Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 522 (2005) 72–77
www.elsevier.com/locate/ejphar

Reduction of voluntary ethanol consumption in alcohol-preferring Alko alcohol (AA) rats by desoxypeganine and galanthamine

Roman Doetkotte [a,*], Klaus Opitz [b], Kalervo Kiianmaa [c], Hilke Winterhoff [a]

[a] *Institute of Pharmacology and Toxicology, Universitätsklinikum Münster, Domagkstrasse 12, D-48149 Münster, Germany*
[b] *Görlitzerstrasse 102, D-48157 Münster, Germany*
[c] *Department of Mental Health and Alcohol Research, National Public Health Institute, P.O. Box 33, FIN-00251 Helsinki, Finland*

Received 24 March 2005; received in revised form 16 August 2005; accepted 26 August 2005
Available online 4 October 2005

Abstract

The effects of desoxypeganine, an alkaloid from *Peganum harmala* L., and of galanthamine, an alkaloid from *Galanthus nivalis* L., on voluntary ethanol consumption were investigated in female Alko alcohol (AA) rats. Desoxypeganine–HCl reduced ethanol intake and ethanol preference dose-dependently at a dose range between 10 and 30 mg/kg body weight when given by gavage. Subcutaneous and intraperitoneal applications of desoxypeganine lead to even more pronounced decreases of ethanol intake and ethanol preference.

The effects of desoxypeganine and galanthamine seem to be additive. A combination of both substances in doses, which were ineffective when administered alone, caused a significant decrease of ethanol preference.

To exclude habituation to desoxypeganine treatment, the substance was given once daily over a period of 16 days. No decreases of the desoxypeganine effects on ethanol intake, total fluid intake, and ethanol preference were observed.

This attenuation of ethanol preference combined with unchanged total fluid intake and food consumption represents a promising activity especially because no acquirement of tolerance after repeated administration was observed.

Keywords: Ethanol preference; Desoxypeganine; Galanthamine; Alko alcohol (AA) rat

1. Introduction

Alcohol is the most common drug of abuse in Germany as well as in other industrial countries. Though moderate ethanol consumption (up to 40 g/day for men and 20 g/day for women) seems to be an advantage (Singer and Teyssen, 2002), the regular intake of large amounts of ethanol increases the hazard of developing addiction and increases the risk of somatic diseases like hepatic, cardiac and central nervous disorders. A high ethanol intake is rather frequent: in 2001 1.6 million people in Germany suffered from alcohol addiction and further 2.7 million were consuming alcohol regularly in amounts representing a health risk (Clade, 2001). Drugs reducing voluntary ethanol consumption would be of prophylactic value.

Goals of the pharmacotherapy of alcohol dependence are the reduction of the risk of relapse, the craving for alcohol and the number of days drinking. Naltrexone and acamprosate are approved therapeutic options, improving the outcome in rehabilitation of alcohol-dependent patients. For naltrexone an attenuation of the reward effects of alcohol is assumed, for acamprosate a blockade of the negative craving of alcohol-dependent patients in the absence of alcohol (Mann, 2004). Yet both drugs have only modest effects on the long-term outcome and the therapy is not effective in all patients (Kenna et al., 2004). The search for new drugs reducing the motivation to consume alcohol is of considerable interest (Anton and Swift, 2003; Swift, 1999). Sertraline, ondansetron, topiramate and aripiprazole are currently being investigated for their usefulness in the treatment of alcoholism, other drugs are tested in pharmacological studies (Colombo et al., 2004; Kenna et al., 2004).

An increased health risk exists for all heavy drinkers, though only some of them develop addiction. Drugs reducing

* Corresponding author. Tel.: +49 251 83 555 10; fax: +49 251 83 555 01.
E-mail address: doetkot@uni-muenster.de (R. Doetkotte).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.038

ethanol consumption would be of prophylactic benefit in such patients as they might be able to prevent alcohol-related diseases and block the developmental processes leading to addiction. No pharmacotherapy with such a prophylactic activity is yet established, experimental studies are under development.

In the present experiments desoxypeganine (1,2,3,9-tetrahydropyrrolo[2,1-*b*]quinazoline), an alkaloid originally extracted from *Peganum harmala* L., which meanwhile is produced synthetically, was studied in female AA rats for a reduction of alcohol preference. Desoxypeganine has been described by Tuliaganov et al. as an inhibitor of acetylcholinesterase like galanthamine, but less toxic (Tuliaganov et al., 1986). In vitro it inhibits butyrylcholinesterase (IC_{50}=2 μM), acetylcholinesterase (IC_{50}=17 μM), and monoamine oxidase A (IC_{50}=2 μM) but not MAO-B (Moormann et al., 2005).

Galanthamine (4a,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6*H*-benzofuro[3a,3,2-ef]benzazepin-6-ol), an alkaloid which occurs naturally in many plants of the Amaryllidaceae family, e.g. Galanthus species, *Leucojum aestivum* L., and various species of narcissus, is a reversible acetycholinesterase inhibitor which also acts as an allosterically potentiating ligand on nicotine acetylcholine receptors (Harvey, 1995; Lopez et al., 2002; Samochocki et al., 2000). Galanthamine reduces ethanol preference in rats as was previously described (Opitz, 1992) and has also been shown to improve learning memory in rats after prolonged alcohol intake (Iliev et al., 1999). For these reasons galanthamine was included in the present study. To get hints on desoxypeganine's mechanism of action, a combination with galanthamine was tested as well.

AA rats consistently consume more ethanol (10% v/v) than tap water in a free choice situation, i.e. they are ethanol-preferring (Eriksson, 1968; Sinclair et al., 1989). Desoxypeganine was tested in AA rats because their ethanol preference is genetically determined, as it is in humans.

To detect a reduction of voluntary ethanol consumption ethanol intake, total fluid intake and, as a calculated criterion, the ethanol preference were compared during control and treatment periods. In addition the daily food consumption was determined.

2. Materials and methods

2.1. Animals

Thirty eight adult female alcohol-preferring AA (Alko alcohol) rats were obtained from the National Public Health Institute, Finland. The animals, generation F_{87}, all of the same age, approximately 90 days at arrival, were single-housed in makrolon® cages type 3 (37×25×16 cm), at a room temperature of 24±1 °C and a 12-h light–dark schedule (lights on at 6:00 am). They had free access to standard food (altromin® 1324) whereas ethanol (10% v/v) and tap water were available only during the dark period (6:00 pm to 6:00 am).

For the investigations, the animals were randomly assigned to two collectives of 19 animals each. In each collective there was one rat which did not drink ethanol in a consistent quantity, these animals were excluded from the tests.

The experimental procedures used comply with the European Community's Council Directive of 24th November 1986 (86/609/EEC) and were officially approved by the local committee on animal care (Regierungspräsident, Münster, A 36/2000).

2.2. Apparatus and experimental settings

All experiments were conducted in the home cages. Two polypropylene bottles per cage (of 300 ml content each) with stainless steel drinking spouts were fitted with double ball valves. The bottles were removed automatically at 6:00 am. The positions of the bottles were changed every few days to avoid the development of a position preference. Four to six weeks after exposition a fairly constant alcohol preference of about 90% was achieved, which remained nearly unchanged for months and allowed repeated testing of the same animals.

2.3. Drugs

Desoxypeganine–HCl and galanthamine–HBr were provided by HF-Arzneimittelforschung GmbH, Werne, Germany. The substances were dissolved in tap water for oral application and in saline 0.9% for parenteral application.

A solution of 10% v/v ethanol was prepared from ethanol 96% v/v (Ethanol Ph.Eur.; obtained from Carl Roth GmbH and Co.; Karlsruhe, Germany) and tap water.

2.4. Procedure

Investigations were carried out parallel in two collectives of 18 animals each. In the first collective studies on acute effects of different doses of desoxypeganine on ethanol preference were performed, in addition different routes of administration were performed in these animals. The other collective was treated repeatedly with 20 mg/kg body weight desoxypeganine–HCl over 16 days, furthermore the combination of desoxypeganine and galanthamine was tested in this same collective.

The drugs were administered at 6:00 pm directly before the beginning of the dark period (n=18 per experimental group). Ethanol intake, total fluid intake and, as a calculated criterion, the ethanol preference were determined during control and treatment periods and the results at different test days obtained in the same collective were compared. If ethanol or water consumption was influenced by the treatment a washout period of at least 48 h or until normalization of these parameters was interposed before the next test. A control test with oral application of water, or parenteral application of saline 0.9%, respectively, preceded each test period. All acute effects of desoxypeganine, galanthamine and the combination were reproduced in both collectives with variable order of the tested

dosages. Both collectives showed a comparable sensitivity to desoxypeganine treatment.

The test parameters were determined at 10:00 pm and at 6:00 am on the next morning, in order to detect also shorter lasting effects. The ethanol preference was calculated by the following formula:

$$\text{Ethanol preference}\,[\%] = \text{intake of ethanol solution}\,(10\%\,\text{v/v})\,[\text{ml}] \,/\,\text{total fluid intake}[\text{ml}] \times 100.$$

If the animals would be drinking only ethanol (10% v/v) and no water at all then their ethanol preference would be 100%, i.e. maximal. If the animals would consume equal amounts of ethanol (10% v/v) and drinking water then their ethanol preference would be 50%, i.e. minimal. If the animals would drink more water than ethanol (10% v/v) the respective value would be less than 50%, meaning that they dislike ethanol (no ethanol preference).

As decreases in food consumption are indicative for toxicity of the administered dose, food intake was determined every day.

2.4.1. Acute treatment

Desoxypeganine was administered orally by gavage in a volume of 10 ml/kg body weight. Ethanol and water intake as well as food consumption were determined following acute treatment. A control period (application of tap water) preceded each test period so each animal acted as its own control thus eliminating interindividual variability.

2.4.2. Effect after repeated treatment over a period of 16 days

To check for acquirement of tolerance, desoxypeganine–HCl was administered orally once daily in a volume of 10 ml/kg body weight at 6:00 pm. Ethanol, water and food consumption were measured as mentioned above. The values for ethanol and total fluid intake were detected over four days before the first administration of desoxypeganine. The average of these values is taken as the control value.

2.4.3. The activity of desoxypeganine following different routes of administration

The effects of oral desoxypeganine doses were compared with those of subcutaneous or intraperitoneal injections. For the parenteral routes desoxypeganine was dissolved in saline 0.9% and injected in a volume of 5 ml/kg body weight. As control treatment water was given by gavage in a volume of 10 ml/kg body weight and saline 0.9% was given i.p. in a volume of 5 ml/kg body weight.

2.4.4. Co-administration of desoxypeganine and galanthamine

First galanthamine–HBr alone was studied in doses of 2.5, 5 and 10 mg/kg body weight (administered by gavage in a volume of 10 ml/kg body weight). Thereafter a combination of a low dose of galanthamine–HBr (2.5 mg/kg body weight) and desoxypeganine–HCl (10 mg/kg body weight) was tested for additive effects.

2.5. Data analysis

Outliers were detected by StatView® statistical software package, version 5.0 (SAS® Institute Inc., USA) by using the boxplot function. Significances of the resulting data were calculated by analysis of variance (ANOVA) with Fischer–PLSD and Student–Newman–Keuls post hoc tests for multiple comparisons. Data are expressed as means ± S.E.M. Statistical significance was set at $^{*/+}P<0.05$ and $^{**/+}P<0.01$, respectively.

3. Results

A fairly constant ethanol preference of 88% to 90% during control periods was achieved by the two-bottle choice paradigm with 12 h free access to alcohol and tap water (limited access

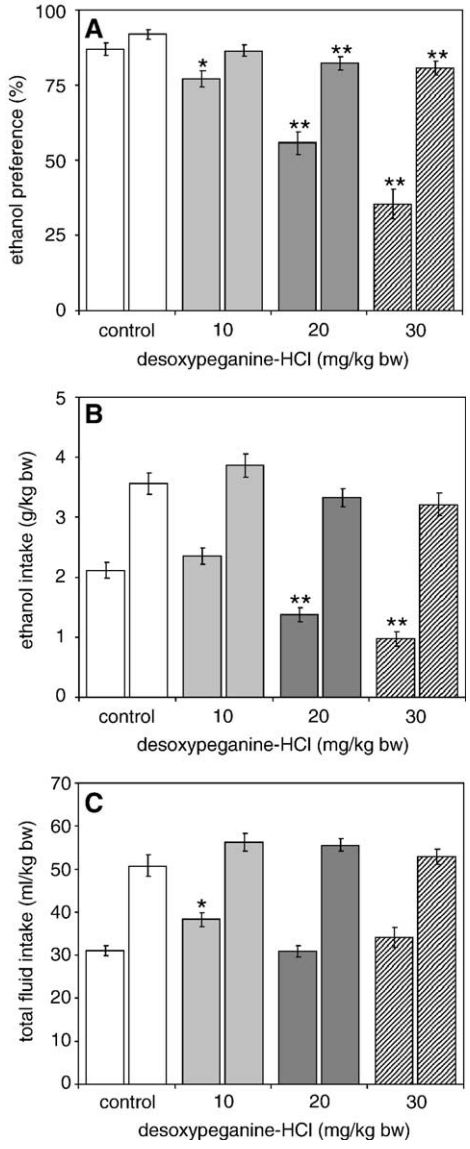

Fig. 1. Dose-dependent effect of desoxypeganine on ethanol intake and ethanol preference. First columns: period from 6 to 10 pm, second ones: period between 10 pm and 6 am next morning. Each point represents the mean ± S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, significance vs. control group.

paradigm) (Sinclair et al., 1992). Desoxypeganine–HCl caused a dose-dependent decrease of ethanol preference in female AA rats at a dose range between 10 and 30 mg/kg body weight during the first 4 h following application (Fig. 1). The ethanol preference (A) was reduced from 87.0±2.1% in the controls to 77.1±2.7% by 10 mg/kg body weight desoxypeganine–HCl (ANOVA: $F(3,61)=43.020$, $P<0.0001$; Fisher–PLSD test: $P=0.0414$; significant according to Student–Newman–Keuls test). After administration of 30 mg/kg body weight desoxypeganine–HCl ethanol preference was abolished, the percentage of ethanol consumption was 35.4±4.9% of the total fluid intake (ANOVA: $F(3,61)=43.020$, $P<0.0001$; Fisher–PLSD test: $P<0.0001$; significant according to Student–Newman–Keuls test). The effect of 10 mg/kg body weight desoxypeganine–HCl did not last longer than 4 h, whereas after application of 20 or 30 mg/kg body weight desoxypeganine–HCl significant reductions of preference were observed in both time intervals (1 to 4 h and 4 to 12 h after treatment; significances for the second time interval as follows: 20 mg/kg body weight: ANOVA: $F(3,60)=6.231$, $P=0.0009$; Fisher–PLSD test: $P=0.0012$; significant according to Student–Newman–Keuls test; 30 mg/kg body weight: ANOVA: $F(3,60)=6.231$, $P=0.0009$; Fisher–PLSD test: $P=0.0002$; significant according to Student–Newman–Keuls test). Four to 12 hours after application of 20 mg/kg body weight desoxypeganine–HCl the values for ethanol preference are 82.2±2.17% and after application of 30 mg/kg body weight 80.6±2.3%, respectively.

A significant reduction of ethanol intake (B) was observed for 20 or 30 mg/kg body weight desoxypeganine–HCl only during the first 4 h (20 mg/kg body weight: ANOVA: $F(3,61)=23.999$, $P<0.0001$; Fisher–PLSD: $P=0.0002$; significant according to Student–Newman–Keuls test; 30 mg/kg body weight: ANOVA: $F(3,61)=23.999$, $P<0.0001$; Fisher–PLSD: $P<0.0001$; significant according to Student–Newman–Keuls test). The total fluid intake (C) was increased during the whole period by 10 mg/kg body weight; the increase during the first 4 h was significant (ANOVA: $F(3,60)=3.810$, $P=0.0144$, Fisher–PLSD test: $P=0.0064$). An insignificant increase following 20 and 30 mg/kg body weight was seen during the second observation period. The food intake was not affected by any of these doses of desoxypeganine (data not shown).

During repeated treatment with 20 mg/kg body weight desoxypeganine–HCl the reduction of ethanol preference remained nearly unchanged, a very slight, insignificant decrease of the desoxypeganine effect indicates that there is no

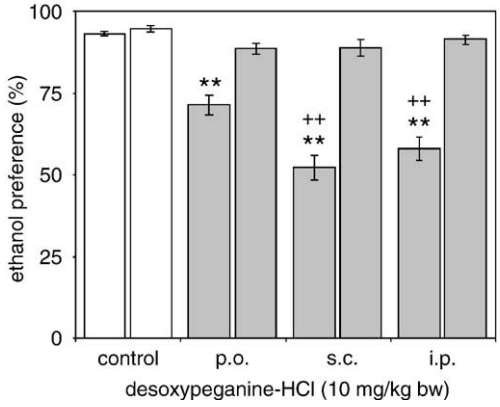

Fig. 3. Activity of desoxypeganine after different application routes. First columns: period from 6 to 10 pm, second ones: period between 10 pm and 6 am next morning. Each point represents the mean±S.E.M. $^{**}P<0.01$, significance vs. control group. $^{++}P<0.01$, significance vs. p.o. group.

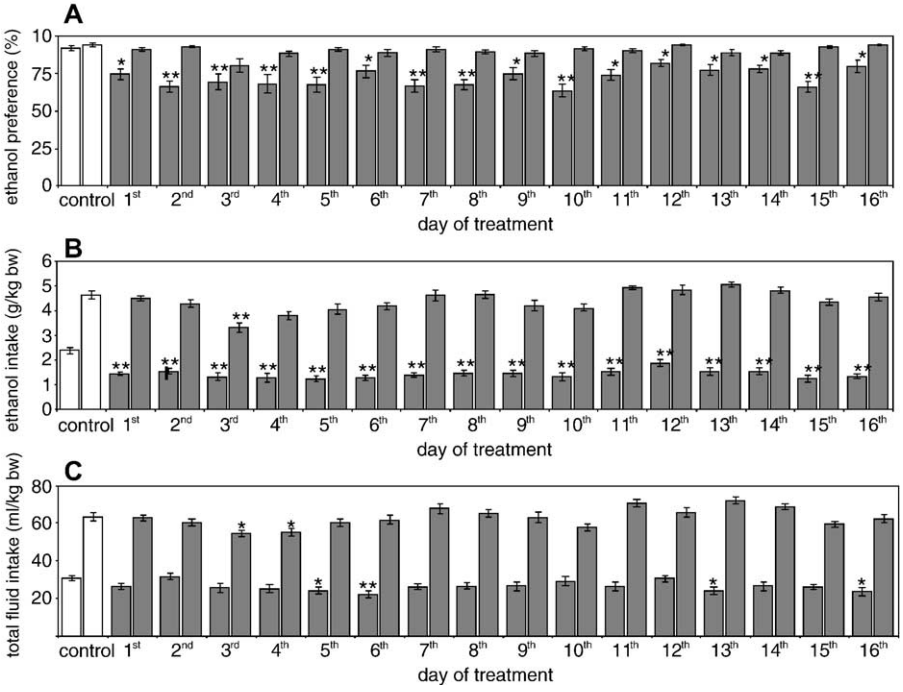

Fig. 2. Repeated treatment with desoxypeganine (20 mg/kg body weight/day) for 16 days, once daily. First columns: period from 6 to 10 pm, second ones: period between 10 pm and 6 am next morning. Each point represents the mean±S.E.M. $^{*}P<0.05$, $^{**}P<0.01$, significance vs. control group.

pronounced acquirement of tolerance. The ethanol intake was also reduced during repeated administration by the same amount as following acute treatment (Fig. 2).

Neither total fluid intake nor food consumption and body weight were affected by the repeated administration of desoxypeganine–HCl in the dose of 20 mg/kg body weight excluding any cumulative toxicity during this treatment period.

In the next figure the effects of different routes of application of desoxypeganine–HCl (10 mg/kg body weight) were compared (Fig. 3). In the first 4 h ethanol preference was reduced from 93.1±0.6% (control) to 71.3±2.9% following oral application (ANOVA: $F(3,58)=35.117$, $P<0.0001$; Fisher–PLSD: $P<0.0001$; significant according to Student–Newman–Keuls test). The effect was clearly more pronounced following parenteral application. After subcutaneous injection, the ethanol preference was reduced to 52.2±3.8%, and to 57.9±.7% after intraperitoneal injection. The difference to the effect of oral application is statistically significant (p.o. vs. s.c.: ANOVA: $F(3,58)=35.117$, $P<0.0001$; Fisher–PLSD test: $P<.0001$; significant according to Student–Newman–Keuls test; p.o. vs. i.p.: ANOVA: $F(3,58)=35.117$, $P<0.0001$; Fisher–PLSD test: $P=0.0025$; significant according to Student–Newman–Keuls test).

Galanthamine given orally reduced the ethanol preference significantly and pronounced as already described (Opitz, 1992). Dose of 2.5 mg/kg body weight did not change ethanol preference in our test animals (preference was 86.1±3.6% during the first four and 87.1±3.8% during the 5th to 12th h as compared to 87.5±1.3% and 93.6±0.7% for the control days). Doses of 5 or 10 mg/kg body weight reduced the ethanol preference dose-dependently and significantly during the first observation period (5 mg/kg body weight 71.2±6.3%; 10 mg/kg body weight 66.9±8.4%; data not shown). The ethanol preference was not affected during the second time interval by these doses of galanthamine–HBr. By the combination of 10 mg/kg body weight desoxypeganine–HCl and 2.5 mg/kg body weight galanthamine–HBr the activity was clearly increased. The reduction of ethanol preference during the first period was more pronounced (64.9%±6.1) and became significant compared not only to controls but also to the actions of the individual substances (DOP+GAL vs. DOP: ANOVA: $F(3,78)=7.018$, $P=0.0003$; Fisher–PLSD test: $P=0.038$; significant according to Student–Newman–Keuls test; DOP+GAL vs. GAL: ANOVA: $F(3,78)=7.018$, $P=0.0003$; Fisher–PLSD test: $P=0.0003$; significant according to Student–Newman–Keuls test) (Fig. 4).

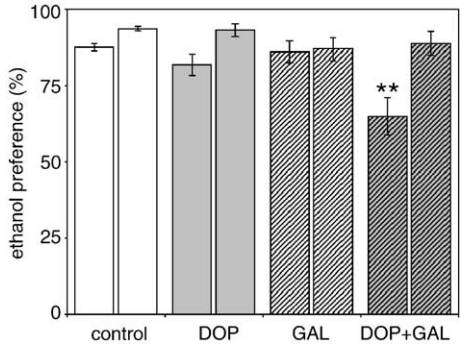

Fig. 4. Effect of desoxypeganine (DOP) 10 mg/kg body weight and galanthamine (GAL) 2.5 mg/kg body weight alone and in combination. First columns: period from 6 to 10 pm, second ones: period from 10 pm to 6 am next morning. Each point represents the mean±S.E.M. **$P<0.01$, significance vs. control group.

4. Discussion

Desoxypeganine–HCl at a dose range between 10 and 30 mg/kg body weight given orally reduced ethanol intake and, more important, ethanol preference dose-dependently whereas food consumption remained unaffected. The effect on ethanol preference remained unchanged during a treatment period of 16 days (desoxypeganine–HCl 20 mg/kg, once daily), excluding a development of tolerance. As food consumption was unaffected after repeated treatment also, toxic effects at this dose range can be excluded.

Stronger effects on ethanol intake and ethanol preference were observed after parenteral application as compared to the oral route due to a larger amount of absorption after i.p. and s.c. application or to a first pass-effect after oral administration.

A reduction of the voluntary alcohol uptake has been shown for isolated compounds as well as for plant extracts e.g. from *Hypericum perforatum* L., St. John's wort (Rezvani et al., 1999), for extracts of *Salvia miltiorrhiza* BGE. (Serra et al., 2003; Vacca et al., 2003), of *Pueraria lobata* WILLD. (Overstreet et al., 1996; Rezvani et al., 2003) and for the isoflavonoid compounds puerarin, daidzein or daidzin and for ibogaine from *Tabernanthe iboga* BAILL. (Rezvani et al., 1995). A short review of plants reducing alcohol intake is given by Carai et al. (2000).

Ethanol preference seems to be correlated with disturbances in different central systems. The serotoninergic system plays an important role in ethanol preference, but other central monoaminergic systems such as the dopaminergic, the noradrenergic and the opioid system, the acetylcholine system and the excitatory amino acids are involved as well. Correspondingly substances of quite different mode of action proved to reduce ethanol preference. For instance, such an activity has been shown for the α-2-adrenoceptor agonist clonidine (Opitz, 1990), the 5-HT_{1A} receptor agonist ipsaspirone (Schreiber et al., 1993), the $GABA_B$ receptor agonist baclofen (Colombo et al., 2004), the cannabinoid antagonist SR-141716 (Colombo et al., 1999), for calcium channel antagonists (Fadda et al., 1992), the opiod receptor antagonist naloxone (Altshuler et al., 1980; Myers and Critcher, 1982), and the dopamine agonist bromocriptine (Andronova et al., 1985).

This diversity in therapeutic options reflects the inhomogeneity of the target population. Allocation to special subgroups increases the probability of the benefit of a special medication and of a better outcome (Addolorato et al., 2005). Therefore new therapies may be reasonable and of advantage for a special subtype of patients.

The finding that desoxypeganine reduces ethanol preference without concomitant effects on total fluid intake and food consumption exhibits a promising couple of properties,

particularly as no marked acquirement of tolerance became obvious. The acute effects were reproduced independent of the order in which the dosages were tested. The duration of the effect was dose dependent, a significant reduction of ethanol preference was seen in the second period (4–12 h after application) only after application of 20 mg/kg desoxypeganine–HCl body weight or more.

Thus from the reduction of ethanol preference by desoxypeganine no hints on the mode of action were obtained, the mechanism of action is still unrevealed. In in vitro studies the substance proved to be an inhibitor of acetylcholinesterase and butyrylcholinesterase as well as a reversible inhibitor of monoamine oxidase A. If one of these activities is responsible for the effect on alcohol intake has still to be clarified. However galanthamine, an inhibitor of acetylcholinesterase, showed comparable dose-dependent effects on alcohol preference, a combination of both substances in subeffective doses was effective, suggesting an additive effect of desoxypeganine with that of galanthamine. This finding suggests a comparable mechanism of action of both drugs, but this remains to be clarified.

Acknowledgments

We appreciate the generous support of parts of this work by the ‘Stiftung Immunität und Umwelt’ (Stifterverband für die Deutsche Wissenschaft e.V., Frankfurt a. M., Germany).

References

Addolorato, G., Abenavoli, L., Leggio, L., Gasbarrini, G., 2005. How many cravings? Pharmacological aspects of craving treatment in alcohol addiction: a review. Neuropsychobiology 51, 59–66.

Altshuler, H.L., Phillips, P.E., Feinhandler, D.A., 1980. Alteration of ethanol self-administration by naltrexone. Life Sci. 26, 679–688.

Andronova, L.M., Stanishevskaia, A.V., Kudriavtsev, R.V., Barkov, N.K., 1985. Effect of bromocriptine in experimental alcoholism in rats of both sexes. Farmakol. Toksikol. 48, 96–101.

Anton, R.F., Swift, R.M., 2003. Current pharmacotherapies of alcoholism: a U. S. perspective. Am. J. Addict. 12 (Suppl. 1), S53–S68.

Carai, M.A., Agabio, R., Bombardelli, E., Bourov, I., Gessa, G.L., Lobina, C., Morazzoni, P., Pani, M., Reali, R., Vacca, G., Colombo, G., 2000. Potential use of medicinal plants in the treatment of alcoholism. Fitoterapia 71 (Suppl 1), S38–S42.

Clade, H., 2001. Alkoholsucht—Soziale Kosten. Dtsch. Arztebl., vol. 98, p. B 477.

Colombo, G., Agabio, R., Lobina, C., Reali, R., Morazzoni, P., Bombardelli, E., Gessa, G.L., 1999. *Salvia miltiorrhiza* extract inhibits alcohol absorption, preference, and discrimination in sP rats. Alcohol 18, 65–70.

Colombo, G., Serra, S., Vacca, G., Gessa, G.L., Carai, M.A., 2004. Suppression by baclofen of the stimulation of alcohol intake induced by morphine and WIN 55,212-2 in alcohol-preferring rats. Eur. J. Pharmacol. 492, 189–193.

Eriksson, K., 1968. Genetic selection for voluntary alcohol consumption in the albino rat. Science 159, 739–741.

Fadda, F., Garau, B., Colombo, G., Gessa, G.L., 1992. Isradipine and other calcium channel antagonists attenuate ethanol consumption in ethanol-preferring rats. Alcohol. Clin. Exp. Res. 16, 449–452.

Harvey, A.L., 1995. The pharmacology of galanthamine and its analogues. Pharmacol. Ther. 68, 113–128.

Iliev, A., Traykov, V., Prodanov, D., Mantchev, G., Yakimova, K., Krushkov, I., Boyadjieva, N., 1999. Effect of the acetylcholinesterase inhibitor galanthamine on learning and memory in prolonged alcohol intake rat model of acetylcholine deficit. Methods Find. Exp. Clin. Pharmacol. 21, 297–301.

Kenna, G.A., McGeary, J.E., Swift, R.M., 2004. Pharmacotherapy, pharmacogenomics, and the future of alcohol dependence treatment, Part 2. Am. J. Health-Syst. Pharm. 61, 2380–2388.

Lopez, S., Bastida, J., Viladomat, F., Codina, C., 2002. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and Narcissus extracts. Life Sci. 71, 2521–2529.

Mann, K., 2004. Pharmacotherapy of alcohol dependence: a review of the clinical data. CNS Drugs 18, 485–504.

Moormann, J.A., Opitz, K., Pena, M.A., Algorta, J., Maraschiello, C., Windisch, M., Mucke, H.A.M., 2005. Pharmacokinetics and metabolism in rats, dogs, and humans of desoxypeganin, a candidate compound for the therapy of alcohol abuse and smoking. Poster Presentation at the Annual Conference of the European Association of Addiction Therapy, Budapest.

Myers, R.D., Critcher, E.C., 1982. Naloxone alters alcohol drinking induced in the rat by tetrahydropapaveroline (THP) infused ICV. Pharmacol. Biochem. Behav. 16, 827–836.

Opitz, K., 1990. The effect of clonidine and related substances on voluntary ethanol consumption in rats. Drug Alcohol Depend. 25, 43–48.

Opitz, K., 1992. Verwendung von Galanthamin zur Behandlung des Alkoholismus, Patent DE 40 10 079.

Overstreet, D.H., Lee, Y.W., Rezvani, A.H., Pei, Y.H., Criswell, H.E., Janowsky, D.S., 1996. Suppression of alcohol intake after administration of the Chinese herbal medicine, NPI-028, and its derivatives. Alcohol. Clin. Exp. Res. 20, 221–227.

Rezvani, A.H., Overstreet, D.H., Lee, Y.W., 1995. Attenuation of alcohol intake by ibogaine in three strains of alcohol-preferring rats. Pharmacol. Biochem. Behav. 52, 615–620.

Rezvani, A.H., Overstreet, D.H., Yang, Y., Clark Jr., E., 1999. Attenuation of alcohol intake by extract of *Hypericum perforatum* (St. John's Wort) in two different strains of alcohol-preferring rats. Alcohol Alcohol. 34, 699–705.

Rezvani, A.H., Overstreet, D.H., Perfumi, M., Massi, M., 2003. Plant derivatives in the treatment of alcohol dependency. Pharmacol. Biochem. Behav. 75, 593–606.

Samochocki, M., Zerlin, M., Jostock, R., Groot Kormelink, P.J., Luyten, W.H., Albuquerque, E.X., Maelicke, A., 2000. Galantamine is an allosterically potentiating ligand of the human alpha4/beta2 nAChR. Acta Neurol. Scand., Suppl. 176, 68–73.

Schreiber, R., Opitz, K., Glaser, T., De Vry, J., 1993. Ipsapirone and 8-OH-DPAT reduce ethanol preference in rats: involvement of presynaptic 5-HT1A receptors. Psychopharmacology (Berl.) 112, 100–110.

Serra, S., Vacca, G., Tumatis, S., Carrucciu, A., Morazzoni, P., Bombardelli, E., Colombo, G., Gessa, G.L., Carai, M.A., 2003. Anti-relapse properties of IDN 5082, a standardized extract of *Salvia miltiorrhiza*, in alcohol-preferring rats. J. Ethnopharmacol. 88, 249–252.

Sinclair, J.D., Le, A.D., Kiianmaa, K., 1989. The AA and ANA rat lines, selected for differences in voluntary alcohol consumption. Experientia 45, 798–805.

Sinclair, J.D., Hyytia, P., Nurmi, M., 1992. The limited access paradigm: description of one method. Alcohol 9, 441–444.

Singer, M.V., Teyssen, S., 2002. Moderater alkoholkonsum: gesundheitsförderlich oder schädlich? Dtsch. Arztebl. 99, A 1103–A 1106.

Swift, R.M., 1999. Medications and alcohol craving. Alcohol Res. Health 23, 207–213.

Tuliaganov, N., Sadritdinov, F.S., Suleimanova, G.A., 1986. Pharmacological characteristics of desoxypeganine hydrochloride. Farmakol. Toksikol. 49, 37–40.

Vacca, G., Colombo, G., Brunetti, G., Melis, S., Molinari, D., Serra, S., Seghizzi, R., Morazzoni, P., Bombardelli, E., Gessa, G.L., Carai, M.A., 2003. Reducing effect of *Salvia miltiorrhiza* extracts on alcohol intake: influence of vehicle. Phytother. Res. 17, 537–541.