EFFECT OF HYPERICUM PERFORATUM CO₂ EXTRACT ON THE MOTIVATIONAL PROPERTIES OF ETHANOL IN ALCOHOL-PREFERRING RATS

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Abstract — Aims: Extracts of Hypericum perforatum (HPE) attenuate voluntary ethanol intake in different lines of alcohol-prefering rats. The present study evaluated the effect of the intragastric (IG) administration of a CO₂ Hypericum perforatum extract (HPCO₂) on operant ethanol self-administration, as well as on voluntary ethanol intake, after a period of ethanol deprivation in genetically selected Marchigian Sardinian alcohol-prefering rats. Methods: HPCO₂ was administered by means of an indwelling IG catheter, 1 h before the tests. For the self-administration experiments, the rats were trained to self-administer 10% (v/v) ethanol in 30-min daily sessions under a fixed ratio 1 schedule of reinforcement. HPCO₂ was also tested on 0.2% w/v saccharin self-administration. For the ethanol deprivation experiments, rats that had a previous experience with voluntary ethanol drinking were deprived of ethanol for 9 days, whereas water and food were freely available; HPCO₂ was given by IG injection 1 h before the ethanol re-presentation. Results: HPCO₂, in doses of 31 or 125 mg/kg but not 7 mg/kg, significantly reduced ethanol self-administration, while it did not modify saccharin self-administration. The same doses of the extract abolished the increased ethanol intake following ethanol deprivation. Conclusions: These findings provide evidence that HPCO₂ markedly reduces the reinforcing properties of ethanol in the self-administration paradigm, as well as the increase of ethanol intake following ethanol deprivation. These findings further support the view that the use of HPE may represent an interesting pharmacological approach in the treatment of alcohol abuse and alcoholism.

INTRODUCTION

Extracts of Hypericum perforatum (HPE), the common plant usually referred to as St John’s wort, are known to exert antidepressant effects in humans (Volz, 1997; Laakmann et al., 1998; Linde and Mulrow, 2000; Vormfelde and Poser, 2000; Barnes et al., 2001; Kasper, 2001; Van Grup, 2002; Muller, 2003; Rodriguez-Landa and Contreras, 2003) and an antidepressant-like action in laboratory animals, in different experimental models (Butterweck et al., 1997; Bennett et al., 1998; Gambbarana et al., 1999, 2001; Nathan, 1999; Perfumi et al., 1999; Greeson et al., 2001). Recent reports have also described the anxiolytic, analgesic and memory enhancing properties of HPE in rats (Kumar et al., 2000, 2001; Vandenbogaerde et al., 2000; Khalifa, 2001; Klusa et al., 2001; Flausino et al., 2002; Skalisz et al., 2004). Catania et al. (2003) have also demonstrated that HPE attenuates nicotine withdrawal signs in mice.

In the last few years, several studies have shown that HPE reduces voluntary ethanol intake in the home-cage two-bottle choice paradigm in alcohol-prefering rats (De Vry et al., 1999; Perfumi et al., 1999, 2001, 2002, 2003; Rezvani et al., 1999; Panocka et al., 2000) and C57BL/6J mice (Wright et al., 2003). The mechanisms accounting for the inhibition of ethanol intake in alcohol-prefering rats and mice by HPE remain unknown. Hyperforin has been suggested to be the primary bioactive ingredient responsible for the effect of HPE on ethanol intake (Perfumi et al., 2001; Wright et al., 2003), as well as for its antidepressant-like action (Bhattacharya et al., 1998; Chatterjee et al., 1998a,b; Laakmann et al., 1998; Muller et al., 1998, 2001; Singer et al., 1999; Vormfelde and Poser, 2000; Nathan, 2001; Cervo et al., 2002; Keller et al., 2003).

In the present work, hyperforin enriched plant extract, namely a CO₂ Hypericum perforatum extract (HPCO₂), was used to further investigate the mechanisms accounting for the control of ethanol intake by HPE, under operant conditions. For this purpose, using Marchigian Sardinian alcohol-prefering (msP) rats, the HPCO₂ extract was tested on the oral self-administration of 10% ethanol under a fixed ratio 1 (FR1) schedule of reinforcement. It is well known that the data obtained under operant paradigms provide a closer measure on the reinforcing value of the self-administered drug, thus offering important advantages over the home-cage two-bottle choice paradigm (Samson et al., 1988, 1990, 1998; Weiss et al., 1993; Vacca et al., 2002; Samson and Czachowski, 2003). Moreover, in order to evaluate the selectivity of the effect of HPCO₂, its ability to affect 0.2% saccharin self-administration was studied.

Finally, to extend our study, we investigated the effect of HPCO₂ on the alcohol deprivation effect (ADE) in the msP rats under the home-cage two-bottle choice condition. ADE is a temporary increase in the voluntary ethanol intake found in different animal species after a period of alcohol abstinence and has been proposed as an experimental model of alcohol relapses in human alcoholics (Spanagel, 2000; Boening et al., 2001; McBride et al., 2002).

MATERIALS AND METHODS

Animals

Male genetically selected alcohol-prefering rats, weight between 250–300 g (at the beginning of the experiment), were used. Animals were bred at the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy) for 41 generations, starting from the
Sardinian alcohol-preferring (sP) rats of the 13th generation provided by the Department of Neurosciences of the University of Cagliari (Gessa et al., 1991). They are referred to as Marchigian Sardinian (msP) rats. The animals were kept in a room with a reverse 12 h:12 h light/dark cycle (lights off at 10:00 a.m.), at a temperature of 20–22°C and a humidity of 45–55%. The rats were allowed free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy).

At the age of 2 months, the 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 provided in graduated drinking tubes equipped with metallic drinking spouts. The rats selected for the following experiments had a 24-h ethanol intake of ~6 g/kg with an ethanol preference [ml of ethanol solution/ml of total fluids (water + 10% ethanol) ingested in 24 h × 100] >80%. All the msP rats (n = 57) that were subjected to this screening procedure reached the selection criteria and were therefore used in the experiments. Different groups of msP rats were used in each experimental procedure.

Animal testing was carried out according to the European Communities Council Directive of 24 November 1986 (86/609/EEC).

**Drugs**

HPCO₂, containing 24.33% hyperforin and 0.08% hypericin, was provided by Indena, Milan, Italy. It was emulsified in 0.1% Tween 80 and tap water, just before administration, and given by intragastric (IG) injection at doses of 7, 31 and 125 mg/kg.

**IG administration**

The rats were implanted with an indwelling IG catheter before the experiments. IG administration by means of an indwelling catheter was adopted to avoid any disturbance to the animal during or just before the experiments.

The rats were anaesthetized by an intramuscular injection of Zoletil 100 (Virbac, Milan, Italy), which contains Tiletamine and Zolazepam. A polyethylene (PE) catheter (PE-50, Clay Adams) was permanently implanted into the stomach, according to the method of Lukas and Moreton (1979). The PE tubing ran subcutaneously to reach the skin between the scapulae, where it was exteriorized. The rats were then allowed a week to recover from the IG surgery. Before the experiments, they were familiarized with the administration procedure.

**The self-administration apparatus**

The self-administration stations consisted of operant conditioning chambers (Med Associate, Inc.) enclosed in sound-attenuating, ventilated environmental cubicles. Each chamber was equipped with a drinking reservoir (volume capacity: 0.2 ml), positioned 4 cm above the grid floor in the centre of the front panel of the chamber, and two retractable levers located 3 cm, one to the right and the other to the left of the drinking receptacle. An infusion pump was activated by responses on the right or ‘active’, lever, while responses on the left, or ‘inactive’ lever were recorded but did not result in activation of the pump. Activation of the pump resulted in the delivery of 0.1 ml of fluid (either ethanol, saccharin or water). During the infusion of ethanol (10% v/v) or saccharin (0.2% w/v), a stimulus light (‘house light’) located on the front panel was turned on for 1.0 s (that corresponds to the duration of the syringe pump activation). Lever presses during this period of time were counted but did not lead to further infusions. A PC station controlled the delivery of fluids, presentation of visual stimuli and recording of the behavioural data.

**Alcohol self-administration training procedures**

The animals were trained to self-administer 10% (v/v) ethanol in 30-min daily sessions under an FR1 schedule of reinforcement, wherein each response resulted in the delivery of 0.1 ml of fluid (Weiss et al., 1993). To facilitate the acquisition of level pressing during the first 3 days of training, the rats were placed on a restriction schedule, limiting the water availability to 2 h/day. During this time, responses at the lever were reinforced by the delivery of a 0.2% (w/v) saccharin solution into the drinking receptacle under an FR1 schedule, throughout the daily 30-min sessions. During all subsequent training and testing, water was freely available in the home cages. After the successful acquisition of saccharin-reinforced responding, the rats were trained to self-administer 10% ethanol. From the first day, the rats began to press for 10% ethanol and a stimulus light located on the front panel (‘house light’) was turned on for 1.0 s.

**Saccharin self-administration training procedures**

For saccharin self-administration, the training procedure was identical to that described for alcohol self-administration, except that lever pressing during the first 3 days of water deprivation was reinforced by water delivery, and after the successful acquisition of operant responding, the animals immediately received 0.2% (w/v) saccharin. From the first day, the rats began to press for saccharin and a stimulus light located on the front panel (‘house light’) was turned on for 1.0 s.

**Effect of HPCO₂ on ethanol self-administration**

During the last 4 days of self-administration training (pre-treatment), the animals were given an IG administration of the drug vehicle in order to familiarize the animals with the injection procedure. After the acquisition of a stable 10% ethanol self-administration baseline (9 days), eight msP rats received HPCO₂ (7, 31 or 125 mg/kg/10 ml) or vehicle in four different experimental sessions (at intervals of 3 days), according to a within-subject design. The IG injection took place 1 h before the 10% ethanol self-administration.

The number of responses at both active and inactive levers was recorded for the entire period of the experiment. Between the drug testing session, ethanol self-administration baseline was always re-established.

**Effect of HPCO₂ on saccharin self-administration**

To control for the behavioural selectivity of the effect of HPCO₂ on ethanol-related responding, this drug was tested on the self-administration of a different reward. For this purpose saccharin was adopted because the sweet taste represents, for msP rats, a reinforcer comparable to ethanol.

Thus, the msP rats (n = 8) were trained to respond for 0.2% saccharin until a stable self-administration baseline (8 days) was reached. As in the previous experiment during the last 4 days of training (pre-treatment), before the self-administration sessions, the animals were given an IG administration of the
vehicle to familiarize them with the injection procedure. Subsequently, the rats received HPCO₂ (7, 31 or 125 mg/kg/10 ml) or vehicle in four different experimental sessions (at intervals of 3 days), according to a within-subject design. The IG injection took place 1 h before the saccharin self-administration. Between the drug-testing session, saccharin self-administration baseline was always re-established. The number of responses at both the active and inactive lever was recorded for the entire period of the experiment.

**Effect of HPCO₂ on voluntary ethanol intake after a period of ethanol deprivation**

For 35 consecutive days (pre-deprivation period), the rats were allowed free home-cage access to 10% ethanol and tap water. Ethanol and water intakes were recorded daily at 10:00 a.m., just before the beginning of the dark phase of the inverse light/dark cycle. The position (to the right or left) of the ethanol and the water drinking tubes was changed daily to avoid the development of preference for a particular side (right or left).

After 35 days, the rats were divided into two groups of similar ethanol intake. A group of 33 animals was deprived of ethanol, but not of water, for nine consecutive days. The other group of eight msP rats had free access to both water and ethanol. After 9 days of ethanol deprivation, the 33 deprived rats were divided into four subgroups that received HPCO₂ (7, 31 or 125 mg/kg/10 ml) or vehicle, according to a between-subject design. The group of non-deprived msP rats received only the vehicle under the same experimental condition.

The IG administration took place at 9:00 a.m., that is, 1 h before the beginning of the dark phase. Ethanol, water, and food intakes were measured 1, 2 and 24 h after ethanol representation.

**Statistical analysis**

Data are reported as mean ± SEM. To evaluate the effect of HPCO₂ on ethanol and saccharin self-administration, the results were analysed by a one-way analysis of variance (ANOVA) with repeated measures (Latin Square design). To evaluate the effect of HPCO₂ on ethanol intake after the deprivation, a split-plot ANOVA, with between-group comparisons for drug treatment and within-group comparisons for time was used. Post hoc comparisons were made by means of the Dunnett’s test. Statistical significance was set at $P < 0.05$.

**RESULTS**

**Effect of HPCO₂ on ethanol self-administration**

During the training period, the rats reached a baseline number of lever presses for ethanol between 40 and 75 in the 30-min sessions. As shown in Fig. 1A, HPCO₂ reduced ethanol self-administration in a dose-dependent manner. The ANOVA revealed a statistically significant treatment effect $F(3,7) = 58.843; P < 0.01$. The dose of 7 mg/kg of HPCO₂ did not significantly modify ethanol self-administration, while the doses of 31 and 125 mg/kg reduced the number of lever presses for ethanol by 37.2 ($P < 0.01$) and 81.8% ($P < 0.01$), respectively. The responses at the inactive lever were almost absent during the training and treatment period, and were not influenced by treatment with HPCO₂ $F(3,7) = 0.368; NS]$. On the first day after the end of treatment, lever pressing for ethanol returned to baseline levels (data not shown).

A closer analysis of the pattern of lever pressing showed that, in the control group, ethanol reinforced responses mostly occurred (~80%) during the first 10-min session bin. During the remaining 20 min, only a few scattered responses were detected. Latency of response to the lever was very short and in all vehicle-treated animals, with the first lever pressing occurring within 10 s from the beginning of the session. In the animals treated with HPCO₂, a delay was observed in the first lever pressing, which, in the rats administered with the highest dose of the drug, occurred within a few minutes from the beginning of the session. In addition, in the animals treated with 31 and 125 mg/kg of HPCO₂, lever pressing was not clustered during the first 10-min bin but, rather, it was homogeneously scattered throughout the entire 30-min session.

Fig. 1. Effect of IG HPCO₂ (7, 31 and 125 mg/kg) or its vehicle (VEH) on: (A) 10% alcohol self-administration in msP rats ($n = 8$); (B) 0.2% saccharin self-administration in msP rats ($n = 8$). Values represent the mean ± SEM of the number of lever presses for ethanol. Difference from vehicles: *$P < 0.01$; where not indicated, the difference was not statistically significant.
Effect of HPCO₂ on saccharin self-administration

As shown in Fig. 1B, IG administration of HPCO₂ (7, 31 or 125 mg/kg) did not significantly modify the number of responses for saccharin self-administration [F(3,7) = 0.572; NS]. Responses at the active lever were almost absent during the whole experiment and were never influenced by HPCO₂ treatment [F(3,7) = 0.572; NS].

Effect of HPCO₂ on voluntary ethanol intake after a period of ethanol deprivation

As shown in Fig. 2A, ethanol deprivation for 9 days induced a statistically significant increase in rats with voluntary ethanol intakes at 1, 2 or 24 h after ethanol representation compared with rats that were not ethanol deprived [F(1,14) = 10.909; P < 0.01]. Moreover, the ANOVA also revealed a statistically significant increase when the post-deprivation intake was compared with that of the same animals in the pre-deprivation period [F(1,14) = 10.648; P < 0.01]. On the other hand, no significant differences in food intake were observed between the pre- and post-deprivation period [F(1,14) = 0.681; NS] (data not shown).

As shown in Fig. 2B, IG treatment with HPCO₂ significantly reduced the increased ethanol intake following a period of ethanol deprivation [F(3,29) = 13.628; P < 0.01]. Post hoc comparisons revealed that the administration of 31 and 125 mg/kg of HPCO₂ significantly reduced the increased ethanol intake induced by deprivation at 1, 2 and 24 h after access to ethanol, while 7 mg/kg of HPCO₂ did not show any significant modification of ethanol intake. The reduction of ethanol drinking elicited by HPCO₂ was no longer evident after 48 h. Nevertheless, no rebound of ethanol drinking was observed in the animals. The concomitant water intake was not modified by HPCO₂ treatment [F(3,29) = 2.87; NS]. The treated rats ingested more water than controls, but this difference never reached statistical significance (data not shown).

Moreover, 24-h food intake of ethanol-deprived rats was not significantly affected by HPCO₂ treatment [F(3,29) = 1.703; NS] (data not shown).

DISCUSSION

Several studies have shown that HPE markedly reduces voluntary ethanol intake in several lines of alcohol-preferring rats (for a review see Rezvani et al., 2003) and alcohol-preferring C57BL/6J mice (Wright et al., 2003). The effect is behaviourally selective, since doses that reduce ethanol intake do not modify the simultaneous intake of food, water or saccharin solution (Perfumi et al., 2001, 2003). Until now, the effect of the extracts has been investigated only under conditions of voluntary access to home-cage ethanol, which does not allow one to fully evaluate the motivation of the animal for ethanol. On the other hand, the operant self-administration represents a paradigm in which the animal is required to express a deeply motivated behaviour to obtain ethanol reinforcement (Samson and Czachowski, 2003).

Another interesting paradigm to evaluate the motivation for ethanol is the voluntary ethanol intake following a period of ethanol deprivation; animals show a pronounced increase in ethanol intake immediately after ethanol representation, which is considered to be an expression of craving for ethanol (Spanagel and Holter, 2000; McBride et al., 2002). Thus, the present study evaluated whether the doses of HPCO₂ that reduce voluntary ethanol intake are also able to reduce ethanol self-administration in operant cages, as well as the increased ethanol intake following a period of ethanol deprivation.

The results clearly showed that, under operant conditions, HPCO₂ reduces ethanol intake over the same range of doses that in previous studies have been shown to reduce home-cage ethanol drinking. The effect observed is behaviourally selective because, under identical experimental conditions,
perforatum that HPE do not bind directly to opioid receptors nor do their reduction of the motivational properties of alcohol by HPE synergistically to induce a pronounced and selective reduction of voluntary ethanol consumption in msP rats (Perfumi et al., 2001). Pharmacological relapse prevention in alcohol dependence: from animal models to clinical trials. Alcoholism: Clinical and Experimental Research 25, 1275–131S.


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It has been widely demonstrated that the opioid receptor antagonists attenuate alcohol drinking by lowering its rewarding and reinforcing properties (Hubbell and Reid, 1990; Gianoulakis, 1993; Hytti, 1993; Di Chiara et al., 1996; Honkanen et al., 1996; Herz, 1997, 1998; Koob et al., 1998; Krishnan-Sarin et al., 1998). Therefore, we speculate that the reduction of the motivational properties of alcohol by HPE and opioid receptor antagonists represents a converging mechanism that can explain the synergism of action of the two classes of compounds. However, it should also be mentioned that HPE do not bind directly to opioid receptors nor do they modulate opioid activity via other mechanisms. Future studies are warranted to investigate the mechanism by which H. perforatum reduces the motivational properties of alcohol.

In conclusion, the results of the present experiments provide evidence that HPCO₂ markedly and selectively reduces the motivation for ethanol as revealed in the self-administration paradigm, as well as in the increased craving for ethanol observed after a period of ethanol deprivation. These results, together with those obtained in voluntary drinking rats, strengthen the idea that the use of HPE may represent an interesting pharmacological approach to treat excessive alcohol drinking and prevent alcohol relapse in human alcoholics.

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HYPERICUM PERFORATUM AND MOTIVATION FOR ALCOHOL

saccharin self-administration was not modified. Consistent responding at the inactive lever was not affected by HPCO₂ at the doses used in the present study. Overall, these results are consistent with the previous reports showing the selectivity of the effect of HPE on the home-cage two-bottle choice ethanol drinking paradigm (Perfumi et al., 2003).

Results of the ADE experiment showed that the msP rats display a pronounced increase in ethanol drinking following a period of deprivation. This finding is consistent with the results obtained in other lines of alcohol-prefering animals (Sinclair and Li, 1989; McKinzie et al., 1998; Agabio et al., 2000; Rodd-Henricks et al., 2000; Serra et al., 2002, 2003; Colombo et al., 2003; Vengeliene et al., 2003) and confirms that the msP rats represent a suitable animal model to study alcohol craving. HPCO₂ also evoked a pronounced and long lasting effect on the increased motivation for ethanol observed after a period of ethanol deprivation. The doses of 31 and 125 mg/kg of HPCO₂ were able to completely abolish the alcohol deprivation effect. Again, the effect was behaviourally selective since food and water intake were not affected.

Overall, the present results, obtained under high motivational conditions (i.e. operant self-administration and alcohol deprivation), demonstrate that HPCO₂ markedly reduces the reinforcing properties of ethanol. A previous study by our group has shown that HPCO₂ and low doses of the opiate receptor antagonists, naloxone and naltrexone, act by our group has shown that HPCO₂ and low doses of the opiate receptor antagonists, naloxone and naltrexone, act synergistically to induce a pronounced and selective reduction of voluntary ethanol consumption in msP rats (Perfumi et al., 2003). It has been widely demonstrated that the opioid receptor antagonists attenuate alcohol drinking by lowering its rewarding and reinforcing properties (Hubbell and Reid, 1990; Gianoulakis, 1993; Hytti, 1993; Di Chiara et al., 1996; Honkanen et al., 1996; Herz, 1997, 1998; Koob et al., 1998; Krishnan-Sarin et al., 1998). Therefore, we speculate that the reduction of the motivational properties of alcohol by HPE and opioid receptor antagonists represents a converging mechanism that can explain the synergism of action of the two classes of compounds. However, it should also be mentioned that HPE do not bind directly to opioid receptors nor do they modulate opioid activity via other mechanisms. Future studies are warranted to investigate the mechanism by which H. perforatum reduces the motivational properties of alcohol.

In conclusion, the results of the present experiments provide evidence that HPCO₂ markedly and selectively reduces the motivation for ethanol as revealed in the self-administration paradigm, as well as in the increased craving for ethanol observed after a period of ethanol deprivation. These results, together with those obtained in voluntary drinking rats, strengthen the idea that the use of HPE may represent an interesting pharmacological approach to treat excessive alcohol drinking and prevent alcohol relapse in human alcoholics.


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