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Oxytocin Attenuates Tolerance Not Only to the Hypothermic But Also to the Myorelaxant and Akinesic Effects of Ethanol in Mice

CHRISTINE JODOGNE,* EZIO TIRELLI,* PHILIPPE KLINGBIEL† AND JEAN-JACQUES LEGROS†

**Laboratoire de Psychologie Experimentale, Université de Liège au Sart Tilman*
5, Boulevard du Rectorat (B32), B-4000 Liège-1, Belgium
†Section de Neuroendocrinologie, Université de Liège au Sart Tilman
Centre Hospitalier Universitaire (B23), B-4000 Liège-1, Belgium

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JODOGNE, C., E. TIRELLI, P. KLINGBIEL AND J. J. LEGROS. *Oxytocin attenuates tolerance not only to the hypothermic but also to the myorelaxant and akinesic effects of ethanol in mice.* PHARMACOL BIOCHEM BEHAV **40**(2) 261–265, 1991.— Inhibition of ethanol tolerance by oxytocin has been demonstrated previously using the hypothermic effect only. The purpose of the present experiment was to investigate the effect of oxytocin on the development of tolerance to ethanol-induced hypothermia, myorelaxation and akinesia in mice. Four groups of mice received daily intraperitoneal injections of saline or oxytocin (0.005 mg) plus saline or ethanol (2 g/kg). The peptide was administered 2 hours before ethanol. For five consecutive days, temperature measurements were performed 20 minutes before and after ethanol injection. Myorelaxation and akinesia were evaluated following the second temperature measure. Oxytocin pretreatment, which had no intrinsic effects, resulted in a robust selective attenuation of tolerance to ethanol-induced hypothermia, myorelaxation and akinesia. These results suggest that the mechanisms for peptide modulation are common to these three typical effects of ethanol.

Ethanol tolerance Oxytocin Hypothermia Myorelaxation Akinesia Mice

SEVERAL studies have suggested that the neurohypophyseal hormones oxytocin and arginine-vasopressin modulate the expression, development or maintenance of tolerance to the central effects of ethanol (11, 12, 18).

Tolerance to ethanol-induced hypothermia and narcosis naturally dissipates within few days after cessation of ethanol administration. However, the response to ethanol has been shown to be maintained at its initial levels over the entire period studied by daily administration of arginine-vasopressin during ethanol treatment and withdrawal period (5), or during withdrawal only (4). In both experiments, oxytocin or cyclo-leucyl-glycine (a cyclic derivative of oxytocin C terminal fragment) were without effect on the expression or the maintenance of ethanol tolerance.

However, more recent results suggest a role for oxytocin in the development of tolerance to ethanol. Oxytocin administered systemically during chronic treatment, prior to each daily ethanol injection, has been shown to attenuate the development of tolerance to ethanol-induced hypothermia in mice (17,19). Once ethanol tolerance had already developed, an acute injection of oxytocin was ineffective in modulating the level of observed tolerance (17).

Similar results have been reported for the C terminal fragment of oxytocin prolyl-leucyl-glycinamide and its fragment analogues Z-prolyl-D-leucine or D-pipecolyl-leucyl-glycinamide (15,16).

Although ethanol induces a variety of central effects in rodent (e.g., hypothermia, narcosis, ataxia, myorelaxation, antinociception, protection against convulsion, akinesia, locomotor activation at low doses), there is only one paper to our knowledge reporting an influence of oxytocin pretreatment on ethanol tolerance for effects other than hypothermia. Pucilowsky and his colleagues (10) found that oxytocin attenuated tolerance to narcosis (loss of righting reflex) induced by ethanol in rats, but tolerance to the hypothermic effect was not clearly attenuated by pretreatment with the peptide.

At this point, it would be valuable to extend the assessment of the effect of oxytocin on ethanol tolerance to other representative measures. Specifically, the present experiment was designed to examine the effect of oxytocin on the development of tolerance to ethanol-induced hypothermia, myorelaxation and akinesia in mice. The same mice were subjected to all three measures. A differential effect of oxytocin can be more surely related to a difference between the phenotypes measured rather than to individual variation in ethanol-oxytocin sensitivity by using a within-subjects design.

METHOD

Subjects

Thirty-two male OF-1 mice (IFFA-CREDO, Oncins, France) weighing between 35 and 40 g (12–15 weeks of age) and born

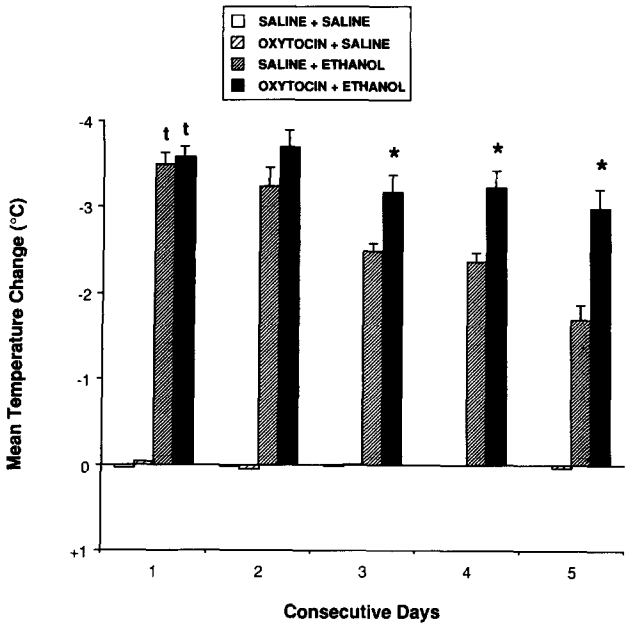

FIG. 1. Attenuation by oxytocin of tolerance to the hypothermic effect of ethanol. Mice received daily intraperitoneal injections of either saline or oxytocin (0.005 mg/animal), and after 2 hours, of saline or ethanol (2 g/kg), for 5 consecutive days. *Significantly different from the saline plus ethanol group at the same day (after a two-way ANOVA followed by Newman-Keuls tests, $p<0.05$). 'Significantly different from the saline plus saline or the oxytocin plus saline group, after separate one-tailed Student's t-tests, at $p<0.001$.

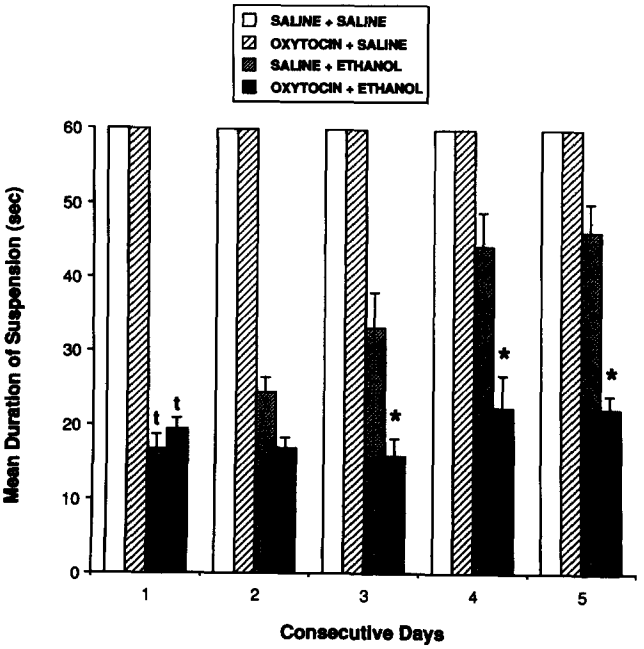

FIG. 2. Attenuation by oxytocin of tolerance to the myorelaxant effect of ethanol. Mice received daily intraperitoneal injections of either saline or oxytocin (0.005 mg/animal), and after 2 hours, of saline or ethanol (2 g/kg), for 5 consecutive days. *Significantly different from the saline plus ethanol group at the same day (after a two-way ANOVA followed by Newman-Keuls tests, $p<0.05$). 'Significantly different from 60 (mean of both saline plus saline and oxytocin plus saline groups which did not exhibit myorelaxation), after separate one-tailed Student's t-tests, at $p<0.001$.

in our laboratory colony, were housed in groups of 8 in opaque plastic cages. The colony room was maintained on a 12-hour light-dark cycle (beginning at 0800 hours) and maintained at a temperature of $23 \pm 2°C$. Throughout the experiment, continuous access was permitted to food and tap water.

Drugs

Exposure to a stressor can facilitate tolerance to the hypothermic effect of ethanol in laboratory rodents (9). Given that intracerebroventricular injection is obviously highly stressful, and can thereby produce important interfering neuroendocrine changes, this route of administration was not adopted. Oxytocin (UCB Bioproduct, Brussels, Belgium) and ethanol were injected intraperitoneally.

Immediately prior to use both drugs were dissolved in 0.9% saline; ethanol (2 g/kg/10 cc) was administered 120 min after oxytocin (0.005 mg/animal/0.2 cc).

Temperature Measurement

Rectal temperature was monitored with an electronic thermometer (RS Components Stk No. 610-067) and a small lubricated probe. The animal was loosely restrained by hand and the probe was inserted 1.5 cm into the rectum until the temperature stabilized (approximately 30 s). Rectal temperature was monitored 20 min prior to and 20 min after ethanol injection. The difference between both values was employed as a measure of ethanol-induced hypothermia. Rectal irritation developed after six days during pilot experiments, therefore the duration of the experiment was limited to five days.

Muscle Tone

Immediately following the second temperature measurement (22 min postethanol treatment), mice were placed in the center of a horizontal wire mesh (17×17 cm). The wire mesh was then turned upside down and maintained 20 cm above the table. The time elapsed between the rotation of the wire and the fall of the animal was recorded. Tests were stopped after 60 s, the maximum score (60) was attributed to mice that did not fall. Because normal animals do not fall, the time taken to fall is dependent upon the degree of myorelaxation induced by ethanol.

Motion

Immediately following tests of myorelaxation, animals were placed in an individual Plexiglas arena ($29 \times 21 \times 10$ cm). Behavioural observation began forty min postethanol treatment. A time-sampling observational technique was employed to assess motion. Mice were observed individually in turn during 60 s, every 10 min, throughout four turns. Within each of these 60-s periods, the duration of akinesia was recorded. The maximal possible score for an animal was 60 s $\times$ 4 turns = 240 s. A mouse was considered akinesic when resting and lying still on ventrum, sometimes with one or more paws elongated on the ground, with complete cessation of motion. Pilot experiments showed that mice exhibiting such an abnormal behavior did not respond to being touched and pushed sideways by the experi-

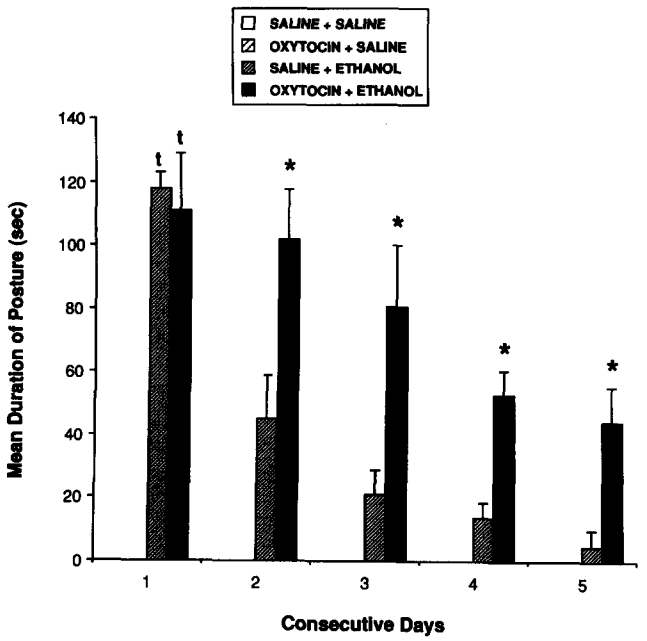

FIG. 3. Attenuation by oxytocin of tolerance to the akinesic effect of ethanol. Mice received daily intraperitoneal injections of either saline or oxytocin (0.005 mg/animal), and after 2 hours, of saline or ethanol (2 g/kg), for 5 consecutive days. *Significantly different from the saline plus ethanol group at the same day (after a two-way ANOVA followed by Newman-Keuls tests, $p<0.05$). 'Significantly different from 0 (absence of akinesia in both saline plus saline and oxytocin plus saline groups), after separate one-tailed Student's t-tests, at $p<0.001$.

menter. In addition, no loss of righting reflex was seen in these mice, indicating that akinesia was not due to narcosis.

Tolerance Development

The same number of subjects in each home cage was randomly allocated to one of the four following treatment conditions ($n=8$): oxytocin plus ethanol, saline plus ethanol, oxytocin plus saline or saline plus saline. Experiments were carried out between 1300 and 1800 hours. Mice were treated daily at approximately the same time for 5 consecutive days. Each day, the animals first received oxytocin or saline, then returned into the colony room until the first temperature measurement, which was performed in the testing room. The testing chamber was illuminated by one 60-W light bulb and maintained at a temperature of 22 ± 1°C. Two hours after oxytocin administration, mice were injected with ethanol or saline and the hypothermic, myorelaxant and akinesic effects of the drug were assessed as described above.

Statistical Analysis

Analysis of variance (ANOVA) was used separately for each parameter under study, taking into account some restrictions. The variances of the control groups, saline plus saline and oxytocin plus saline, were 0 every day for myorelaxation and akinesia and at the fourth and fifth days (only saline plus saline) for hypothermia. Therefore, data considered in a design involving all the possible conditions for each parameter did not meet the fundamental assumptions of homoscedasticity for ANOVA. In this case, no transformation is able to reduce the heterogeneity of variances. Consequently, data were analyzed without saline plus saline and oxytocin plus saline groups, with a two-way ANOVA involving oxytocin (two levels) as a between-subject factor and consecutive days (5 levels) as a within-subject factor. These analyses assessed the effect of oxytocin on ethanol tolerance, which is the main purpose of the present work. Meaningful differences between means were evaluated subsequently with Newman-Keuls tests, derived from the appropriate error mean squares (21).

To ascertain that ethanol did induce significant levels of hypothermia, myorelaxation and akinesia on the first day (basic condition), separate one-tailed Student's t-tests were conducted, comparing each ethanol group to its respective saline group (13). The population mean specified by the null hypothesis was taken as 0.05 (saline plus saline) or -0.0375 (oxytocin plus saline) for hypothermia, 60 (both groups) for myorelaxation and 0 (both groups) for akinesia. These numbers were the actual means obtained for the control groups (see Figs. 1–3).

All significance levels were established at alpha $=0.05$.

RESULTS

As can be seen in Figs. 1, 2, and 3, the effects of oxytocin on the development of ethanol tolerance presented a comparable profile for the three parameters under study. The obvious hypothermic, myorelaxant and akinesic effects of ethanol at the first day (saline plus ethanol group) were sustained by Student's t-tests (all differences at $p<0.001$).

ANOVA applied on saline plus ethanol and oxytocin plus ethanol data revealed an interaction between oxytocin and consecutive days in hypothermia, myorelaxation as well as akinesia, $F(4,56)=4.37$, $p=0.0038$; $F(4,56)=7.98$, $p=0.0001$; $F(4,56)=2.56$, $p=0.0486$, respectively. As shown on the graphs, the response to ethanol developed differently in the oxytocin plus ethanol groups compared to the saline plus ethanol groups as a function of the consecutive days. The saline plus ethanol group showed less hypothermia and myorelaxation (Figs. 1 and 2) during the last three days and less akinesia (Fig. 3) during the last four days than were seen after the first ethanol administration. These differences indicated tolerance development for the three parameters under study. In each measure, tolerance to ethanol was tempered by oxytocin. Specifically, the oxytocin-pretreated group displayed a greater ethanol responsivity than the saline-pretreated group did each consecutive day after the second day for hypothermia and myorelaxation (Figs. 1 and 2), and after the first day for akinesia (Fig. 3). In addition, oxytocin blocked the development of tolerance as determined during the five-day investigation for hypothermia and myorelaxation (Figs. 1 and 2). However, the attenuative effect of oxytocin did not exclude the development of some tolerance to ethanol-induced akinesia: the oxytocin-pretreated group exhibited less akinesia during the last two days than after the first ethanol administration (Fig. 3).

There was no change in responsivity throughout the five daily tests for the two control groups (saline plus saline and oxytocin plus saline). This indicates that chronically injected oxytocin did not influence per se thermoregulation, muscle tone and motion in our experimental conditions.

DISCUSSION

The results of the present experiment indicate that daily ethanol injection over 5 days led to the development of tolerance to the hypothermic, myorelaxant and akinesic effects of ethanol. Oxytocin administration prior to each ethanol injection attenuated the development of tolerance to the three effects under study.

Our data concerning body temperature substantially agree with the results of Szabó and co-workers (18). The multiple oxytocin-ethanol injections paradigm used by these authors has been chosen in this experiment, as well as the 2-hour interval between peptide and ethanol administration. Such a delay seems to be particularly suitable because it allows the assessment of tolerance without direct effects of the peptide itself nor interaction with the acute response to ethanol (3). This was confirmed in this experiment by the fact that oxytocin did not affect body temperature of saline-treated mice nor hypothermia induced by the first ethanol injection. Oxytocin was also without intrinsic effect on muscle tone and motion (oxytocin plus saline groups).

In addition to species, dose and route of administration differences, the relative shorter length of the oxytocin-ethanol interval (10 min) may explain why Pucilowski and co-workers (10) did not find selective effect of oxytocin on tolerance to hypothermic effect of ethanol in rats. They reported that oxytocin administered following this schedule tended to counteract hypothermia induced by acute ethanol administration, and that this effect reached significance for one of the oxytocin doses used.

In our study, some differences in time course and amplitude of tolerance appeared between the three measures. In the saline plus ethanol group, there was a significant decrement in hypothermic and myorelaxant effects from the third day (of 28% and 49%, respectively, as compared to the first day). Tolerance to the akinesic effect of ethanol was less progressive, since a sharp decrease (of 63%) in akinesia can be seen as soon as the second injection. The attenuative effect of oxytocin took place at the same time tolerance developed. In oxytocin plus ethanol group, while no substantial change appeared between days in hypothermia and myorelaxation, a significant tolerance was still present in akinesia. In addition, oxytocin seemed to induce an apparent stronger action on akinesia, since, for example, mice receiving oxytocin before ethanol exhibited degrees of akinesia at the second day 2–2.5-fold higher than those of saline plus ethanol group, whereas this difference was minimal in the other two measures. All this could suggest a more rapid and pronounced tolerance for the akinesic effect of ethanol. However, the scales of measurement were not the same for the three parameters, and possible differences of sensitivity of the scale might explain the apparent sharper tolerance to the akinesic effect.

In the ethanol-treated mice, as tolerance developed to lying still, an unexpected less severe form of akinesia, consisting of immobilization while sitting or standing still, emerged, which was not quantified. This immobilization resembles the behavioral effect of ethanol Smoothy and Berry (14) have reported in mice. In this paper, an increase in the total duration and the number of bouts of immobility without lying, that can coexist with an increase in locomotion, was observed after an acute administration of intermediate and high doses of ethanol. Then, if akinesia were evaluated using the duration of immobility while lying, sitting or standing still instead of the duration of lying only, tolerance to the akinesic effect would have been less pronounced and probably less rapidly developed. In addition, the fact that motion assessment began 20 minutes later, and lasted for a longer time than hypothermia and myorelaxation measurement, could also contribute to an apparent greater sensitivity to tolerance development for the measure of akinesia. Tolerance may not be due only to a decrease in response amplitude but also to a diminished duration of the drug effect. Then, when different drug effects are compared, it is difficult to distinguish the differences related to the response per se from those inherent to the type of measurement.

Brain processes underlying the different CNS effects of ethanol are not yet clearly understood, but the results of genetic and pharmacological experiments indicated that they can be differentiated [e.g., (1, 2, 8)]. Nevertheless, the results of experiments studying the influence of pharmacological manipulation on tolerance to distinct effects of ethanol suggested that ethanol-related phenotypes may share common tolerance processes [e.g., (5, 6, 20)]. The present results, showing that oxytocin attenuates tolerance development to hypothermia, myorelaxation and akinesia, support this notion and suggest a neuropeptidic modulation common to the three different effects of ethanol.

Clinical implications of such an action of oxytocin in humans remain to be defined. It is, however, interesting to note that a change in serum levels of neurophysin II, part of oxytocin precursor, has been found during alcohol withdrawal therapy (7). In about half of these patients, the levels of neurophysin II were higher than normal at the beginning of the withdrawal period and faded progressively within the first week. The peptide levels were correlated with most of the alcoholism blood markers and these patients admitted to greater alcohol consumption immediately before admission, thus suggesting a stimulatory effect of alcohol on oxytocin release. However, neurophysin II levels and mean daily alcohol intake before hospitalization were not correlated.

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