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

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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

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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