ADRENALECTOMY PROTECTS ETHANOL-WITHDRAWN RATS FROM HARMINE-INDUCED TREMOR

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Abstract — A growing number of studies have implicated the hypothalamic-pituitary-adrenal (HPA) axis in acute and chronic alcoholization and in ethanol withdrawal. In order to study the ethanol/HPA axis interaction during alcohol withdrawal, we performed experiments using adrenalectomized (ADX) male rats alcoholized by a chronic pulmonary alcoholization procedure. Eight hours after the 3 weeks of the alcoholization procedure, the rats were evaluated for a tremor activity. In order to reduce the great variability of the withdrawal tremors, we estimated the supersensitivity of the withdrawn rats to the tremorogenic compound harmine. We also studied the effect of a hydrocortisone treatment given in the drinking bottle during the alcoholization procedure on the harmine-induced tremors of ADX and sham rats. Alcohol withdrawal resulted in increased tremor response to 10 mg/kg harmine, and a protective effect of adrenalectomy on this effect was observed. Hydrocortisone administration to ADX or sham rats did not affect the tremor profile of the alcohol withdrawn rats.

INTRODUCTION

Acute or chronic ethanol administration to experimental animals and man alters the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis. Thus acute exposure to alcohol produces a potent activation of the HPA axis marked by an increased plasma glucocorticoid level in rodents (Ellis, 1966; Kakihana et al., 1971: Tabakoff et al., 1978; Pohorecky et al., 1980) and in man (Jenkins and Connolly, 1968; Thiagarajan et al., 1989). This adrenal response is dependent upon an increased pituitary adrenocorticotropic hormone (ACTH) secretion (Ellis, 1966) which is stimulated by the corticotropin-releasing factor (CRF) from the hypothalamus (Rivier et al., 1984). In vitro, a direct stimulatory effect of acute ethanol was also evaluated on the hypothalamus, the pituitary gland and the adrenal cortex but the outcome of the studies varied depending on the experiments used (Wand, 1993).

The effects of chronic alcoholization on the HPA axis seem to be controversial. The plasma corticosterone concentration changes vary according to the alcoholization procedure, even if a similar blood ethanol concentration is reached (Koranyi et al., 1987). Some studies underline the persistence of an enlarged HPA axis activity (Ellis, 1966; Tabakoff et al., 1978; Wilkins et al., 1992) while other studies show a tolerance development of the corticosterone response to ethanol (Knych and Prohaska, 1981; Spencer and McEwen, 1990). Alcohol intoxication also led to disturbance of the normal diurnal variation of plasma glucocorticoids in rodents (Kakihana and Moore, 1976) and humans (Adinoff et al., 1991) during chronic alcoholization and during alcohol withdrawal. The corticosterone (rodent) or cortisol (man) concentration perturbations consisted of a shortening in the length of the cyclic secretions. Also an increased adrenal weight of up to 25% and a thymus involution are observed after chronic alcohol intoxication (Spencer and McEwen, 1990).

Alcohol abstinence is accompanied by increased plasma glucocorticoids in rodents (Tabakoff et al., 1978; Roberts et al., 1992; Wilkins et al., 1992) and man (Adinoff et al., 1991). The glucocorticoids reach peak values on the first day of alcohol withdrawal when ethanol is no longer detectable in the blood. This increased HPA axis activity coincides with the behavioural and physiological symptoms of the withdrawal syndrome (Tabakoff et al., 1978; Adinoff et al., 1991).
Corticosterone administration was demonstrated to increase ethanol withdrawal symptoms (Roberts et al., 1991). Adrenalectomy decreased the incidence of withdrawal seizures in animals. In mice the functional protective effect of adrenalectomy was present in terms of a reduced sensitivity to audiogenic seizures during ethanol withdrawal (Sze et al., 1974; Sze, 1977).

In the present study, adrenalectomized (ADX) rats were used to examine the involvement of the HPA axis in the alcohol withdrawal-induced tremors. More precisely, following chronic ethanol inhalation, we analysed the sensitivity of the animals to alcohol withdrawal-induced tremors in adrenalectomized rats and in adrenalectomized rats treated with hydrocortisone. Because withdrawal-induced tremor is very variable between animals over time (Meert and Huysmans, 1994), we decided to characterize the supersensitivity of withdrawal by injecting subthreshold doses of the tremorogenic agent harmine, a beta-carboline (Gothoni, 1985; Meert et al., 1992). Alcohol-withdrawn rats have been demonstrated to be supersensitive to the tremorogenic efficacy of this compound at low doses (Meert, 1994).

**MATERIALS AND METHODS**

**Animals**

Male Wistar rats weighing 150 g at the start of experiments were used. The rats were separated into two groups: an ADX group and a sham group. For the operation, the rats were anaesthetized with chloral hydrate (400 mg/100 g body weight). Bilateral adrenalectomy was performed by means of two dorsal incisions. Sham surgery consisted of bilateral incisions, and localization of the adrenal glands without extraction. Following surgery, all animals were supplied with a 1% (w/v) saline solution as drinking fluid. Completeness of ADX was verified at the end of the experiment by radioimmunoassay (RIA, Becton Dickinson Immunodiagnostics: Cortisol Solid Phase Radioimmunoassay [125I]).

**Experimental treatment**

The ADX and sham groups were subdivided so as to obtain six experimental groups: the chronic alcoholized ADX, the chronic alcoholized ADX + hydrocortisone, the non-alcoholized ADX, the chronic alcoholized sham, the chronic alcoholized sham + hydrocortisone, and the non-alcoholized sham group.

Hydrocortisone (Solu-cortef, Upjohn) was given per os in the drinking bottle (85 mg/l) during the whole alcoholization procedure at a daily dose of 2.5 mg/rat. The hydrocortisone solution was replaced every 2 days and the drinking bottle was sheltered from light. The rats’ intake was assessed to ensure a constant drug administration. The hydrocortisone treatment was stopped at the end of the alcoholization period.

**Chronic alcohol administration**

For alcoholization, rats were individually housed in standard rodent living cages, placed in an isolated plastic chamber (160 x 60 x 60 cm). The chamber was equipped with a mixing system able to pulse alcohol and air mixtures into the chamber. The quantity of alcohol was gradually increased every 2 days during the whole experimental procedure from 0 to 25 mg/l (Le Bourhis, 1975). The rats were kept for a total of 20 days in the alcoholization chamber.

**Blood ethanol determination**

The blood alcohol levels of control and treated rats were regularly determined (between 08:00 and 10:00) during the alcoholization procedure. To do so, samples of plasma (0.1 ml) obtained from blood sampling in the caudal portion of the tail were analysed by an alcohol dehydrogenase-based method (Boehringer Mannheim kit).

**Tremor measurement**

The apparatus used has been described in detail by Meert et al. (1992). Briefly the apparatus consisted of four test cages in plexiglass (25 x 24 x 25 cm). The floor consisted of a flexible plexiglass plate (45 x 23 x 0.6 cm) upon four corners so that it could bend freely. Underneath the middle of the plate, two pieces of piezo-film (200 x 100 x 0.025 mm, polymeric PVF2) were glued and connected with an amplifier, so that the deformations of the cage floor result in a piezoelectric response of each of the piezo-films. These electric signals are first summed by one differential amplifier and the output of the signal is then filtered by a bandpass filter between 6 and 12 Hz (18 db/oct slope). This end signal is sent to a 4-channel Mac Lab connected to a Macintosh II computer. A chart file enables quantification of
the amplitude and frequencies of the pulses. Two pulses were judged comparable if: (1) the amplitude of both pulses was ≥1 V; (2) the difference between the two pulses was <50%; (3) the interpulse interval was between 6 and 12 Hz. The selection of these parameters was based on the assumption that bursts of tremor activity produce a relatively constant bending of the cage floor, resulting in relatively constant output signals. A series of five comparable pulses resulted in one tremor count. Data are presented in terms of the mean (±SEM) number of tremor counts.

The test procedure used to assess the supersensitivity to harmine-induced tremor consisted of a subcutaneous injection of 5 mg/kg of harmine (1 ml/100 g body wt) to individually-housed rats. Five minutes later, the rats were placed into a test cage and activity was measured for 5 min. Directly after this session, the same animals received a second injection of 5 mg/kg harmine (resulting in a cumulative dose of 10 mg/kg, as referred to in the results). After 5 min, a second 5 min recording was performed. After each measurement, the test cages were cleaned. Based on earlier studies, an alcohol withdrawal period of 8 h was used to assess the supersensitivity to harmine-induced tremor (Meert et al., 1992; Meert and Huysmans, 1994).

Statistical analysis

The Mann–Whitney U-test (two-tailed) was used to evaluate differences between experimental groups.

RESULTS

The ADX rats were more sensitive to chronic alcohol intoxication than the corresponding sham controls. Indeed, eleven out of eighteen ADX animals died during the alcoholization and one during the withdrawal. In the sham group (n = 9), only one rat died during the alcohol intoxication. The sensitivity of the ADX rats for alcoholization was also reflected in the average body weight at the end of the experiments. The mean (±SEM) body wt of the ADX and sham groups were respectively 213 ± 8 and 307 ± 12 g. Compared to the preoperative starting level, this represents an increase of 63 and 157 g respectively. In the hydrocortisone-treated ADX group (n = 10), one ADX died during the alcoholization and two during the withdrawal. No death was observed in the hydrocortisone alcoholized sham group (n = 9), nor in both the unalcoholized groups (n = 5 each).

Fig. 1. Blood alcohol levels in sham and adrenalectomized rats. Some sham and adrenalectomized rats were treated with hydrocortisone orally. Blood samples were analysed from day 12 after the start of the chronic alcoholization.
Blood alcohol levels (BAL) were assessed repeatedly over time in order to quantify the alcoholization procedure and to detect differences in the BAL between groups (Fig. 1). There were no differences in the BAL values between the four alcoholized groups. The within-group variations were important and are not shown. At the end of the alcoholization period, mean blood alcohol levels of 148 ± 65 mg/dl were reached.

The sensitivity of the rats during alcohol withdrawal to a tremorogenic compound was evaluated by the injection of harmine (5 and 10 mg/kg) into rats out of the alcoholization chamber for 8 h. There were no significant differences between the various groups after 5 mg/kg harmine ($P > 0.05$). However, after 10 mg/kg harmine administration, significant differences were present. Thus, as shown in Fig. 2, the alcoholized sham rats appeared more sensitive to the harmine-induced tremor than all the other groups. Their increased tremor activity made this group significantly different from the unalcoholized sham rats ($P < 0.005$) and from all the ADX groups (sham/unalcoholized ADX $P < 0.005$; sham/ADX alcoholized and treated with hydrocortisone $P < 0.005$; sham/unalcoholized ADX $P < 0.001$). The tremor level of the hydrocortisone sham rats was intermediate between the alcoholized ($P > 0.05$) and the unalcoholized sham animals ($P > 0.05$). The ADX groups had the lowest tremor counts. There were no significant differences between the three ADX groups (for each comparison, $P > 0.05$). The alcoholized ADX rats did not show a greater tremor activity than the corresponding unalcoholized group. Therefore, the hydrocortisone treatment appears not to modify the sensitivity to the harmine-induced tremor.

**DISCUSSION**

A growing number of studies implicated the involvement of the HPA axis in acute and chronic alcoholization and in ethanol withdrawal. In order to study the alcohol/HPA axis interaction, we...
performed a series of experiments using adrenalectomized male rats. The adrenalectomy makes these animals more sensitive to the chronic pulmonary alcoholization as shown by the severe mortality rate of ADX and body weight changes during chronic alcohol exposure.

Alcohol withdrawal results in a series of phenomena, including some tremorogenic activity. In order to decrease the intra-group variability of the withdrawal-induced tremor, we quantified the tremorogenic state of the animals by injecting low doses of the tremorogenic compound harmine. Gothoni (1985) reported an increased harmine-induced tremor intensity following alcohol withdrawal. Meert et al. (1992) confirmed this potentiation of the tremorogenic properties of harmine after withdrawal of a liquid diet containing alcohol (10%, v/v). Our results confirm the use of 10 mg/kg harmine during withdrawal as a tool to induce tremor in alcoholized sham rats after being intoxicated by inhalation of ethanol vapour for 3 weeks.

The ADX rats failed to show an increased tremor activity upon the injection of 10 mg/kg harmine. ADX thus appears to overcome the supersensitivity to tremorogenic agents during ethanol withdrawal. A protective effect of adrenalectomy has previously been observed against audiogenic seizures upon ethanol withdrawal in mice (Sze et al., 1974; Sze, 1977).

There are several ways to explain the protective effects of ADX on alcohol withdrawal. The reduction of the ethanol withdrawal symptoms after bilateral adrenalectomy might for instance be explained in terms of a protection towards hippocampal neuronal loss induced by chronic alcoholization. As a result, there can be a reduction in alcohol withdrawal-induced tremor. The hippocampus is the principal target for neuronal loss induced by insults such as seizures or hypoxic ischaemia in man (Zola-Morgan et al., 1986; Petito et al., 1987) and in rodents (Ito et al., 1975; Kirino, 1982). This hippocampal neuronal death appears to be exacerbated by glucocorticoid over-exposure in rodents (Sapolsky and Pulsinelli, 1985; Sapolsky, 1990; Morse and Davis, 1990; Packan and Sapolsky, 1990) and ameliorated by adrenalectomy (Sapolsky et al., 1985; Sapolsky, 1986; Stein and Sapolsky, 1988). Chronic alcoholization has been demonstrated to result in a profound damage of hippocampal neurons (for review, see Walker et al., 1993). Furthermore, chronic alcohol exposure stimulates glucocorticoid secretion. Based on these observations, it could be hypothesized that the increased glucocorticoid concentration following chronic alcoholization may be responsible, at least in part, for the enlarged hippocampal neuronal death, as for instance seen during hippocampal damage after insults such as by hypoxic-ischaemia.

If the neuronal hippocampal damage is implicated in some ethanol withdrawal symptoms such as tremors, adrenalectomy may present an effective protection towards ethanol withdrawal-induced tremor. This is, at least in our experiments, confirmed in terms of supersensitivity to tremorogenic agents. Further experiments are needed to fully clarify this issue.

A second explanation may be found in the fact that adrenalectomy and glucocorticoid administration may act by a direct interaction with harmine, so reducing the tremorogenic activity of this compound. The variation in concentration of glucocorticoid hormones induces modifications of different neuromediators. Adrenalectomy and glucocorticoid administration in rodents and in humans modify dopaminergic (DA) and serotonergic (5-HT) functions (Rothschild et al., 1984; Biegon et al., 1985; Wolkowitz et al., 1986; Bagdy et al., 1989; Faunt and Crocker, 1989; Biron et al., 1992). Because harmine provokes a DA/5-HT imbalance, suggesting a direct implication of these neurotransmitter mechanisms in some of the ethanol withdrawal symptoms (Meert et al., 1992), a direct pharmacological interaction with the activity of harmine cannot be excluded as well as alteration of the hepatic metabolism of harmine by glucocorticoids. More studies are needed to clarify these ethanol/DA/5-HT/glucocorticoid linkages during alcohol withdrawal.

Surprisingly, hydrocortisone given during the alcoholization procedure to the ADX rats did not affect the harmine-induced tremor. The hydrocortisone supplement given to rats with an intact functional HPA axis produced a small, but insignificant, decrease in the tremor counts. This lack of effect of hydrocortisone may be caused by the incomplete or absent transformation of hydrocortisone to corticosterone, the main circulating glucocorticoid in rats. An alternative explanation of the absence of action of hydrocortisone is that
it requires the integration of the HPA axis to exert its action. This latter proposition, however, seems unlikely taking the results of Sze et al. (1974) and Sze (1977) into account. These authors observed that the susceptibility to audiogenic seizures of ethanol-withdrawn adrenalectomized mice was reinstated by a corticosterone treatment. Moreover the hydrocortisone treatment of our sham rats did not produce any significant changes in the harmine-induced tremor.

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REFERENCES


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