INTRODUCTION

Recent meta-analyses and overviews of randomized clinical trials consistently show that extract of Hypericum perforatum (HPE), the common plant usually called St John’s wort, displays a clear-cut antidepressant action (Ernst, 1995; Linde et al., 1996; Volz, 1997). The antidepressant-like action of HPE has been demonstrated also in animal models including the forced swimming test (FST) (Porsolt et al., 1977; Borsini and Meli, 1988; Willner, 1991; Butterweck et al., 1997, 1998; Ozturk, 1997).

Several reports indicate co-morbidity between depression and alcohol misuse (Deykin et al., 1987; Neighbors et al., 1992; Grant and Harford, 1995; Markou et al., 1998; Merikangas et al., 1998; Swendsen et al., 1998). Depression may be secondary to ethanol misuse and can be exacerbated by ethanol withdrawal (Baving and Olbrich, 1996). However, according to the ‘self-medication hypothesis’ of addictive disorders (Khantzian, 1985), ethanol may be misused to relieve a pre-existing depression (Deykin et al., 1987; Weiss et al., 1992). Co-morbidity, together with the observation that depression and alcoholism might imply similar changes in the regulation of some central neurotransmitters (Markou et al., 1998), raise interest in the potential therapeutic effects of antidepressant drugs and of HPE in alcoholism.

Genetically selected alcohol-preferring rats represent an interesting animal model for studies concerning mechanisms involved in ethanol intake control. A line of genetically selected alcohol-preferring rats has been bred in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy) from Sardinian alcohol-preferring (sP) rats (Agabio et al., 1996; Colombo, 1997; Lobina et al., 1997), the strain is referred to as Marchigian sP (msP) rats. In a recent study (Ciccocioppo et al., 1999a), it was observed that ethanol-naïve msP and sP rats display longer immobility in the FST in comparison to Sardinian alcohol-non-preferring rats. A positive association between high alcohol intake and a depression-like state has been suggested also in genetically selected alcohol-preferring AA rats (Kiiianmaa et al., 1991; Vigiinskaya et al.,...
1995) and in fawn-hooded rats (Overstreet et al., 1992). Moreover, Cicconioppo et al. (1999a) showed that voluntary ethanol drinking or intragastric (i.g.) ethanol administration to sP or msP rats markedly reduces their immobility score in the FST. These findings raise the question of whether the high ethanol preference and intake of sP and msP rats might somehow be related to the antidepressant-like action of ethanol, and whether antidepressant drugs in these rats are able to reduce ethanol consumption.

On the basis of these findings, the present study was aimed at evaluating the effect of HPE on ethanol intake in msP rats.

MATERIALS AND METHODS

Animals

Male genetically selected msP rats were employed. They were bred in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy) for 25 generations from 13th-generation sP rats, provided by the Bernard B. Brodie Department of Neurosciences of the University of Cagliari, Italy. At the time of the experiments, the rats’ body weights ranged between 400 and 450 g. They were kept in a room with a reverse 12 h:12 h light/dark cycle (lights off at 10:00), a temperature of 20–22°C and a relative humidity of 45–55%. Rats were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy), except where noted. All animal testing was carried out according to Italian ethical rules on animal care.

At the age of 2 months, msP rats were selected for their preference for 10% (v/v) ethanol solution, offering them a free choice between water and 10% ethanol 24 h a day for 15 days. Water and 10% ethanol were offered in graduated drinking tubes equipped with metallic drinking spouts. The rats employed in the following experiments had a 24-h ethanol intake of 6–7 g/kg with an ethanol preference [100 × ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h] higher than 90%.

Drugs

The HPE employed in the present study was a generous gift from Indena S.p.A., Milan, Italy. It was a dry extract (code no: 4149040, prepared in October 1997) containing 0.3% hypericin. It was dissolved in distilled water, just before administration.

Administration i.g.

Rats were anaesthetized by i.p. injection of 100–150 μl/100 g body wt of a solution containing ketamine (86.2 mg/ml) and acepromazine (1.3 mg/ml). A polyethylene catheter (PE-50, Clay Adams) was implanted permanently in the stomach, according to the method of Lukas and Moreton (1979). The PE tubing was run s.c. to reach the skin between the scapulae, where it was exteriorized. Rats were allowed a week to recover from surgery.

The i.g. PE catheter was adopted for extract administration, in order to avoid any possible disturbance to the animal at the time of the experiment. Before the experiments, rats were familiarized with the administration procedure.

Experimental procedure

Experiment 1: effect of acute i.g. administration of HPE on ethanol intake in msP rats, offered 10% ethanol for 2 h/day. A 10% ethanol solution was offered for 2 h/day at the beginning of the dark phase (10:00) of the reverse light/dark cycle, while water and food were freely available during the entire day. Rats were familiarized with this schedule of access to ethanol for 3 weeks following surgery. This period was sufficient to produce a stable 2-h ethanol intake; during the 3 days before the start of the experiments, the mean ± SEM 2-h ethanol intake of the msP rats employed ranged from 1.33 ± 0.1 to 1.50 ± 0.07 g/kg.

Fifteen rats were used for this experiment. According to a within-subject design, each rat received 125, 250 or 500 mg/kg HPE i.g. at intervals of 3–4 days. The i.g. administration of HPE was given 1 h before access to ethanol; a preliminary study revealed that a shorter time interval between i.g. administration and access to ethanol results in a smaller effect of HPE on ethanol intake, at least in the first 30 min of access.

Experiment 2: effect of acute i.g. administration of HPE on food intake in food-deprived msP rats. In Experiment 1, immediately after 10% ethanol presentation, msP rats were strongly motivated for ethanol, but showed low food intake and negligible water intake. To assess the selectivity of HPE on ingestive behaviour, the present experiment
evaluated the effect of HPE on food intake of msP rats following a period of food deprivation.

Rats were food-deprived for 22 h, from 12:00 to 10:00; they had water, but no ethanol was available, during the deprivation period. HPE or vehicle were given i.g. at 09:00, i.e. 1 h before access to food. Food intake was determined by weighing the pellets remaining in the food cup and taking into account spillage; food-associated drinking was determined by reading the volume of water in the graduated burette. Both food and food-associated drinking were measured at 30, 60, 90, and 120 min after access to food. The 10% ethanol solution was offered at the end of the experiment.

Nine rats were employed for this experiment. According to a within-subject design, each rat received both vehicle and HPE, 125 or 250 mg/kg, at intervals of 7 days. The dose of 500 mg/kg was not included in the experiment, since, in Experiment 1, it proved to evoke immobility and in preliminary tests it produced a general inhibition of ingestive behaviour.

**Experiment 3: time course of ethanol intake following acute i.g. administration of HPE in msP rats, offered 10% ethanol for 12 h/day.** In Experiment 1, rats treated with HPE showed a lower ethanol intake, in comparison to controls, during the entire 2-h period of observation. In the present experiment, 10% ethanol solution was offered for 12 h/day at the beginning of the dark phase (10:00) to evaluate the time course of ethanol intake following HPE administration over a longer period. Food and water were freely available during the entire day. Rats were made familiar with this schedule of access to ethanol for 3 weeks after i.g. surgery.

Two groups of msP rats were employed: one group received a single i.g. vehicle dose, whereas the other group received 250 mg/kg HPE i.g. The i.g. administration took place 1 h before access to ethanol.

**Experiment 4: effect of i.g. administration of HPE on the immobility time of msP rats in the FST.** The swimming sessions were conducted by placing the rat in individual glass cylinders 30 cm in diameter, containing water at a temperature of 23–25°C. The water was 30-cm deep rather than 18-cm deep, as reported in the original method of Porsolt et al. (1977). This change was adopted according to a recent study by Detke and Lucki (1995), showing that a deeper water level eliminates the false negative of selective serotonin reuptake inhibitors. At this water depth, rats could touch the bottom of the jar with their tail, but they could not support themselves with their hindlimbs. The first 15-min swimming session (pretest) was conducted between 10:00 and 12:00; 24 h later, rats were again placed in water for the 5-min test. Following each swimming session, rats were removed from the cylinder, dried with paper towels, placed in a heated chamber for 20 min and then returned to their home cages. Test sessions were video-taped (Canon VC-20 colour videocamera) and analysed by means of a Panasonic (NV-HD650EG) video-cassette recorder. The time spent immobile was measured by an experienced observer who was blind to the treatment conditions.

The rats employed in this experiment did not have access to ethanol for 2 weeks before the FST. HPE was given i.g. during the period between the two swimming sessions. In the first part of the experiment, it was given in a single i.g. dose of 250 mg/kg, 1 h before the FST. In the second part of the experiment, the HPE was administered at doses of 125 or 250 mg/kg, given three times (24, 12, and 1 h) before the test. A multiple dosing regime was employed, since a single drug administration is usually not sufficient to reveal the anti-immobility effect of antidepressant drugs.

**Experiment 5: effect of i.g. administration of HPE on the locomotor activity of msP rats.** The open field, used to measure locomotor activity, consisted of a wooden chamber (45 cm high) with a circular base (75 cm diameter). The floor was divided into 12 sections of similar area by two concentric circles and radial segments. The apparatus was placed in a sound-proof room, illuminated by a white 80-W lamp placed 200 cm over the centre of the arena.

Two groups of seven and eight msP rats were used; 1 day before the test, rats were confined in the open arena for 20 min to allow them to habituate to the testing apparatus. Afterwards, animals received three i.g. administrations of HPE, 250 mg/kg, or vehicle, 24, 12, and 1 h before the test. Rats were placed in the open field for 10 min; the rat’s behaviour in the test session was video-taped, analysed, and scored. The following parameters were measured: number of line crossings (number of sections entered with the four limbs); time spent in locomotor activity; and number of rearing reactions.
Experiment 6: effect of acute i.g. injection of HPE on blood-alcohol levels (BAL) following i.g. ethanol administration in msP rats. Twelve msP rats were employed; food was removed from their cages at 07:00, i.e. 2 h before the beginning of the experiment. At 09:00, six rats received an i.g. dose of 250 mg/kg HPE, while the other six received an i.g. intubation of vehicle. One hour later, all the rats employed received i.g. administration of a 0.7 g/kg dose of ethanol, as a 10% solution; this is the amount voluntarily ingested by msP rats shortly (2–5 min) after access to 10% ethanol, when this solution is offered for 2 h/day (Ciccocioppo et al., 1999b). Blood samples (50–100 µl) were taken from the tail vein 15, 30, 60, and 120 min after i.g. ethanol administration. BAL were measured by gas chromatography according to the method of Cingolani et al. (1991).

Validation of the i.g. cannula

After completion of the experiments, rats were killed with an overdose of anaesthetic and the placement of the i.g. cannula was evaluated.

Statistical analysis

Data from the first two experiments were analysed by ANOVA, with repeated measures. Data from Experiments 3 and 6 were analysed by split-plot multifactorial analysis of variance, with between-group comparisons for drug treatment and within-group comparisons for time after treatment. Post-hoc comparisons were made by means of Dunnett’s test. The results of Experiment 4 were analysed by one-way ANOVA, followed by the Newman–Keuls test. The results of Experiment 5 were analysed by Student’s t-test. Statistical significance was set at \( P < 0.05 \).

RESULTS

Experiment 1: effect of acute i.g. administration of HPE on ethanol intake in msP rats, offered 10% ethanol for 2 h/day

As shown in Fig. 1, the i.g. administration of HPE dose-dependently reduced ethanol intake in msP rats. The analysis of the cumulative ethanol intake revealed significant treatment effect \( F(3,42) = 29.9; P < 0.001 \), time effect \( F(3,42) = 83.5; P < 0.001 \), and treatment–time interaction \( F(9,126) = 3.13; P < 0.001 \). Following each of the three doses tested, a pronounced and statistically significant reduction of cumulative ethanol intake was observed during the first 30 min and the cumulative intake of treated rats was significantly lower than that of controls for up to 2 h.

Analysis of ethanol intake data at each time interval revealed significant treatment effects during the first 30 min \( F(3,56) = 11.2; P < 0.001 \), whereas in the subsequent periods of observation (30 to 60, 60 to 90, and 90 to 120), ethanol intake of treated rats was not significantly different from that of controls.

In response to 500 mg/kg HPE, but not at the lower doses tested, rats appeared somewhat sedated and immobile during the first 2 h after administration.

Experiment 2: effect of acute i.g. administration of HPE on food intake in food-deprived msP rats

The i.g. dose of 250 mg/kg HPE produced only a modest reduction in food intake (Fig. 2A) and in food-associated drinking (Fig. 2B) in food-deprived rats. ANOVA revealed no statistically significant effect either on food intake \( F(1,8) = 1.7; P > 0.05 \) or on food-associated drinking \( F(1,8) = 3.9; P > 0.05 \).
Experiment 3: time course of ethanol intake following acute i.g. administration of HPE in msP rats, offered 10% ethanol for 12 h/day

As shown in Fig. 3A, the i.g. administration of 250 mg/kg HPE markedly reduced alcohol intake. ANOVA revealed significant treatment [$F(1,14) = 9.3; P < 0.01$] and time [$F(5,70) = 155.9; P < 0.01$] effects, but non-significant treatment–time interactions. Pairwise comparisons revealed that the cumulative ethanol intake of treated rats was significantly lower than that of controls up to 10 h after access to ethanol, but not at 12 h.

However, ANOVA revealed neither a significant effect of HPE, 250 mg/kg, on food intake during the 12 h of access to ethanol [$F(1,14) = 2.3; P > 0.05$], nor a significant treatment–time interaction [$F(5,70) = 1.7; P > 0.05$] (Fig. 3B).

Experiment 4: effect of i.g. administration of HPE on the immobility time of msP rats in the FST

Following acute i.g. administration of 250 mg/kg HPE, given 1 h before the FST, the immobility time of treated rats was not significantly different from that of controls (Fig. 4A).

Following three administrations of HPE 250 mg/kg the immobility time in the FST was significantly reduced in comparison to controls; three administrations of 125 mg/kg did not evoke a statistically significant effect (Fig. 4B).

Experiment 5: effect of i.g. administration of HPE on the locomotor activity of msP rats

Statistical analysis revealed no significant difference between controls and treated rats as far as number of line crossing (mean ± SEM) ($35.8 ± 3.8$...
HYPERICUM AND ALCOHOL INTAKE

Experiment 6: effect of acute i.g. injection of HPE on BAL levels following i.g. ethanol administration in msP rats

The mean BAL in controls and in HPE-treated rats, following i.g. administration of a 0.7 g/kg dose of ethanol, are reported in Table 1. ANOVA revealed neither a statistically significant effect of HPE treatment \([F(1,10) = 1.1; P < 0.05]\), nor a significant treatment–time interaction \([F(3,30) = 0.7; P > 0.05]\), but a significant time effect \([F(3,30) = 25.4; P < 0.001]\).

**DISCUSSION**

The results from the present study show that i.g. administration of HPE markedly reduces ethanol intake in msP rats. The effect is reproducible and of remarkable intensity. Moreover, following a single i.g. administration of 250 mg/kg HPE, the cumulative ethanol intake of treated rats was significantly lower than that of controls for up to 10 h. In addition, the present results show that the effect of HPE is highly selective from a behavioural point of view. The dose of 250 mg/kg HPE, that markedly reduced ethanol intake, did not significantly modify either food intake or food-associated drinking. The same dose did not modify the rats’ gross behaviour in the open field test.

The finding that HPE does not significantly affect food intake, at doses that reduce ethanol intake, may represent an interesting advantage in comparison to selective serotonin reuptake inhibitors. The latter reduce ethanol intake in both humans and rats, but their effect is usually associated with a pronounced inhibitory effect on food intake (Ciccocioppo et al., 1997), which is an undesirable effect in the treatment of alcoholic patients.

Another interesting finding of the present study is that the effect on ethanol intake is not strictly related to the antidepressant-like effect of HPE in msP rats. As a matter of fact, the two effects can be dissociated: the first can be observed after a single administration of 125 mg/kg, while the latter requires repeated HPE treatment at doses higher than 125 mg/kg.

As far as the mechanism of action is concerned, the only information provided by the present study is that the effect of HPE on ethanol intake is not related to changes in the pharmacokinetics of ethanol.

The present study employed whole HPE, which is known to contain at least seven ‘classes’ of compounds (phenylpropanes, flavonol glycosides, biflavones, proanthocyanidins, xanthones, naphthodianthrone, phloroglucinols) (Nahrstedt and Butterweck, 1997). The fractions containing flavonoids and the naphthodianthrone, hypericin, were found to be the most active on immobility
time in the FST by Nahrstedt and Butterweck (1997); moreover, recent studies by Chatterjee et al. (1998a,b) suggested that the phloroglucinol derivative hyperforin may represent an interesting antidepressant component of HPE. However, at present we do not know which compounds are responsible for the effect of HPE on ethanol intake.

A variety of neurochemical and biochemical effects have been reported for HPE in the literature. It can reduce serotonin reuptake (Perovic and Muller, 1995; Muller et al., 1997); the flavonoids of the HPE can cause monoamine oxidase inhibition (Cott, 1997; Nahrstedt and Butterweck, 1997). It is also noteworthy that components of HPE show remarkable affinity for 5-HT$_{1A}$ receptors (Cott, 1997). All these effects can influence serotonin neurotransmission. HPE has been shown to reduce not only serotonin reuptake, but also noradrenaline, dopamine and L-glutamate reuptake (Muller et al., 1997; Chatterjee et al., 1998a,b). Butterweck et al. (1997) showed that the effects of HPE in the FST may be mediated, at least in part, by activation of dopaminergic and opioid mechanisms. Finally, Cott (1997) reported affinity of crude HPE for GABA$_{A}$, GABA$_{B}$, adenosine, and benzodiazepine receptors; the same author also reported that hypericin shows affinity for the N-methyl-D-aspartate receptor in the micromolar range.

Further studies are under way to establish whether the effects of HPE on ethanol intake are due to a particular active principle with a selective mechanism of action. The identification of the active principle might allow the development of new pharmacological and pharmaceutical research; it might also allow the administration of an active principle without the simultaneous administration of other compounds of HPE that may be responsible for adverse side-effects, such as photosensitivity (Brockmoller et al., 1997).

However, it could also be hypothesized that the effects of HPE on ethanol intake may be the result of the effect of a cocktail of compounds present in the extract and that the overall effects of HPE may be due to interaction with many (if not all) of the neurochemical systems mentioned above. Several studies have documented that pharmacological manipulation of the central serotoninergic, dopaminergic, opioid, GABAergic, and glutamatergic mechanisms can influence ethanol consumption (for review, see Weiss and Koob, 1991).

Acknowledgements — The work was supported by grants from the University of Camerino (Fondi 60%). The authors wish to thank Indena S.p.A., Milan, Italy, for a generous gift of Hypericum perforatum extract and Professor R. Froldi and Mr M. Cippitelli, Institute of Legal Medicine of the University of Macerata, Italy, for determination of blood-alcohol levels.

REFERENCES


