BUSPIRONE AS AN INHIBITOR OF VOLUNTARY ETHANOL INTAKE IN MALE RATS

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Abstract — The effect of buspirone, a drug with mainly 5-HT1A-agonist activity, on voluntary ethanol intake was tested in a rat model of alcoholism. In this model the treatment consists of an injection of ethanol (2.0 g/kg) or saline once a week, preceded by a 24 h choice between water and ethanol (10%, w/v). This weekly injection of ethanol reduces voluntary ethanol intake in male rats. Maximal inhibition is seen after 5-6 weeks. At this maximal inhibition buspirone or saline was injected prior to the voluntary 24 h intake of ethanol in both the ethanol- and saline-injected groups. The tested doses were 5 mg/kg (week 5) and 20 mg/kg (week 6). There was no reduction in ethanol intake in the buspirone-injected groups when compared with their corresponding controls. A second experiment with buspirone was performed during the evaluation period following treatment with ethanol. This treatment consisted of a choice between water and ethanol (10%, w/v) for 1 day each week, followed by an injection of ethanol (2.0 g/kg) and lasted for 52 weeks. During the evaluation period the rats had a continuous choice between ethanol and water for 37 weeks and no injections were given. In this situation, with a longer exposure to ethanol, a dose of 20 mg/kg of buspirone in week 90 reduced ethanol intake by approximately 40%, when compared with controls. The effect of this buspirone dose lasted at least a week. This indicates that the long-term exposure to ethanol in the second experiment induces changes that affect the serotonergic transmission, and that this changed neural system is involved in the regulation of voluntary ethanol intake.

INTRODUCTION

Ethanol is perhaps the drug of abuse with the greatest cost to society, not to mention its effects on the abuser. Much money and effort have been put into the development of drugs that can reduce ethanol intake in alcoholics and that can counteract the symptoms of ethanol withdrawal. Changes in the serotonergic system have been reported after ethanol exposure (Ballenger et al., 1979; Simonsson and Alling, 1988; Alexi and Azmitia, 1990). It has also been shown that rats with an inherent serotonin deficiency consume more ethanol (Overstreet et al., 1992). These facts indicate that drugs affecting the serotonergic system may also affect ethanol intake. Buspirone is an anxiolytic that has been in clinical use for some years. It has presynaptic agonistic and postsynaptic partial agonistic properties at the 5-HT1A-receptor (for further description, see Napoliello and Domantay, 1991). It has been shown to reduce ethanol intake in monkeys and rats (Collins and Myers, 1987; Privette et al., 1988). Similar effects have also been shown for other 5-HT1A-agonists (Kostowski and Dyr, 1992; Meert, 1993; Svensson et al., 1993; Blomqvist et al., 1994). Clinical trials have shown a beneficial effect of buspirone after ethanol withdrawal, especially in anxious alcoholics (Bruno, 1989; Kranzler and Meyer, 1989; Olivera et al., 1990; Tollefson et al., 1992). The major effect of buspirone in these studies in man has been a reduction of anxiety, and little or no direct effect has been shown on ethanol intake. However, buspirone treatment without effects has also been reported (Malcolm et al., 1992). Clinical trials have found buspirone effective in the acute state of ethanol withdrawal (Dougherty and Gates, 1990).

The present experiments were intended to examine if buspirone could reduce ethanol intake in animals according to our rat model of alcoholism (Wahlström, 1983, 1987, 1994a, b). Buspirone was first given in the early phase of the intermittent treatment, used to induce psychological dependence. This exposure to ethanol resembles the drinking pattern of people in Nordic countries.
with heavy drinking at weekends. Buspirone was then given in the following evaluation period, where the animals had undergone a long and continuous exposure to ethanol. At this stage, the rats, in a continuous choice situation, take the same dose of ethanol independent of the offered ethanol concentration. Thus, in this model we have an empirical sign of psychological dependence to ethanol, which has been induced by the intermittent treatment.

Psychological dependence must be separated from physical dependence. The physical dependence to a drug is the mainly adaptive changes of, for instance, receptor systems, that develop during exposure to a drug. The physical dependence is responsible for the main part of the symptoms seen during the first part of withdrawal. These symptoms have a limited duration. The psychological type of dependence is more of a 'memory' of the effect of the drug, and is responsible for the craving. This part of dependence does not end after a period of withdrawal, but persists for a much longer time. In the present experiments, single doses of buspirone were tested on voluntary ethanol intake, both early in the treatment, with no dependence, and in 'psychologically' dependent rats.

**MATERIALS AND METHODS**

**Animals**

Male Sprague–Dawley rats (Mol:SPRD, Möllegaards Breeding Centre Ltd, Li Skensved, Denmark) were kept in individual cages in a room with a reversed light/dark schedule (light on 19:00–07:00) and at a room temperature around 23°C. Each cage was equipped with two drinking bottles. One of the bottles always contained tap water, and the other contained either water or an ethanol solution. The concentration of the ethanol solution was 10% (w/v) during the treatment period in both experiments. During the evaluation period of the second experiment, it was varied between 0% and 20% to test for psychological dependence. The buspirone treatment in the present continuous exposure experiment was given to rats age/weight curve (supplied by the breeder) and was ~10 weeks at the start of the short experiment and 5 weeks at the start of the long experiment.

**Chemicals**

Ethanol (AB Svensk Sprit) as a drinking fluid was mixed with tap water to a 10% (w/v) solution. Ethanol for injection was dissolved in 0.9% (w/v) saline and administered intraperitoneally (i.p.) in a dose of 2.0 g/kg at a concentration of 10% (w/v). Control animals were given isotonic saline. Buspirone (Bristol-Myers Squibb) was dissolved in 0.9% NaCl and injected i.p. at the concentrations of 5 mg/ml (5 mg/kg) and 20 mg/ml (20 mg/kg).

**Experimental design**

The experimental design used in the two experiments presented here has been described in detail in earlier publications (Wahlström, 1983, 1987, 1994a, b). Three prerequisites founded on experiences in humans are involved: (1) intoxication once a week; (2) chronic treatment; (3) oral intake (Wahlström 1994a). The basic experiment incorporating these prerequisites consisted of a treatment period and an evaluation period.

During the treatment period, the weekly ethanol intake consisted of a voluntary oral choice for 24 h which is ended by an ethanol injection (2.0 g/kg i.p.). Thus the first prerequisite was fulfilled. With such a treatment schedule, an inhibition of voluntary ethanol intake develops during the first 6 weeks of the treatment. The buspirone treatment in the present experiment with intermittently treated rats was performed at this minimum in the ethanol-injected groups. If the treatment is continued the inhibition of ethanol intake gradually disappears. In a basic experiment, the treatment is pursued for 1 year. Thus the second prerequisite is fulfilled.

During the following evaluation period, all ethanol exposures are voluntary and given as a continuous oral choice between ethanol and water. Thus the third prerequisite is fulfilled. The concentration of the ethanol solution offered is changed (tested range 5–20%) every third week, and our empirical definition of psychological dependence is that the voluntary daily dose of ethanol (in g/kg) is not changed by this manipulation. The buspirone treatment in the present continuous exposure experiment was given to rats.
during the evaluation period. These rats had fulfilled the "psychological" dependence when tested with changing concentrations of ethanol prior to the administration of buspirone.

**Intermittent treatment.** Using the first letters of the words Ethanol, Buspirone and Saline, we randomly divided the rats into the four groups SS, SB, ES and EB as shown in Fig. 1. All handling of the rats was conducted between 07:00 and 09:00. In weeks 1–7, the rats had during the first 6 days two bottles of water available. At the start of day 7, a bottle of ethanol (10% w/v) was inserted instead of the bottle from which the rat had consumed most water during days 1–6. The next morning, when the bottles were changed to two bottles containing fresh water, the rats were injected with either 2.0 g/kg ethanol i.p. [10% w/v, volume injected (7–12 ml) determined by weighing the rats], a dose that causes moderate intoxication, or a corresponding volume of saline.

In week 5, the rats were given saline or buspirone in a dose of 5 mg/kg at the start of day 7. In week 6, the rats were given saline or buspirone in a dose of 20 mg/kg at the start of day 7. At the end of week 7, a final injection of ethanol or saline was given. In week 8, the rats had a continuous choice between water and ethanol and no injections were given.

At the time of the maximal difference between the groups it is possible to detect both stimulatory and inhibitory effects of a drug on voluntary ethanol intake. Buspirone was tested at the above two levels of ethanol intake.

**Continuous exposure.** In the treatment part of the second experiment the rats were allowed to choose between water and ethanol for 1 day each week, and were at the end of the 24 h period injected with ethanol (2.0 g/kg). This treatment was given during weeks 1–52. After this treatment the rats had, during an evaluation period, a continuous choice between water and ethanol. This period lasted for 37 weeks (weeks 53–89). During the evaluation period, the ethanol concentration was varied (according to experimental conditions not involved in the present experiment) between 0% and 20% (w/v). These conditions were used to evaluate the psychological dependence. There were two patterns of changes in ethanol concentration in the two groups participating in this experiment. For this reason buspirone was given to half the rats in each group, yielding four different groups. The rats in each group were randomly given either buspirone or saline, but since there were no differences between the rats with different exposure patterns, neither in pre-injection ethanol intake nor in the response to buspirone or saline, both the groups given buspirone (total n = 8) and both the groups given saline (total n = 10) were combined. In the first day of week 90, the rats were injected with buspirone.
(20 mg/kg) or saline. The rats had a continuous choice between water and a 10% (w/v) ethanol solution during 3 weeks prior to the buspirone treatment and the period following the injection. A test of dependence, as described in earlier experiments with this model (Wahlström, 1987, 1994a, b), with 20% ethanol during weeks 61–63 showed that the groups had developed a 'psychological' dependence. The rats may also at this time have been physically dependent, but no tests were performed.

Statistical methods

Conventional statistical methods were used. Differences between two groups were tested with Student's t-test. \( P < 0.05 \) was used as the basic level of significance; NS denotes value outside this range. \( n \) denotes numbers of observations. Values are given as means ± SEM.

RESULTS

Fluid intake

The total intake of fluid (data not shown) was not affected by buspirone in any significant way in any of the experiments.

Intermittent treatment

Week 1. There was no significant difference in ethanol intake between the four groups (Fig. 2).

Weeks 2–4. The difference between the groups injected with ethanol (ES and EB) and the groups injected with saline (SS and SB) reached a statistically significant level \( (P < 0.05) \) in week 3, and this difference remained throughout the experiment (Fig. 2).

Weeks 5–6. In weeks 5 and 6 the EB and SB groups were injected with buspirone i.p. prior to the 24 h choice between water and ethanol. The groups ES and SS were injected with a corresponding volume of saline i.p. There was no difference between the groups given buspirone and their corresponding controls in their intake of ethanol (Fig. 2).

Weeks 7–8. At the end of week 7, there was a final 24 h choice between water and ethanol, but no significant difference between the rats that had received buspirone or saline was detected. In week 8, the rats had a continuous choice between water and ethanol (Fig. 2). When the rats had a continuous access to ethanol a significant \( (SS, P < 0.01; SB, P < 0.001; ES, P < 0.05; EB, P < 0.001) \) reduction in daily ethanol intake was seen compared to the intakes in week 7, when the rats had access to ethanol for a single day. However, in week 8, there was also a significant difference \( (P < 0.05) \) in ethanol intake between group ES (0.46 ± 0.07 g/kg) and group EB (0.37 ± 0.05 g/kg). Whether this small difference has any biological relevance is an open question.

Continuous exposure

In the first day of week 90 in the second experiment, an injection of buspirone (20 mg/kg i.p.) or saline was given. When comparing the weekly ethanol intake during weeks 88–93, the only week with a significant \( (P < 0.05) \) difference between the saline- and the buspirone-injected animals was week 90, which is the week following the treatment (Fig. 3).

A more detailed analysis of the treatment with 20 mg/kg buspirone showed that ethanol intake was significantly \( (P < 0.01, n = 8, \text{ paired } t\text{-test}) \) week 89 vs week 90) reduced during the day of injection (Fig. 4A). There was no significant correlation between the intake of ethanol on these two occasions, but all rats decreased their intake after the buspirone treatment (Fig. 5A). No corresponding decrease was seen in the saline-injected animals (Fig. 4A).

A significantly \( (P < 0.01) \) decreased ethanol intake was also seen during the remaining part (days 2–7) of week 90 in the previously buspirone-injected rats (Fig. 4B), but since the intake is a mean daily intake during days 2–7 of week 90, it is not possible to tell how this inhibition of ethanol intake changes with time. There was a significant \( (P < 0.05) \) correlation during days 2–7 of week 90 with the corresponding intake during days 2–7 of week 89. Since the regression coefficient did not deviate from 1.0 (0.91 ± 0.27), the reduction in intake was probably not influenced by a high and a low intake in the week prior to injection (Fig. 5B). Also during this part of the experiment, no corresponding changes were seen in the controls (Fig. 4B).

DISCUSSION

Effects of buspirone on ethanol intake in the present experiment were only found in rats with a heavy exposure to ethanol. This indicates that
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Fig. 2. Weekly ethanol intake during the first experiment. Group SS is indicated by open squares, group SB by solid squares, group ES by open circles and group EB by solid circles. The marks along the abscissa are identical to those given in Fig. 1. Error bars are plotted only in one direction and indicate 1 SEM. No error bar indicates an SEM smaller than the corresponding symbol *P < 0.05 when comparing groups SS and SB, or groups ES and EB. Significance between the ethanol-injected and the corresponding saline-injected groups is given in the text.

ethanol exposure induces changes in a neural system regulating ethanol intake which, due to the main action of buspirone, includes a part where serotonergic transmission is involved. The negative results of our first experiment indicate that these changes have not yet developed in the early stages of the treatment part of the present model. Since the treatment used here can induce 'psychological' dependence (Wahlström 1994a) it is possible that the effects of buspirone reported in the experiment with continuous exposure could have influenced this state. This means that psychological dependence could be connected to neural changes in the serotonergic system. Another explanation for the changed response to buspirone after long-term ethanol exposure could be that the rats in our second experiment have grown old, or that there exists a combined effect of ethanol exposure and ageing. However, the younger age of the rats in the other experiments with 5-HT_{1A} agonists, where a decreased ethanol intake was seen (Kostowski and Dyr, 1992; Svensson et al., 1993; Meert, 1993) excludes age as an important factor.

In two earlier experiments (Kostowski and Dyr, 1992; Meert, 1993) effects of buspirone on ethanol preference or intake have been shown in rats. However, these effects were only obtained in rats selected for high ethanol intake, recorded after forced exposure to ethanol. Other 5-HT_{1A}-agon-
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Fig. 4. Effect of a single dose of buspirone (20 mg/kg, i.p.) given at the start of day 1 of week 90 on ethanol intake. Controls were given saline. The intake of ethanol in week 90 is compared with the corresponding pretreatment intake recorded in week 89. Points indicate week 89 and diagonal lines indicate week 90. Error bars indicate 1 SEM. (A) Ethanol intake during day 1. (B) Daily ethanol intake during days 2–7.

Fig. 5. Correlations between ethanol intake in week 89 and the corresponding intake in week 90, after a single dose of buspirone (20 mg/kg i.p.) given at the start of day one of week 90.
The unbroken lines indicate identical intake of ethanol in both weeks. (A) Ethanol intake during day 1. (B) Daily ethanol intake during days 2–7. The dashed line is the estimated regression line (correlation coefficient = 0.81, regression coefficient = 0.91, n = 8, P < 0.05).

The present experiment, both at an early stage (Fig. 2) and with heavily exposed rats (Fig. 5), no difference in effects of buspirone was seen between rats with a high and a low pretreatment intake of ethanol.

In the second experiment, the effect of a single buspirone injection lasted for at least a week. This is far longer than would be expected from the elimination rate in rats, where buspirone has a plasma half-life of ~0.5 h (Per Högberg, personal communication). The explanation for this could be that a single dose of buspirone causes lasting changes in the serotonergic system, but other explanations cannot be excluded by the results of the present experiment.

Since buspirone has been shown to counteract...
the anxiogenic effects of ethanol withdrawal in rats (Lal et al., 1991), and since buspirone also shows the best results in 'anxious alcoholics' (Tollefson, 1991), the effects of buspirone on ethanol intake could be secondary to the anxiety often coupled with ethanol withdrawal. In experiments with anxious alcoholics, the major clinical effect has also been a reduction of anxiety, rather than a primary decrease in ethanol intake. An important difference between a clinical trial and an animal model is that the clinical trial can produce a more accurate measure of anxiety in the patients, while the objective ethanol intake can be more easily measured in the animal model. If anxiety affects ethanol intake, it is difficult to discriminate between drugs that only affect anxiety, and drugs that affect both anxiety and ethanol intake. However, since there is a gradual development of the clinical anxiolytic effect of buspirone, the long duration of the effect seen in the second experiment could indicate that similar mechanisms are involved.

Several investigators have reported changes in the serotonergic system following ethanol exposure. These changes could eventually lead to an explanation of how ethanol affects the serotonergic transmission. Alexi and Azmitia (1990) have reported that ethanol stimulates the production of 5-HT. An increase in the plasma levels of the total amount of 5-HT degradation products (5-HIAA and 5-HTOL) has been reported by Helander et al. (1993) following acute ethanol exposure. A down-regulation of postsynaptic receptor function has been described by Simonsson and Alling (1988) in platelets of alcoholics. Ballenger et al. (1979) have reported a decrease in 5-HT production following ethanol withdrawal in humans. Fawn-Hooded rats, which have a decreased 5-HT function, have both a high preference for ethanol and show signs of depression (Rezvani et al., 1991; Overstreet et al., 1992). Drugs affecting the serotonergic system show less effect on ethanol intake in these rats.

If our results are combined with these observations, it is evident that there is an interaction between the serotonergic system and voluntary ethanol intake. However, the present results indicate that it is not a simple relationship, and furthermore this relationship must be put in context with other transmitter systems that affect voluntary ethanol intake.

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