), also after the inclusion of an AD period, which is known to promote EtOH drinking. This pattern contrasts to that reported for several other substances, e.g. selective serotonin (5-HT) reuptake inhibitors and 5-HT1A receptor agonists, which often have prompt onsets of action on EtOH consumption but then lose effect after one or two weeks of treatment (Hedlund and Wahlström, 1996, 1998). The suppressive effect of Org 25935 on EtOH consumption appeared robust and dose-related, and the EP>60 animals fully returned to their
baseline drinking level after Org 25935 withdrawal, indicating that the effect is not due to chance fluctuations of EtOH intake.

In consensus with previous studies (Holter et al., 1998; Spanagel and Holter, 1999; Rodd-Henricks et al., 2001; Samson and Chappell, 2001; Vengeliene et al., 2003; Thileen et al., 2004), EtOH intake increased after an AD period. This was true both for vehicle- and Org 25935-treated animals, even though EtOH intake remained significantly lower in the Org 25935 group. Total fluid intake increased in both groups of EP>60 rats. In the control group, this increase was mainly accounted for by the increased EtOH intake, which is hypothesized to be due to an increased drive to consume EtOH in order to experience its pharmacological effects. However, in Org 25935-treated EP>60 rats the increased drinking drive was equally expressed in EtOH and water intake. This could indicate that the animals no longer can discriminate between the water and EtOH bottle, e.g. due to an alternation of taste, or that the pharmacological effect of EtOH is attenuated, making the rats search for the effect from the alternative bottle. EtOH preference was also increased after the AD period, but only in EP<60 rats. This difference is probably explained by a ceiling effect in the EP>60 rats, since the mean ethanol preference was ~90% in this group at baseline. Org 25935 treatment significantly decreased EtOH preference after the AD period, both in EP>60 and EP<60 rats.

Org 25935 tended to reduce mean rat weight in Experiment 1. Therefore, rat weight and daily food intake was carefully monitored in Experiment 2 (EP<60 animals). Here, both mean rat weight and food intake was lower in the Org 25935-treated group, and one explanation may be that the drug influences appetite and/or eating behaviour. However, even though the mean rat weight was lower in the experimental group compared with controls, it was not significantly reduced over time during in the Org 25935 group, but rather maintained at the same level. This could indicate a counteraction of a tentative over-eating in laboratory rats, which have unlimited supply of food. However, the reduced food intake was abolished after the AD period and the rats subsequently gained weight during and after the AD period, indicating that this may be an initial effect only.

Water intake remained stable or increased throughout both test periods for EP<60 and EP>60 rats, making it less probable that, toxicological effects are present. This is also reinforced by the fact that Org 25935 did not induce a weight loss compared with baseline but merely prevented the weight gain observed in controls. Also, as judged from gross observation all rats appeared healthy and behaviourally unaffected by the doses of Org 25935 used in these studies, which were selected based on earlier experiments performed by Organon R & D. However, Org 25935 probably produced a slight sedative effect early on during treatment, as indicated by the decreased water intake observed at this time-point in EP<60 rats. Apparently tolerance developed to this effect, since later on during treatment, as well as after the AD period, Org 25935-treated EP<60 rats maintained their water intake at levels comparable to the vehicle group. In contrast, and as pointed out above, there was no tolerance development to the Org 25935-induced effect on EtOH intake, indicating that this effect is not due to sedation affecting drinking behaviour in general. This interpretation is further reinforced by the increased total fluid and water intake observed in Org 25935-treated EP>60 rats after the AD period, and that the total fluid intake at this time was almost identical to that in the vehicle-treated group.

Functional GlyRs are present in the nAc, a major target of the mesolimbic DA system, and bilateral administration of glycine directly into this area may increase DA release and reduce EtOH consumption (Molander et al., 2005; Molander and Soderpalm, 2005b). We have suggested that this DA liberating effect results from interference with inhibitory GlyRs located on the soma of inhibitory GABAergic feed-back neurons projecting from the nAc to the VTA (Holter and Soderpalm, 2005a, b). Indeed glycine has previously been shown to produce disinhibition of VTA dopaminergic neurons and of nigrostriatal DA neurons, via reduction of GABAergic inhibitory activity (Mendez et al., 1976; Pycock et al., 1981; Zheng and Johnson, 2001), although these effects probably were due to interference with GABAergic terminals in the VTA. Against this background we hypothesize that the significant reduction of EtOH intake produced by the GlyT1 inhibitor Org 25935 is mediated via modulation of mesolimbic DA activity. Thus, Org 25935 is hypothesized to increase endogenous glycine levels, leading to an interference with GlyRs in the nAc and/or in the VTA, which disinhibits mesolimbic DA neurons by turning off inhibitory GABAergic neurons. This DA activation may in turn, as in our previous study, be associated with a decrease in EtOH consumption. Alternatively, the reduction in EtOH intake may be more directly related to activation of GlyRs in the nAc. In our previous acute study it was suggested that the decrease in EtOH intake produced by glycine may be due to glycine substituting for ethanol and/or that glycine by desensitizing GlyRs prevents further EtOH-induced DA activation. The same could be suggested for Org 25935, a hypothesis that is currently challenged in our laboratory using in vivo microdialysis techniques.

However, Org 25935 may also produce its effect on EtOH intake by other mechanisms. Thus, enhanced extracellular glycine levels induced by Org 25935 are likely to interfere also with the glycine site of NMDA receptors. Indeed, it has been suggested that acamprosate, an agent used in the clinic to reduce EtOH consumption, produces its effect via interference with NMDA receptors (al Qatari et al., 1998; Naassila et al., 1998). In addition, EtOH interacts with the NMDA receptor (Spanagel and Zieglgansberger, 1997; Ren et al., 2003), and perhaps especially with the glycine site of the receptor (Naassila et al., 1998), even though this issue remains open to debate. Clearly, additional pharmacological studies are required in order to better understand by which mechanisms Org 25935 produces the effects on EtOH consumption observed in the present studies.

In conclusion, the selective GlyT1 inhibitor Org 25935 produced a robust, long-lasting and reversible decrease in EtOH intake and preference in a two-bottle free choice model in the male Wistar rat. Also food intake was transiently reduced. The EtOH intake reducing effect of Org 25935 could tentatively be related to an increase of extracellular glycine levels and a subsequent modulatory effect on brain GlyRs. Clinical studies examining the effect of GlyT1
inhibitors in the treatment of alcoholism should be of high priority.

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