Involvement of GABA-A receptor chloride channel complex in isolation stress-induced free choice ethanol consumption in rats

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The present study revealed the effect of diazepam, a benzodiazepine, and progesterone, a pregnancy precursor of neurosteroids, which act via modulating GABA-A chloride channel complex on the isolation stress-induced free choice ethanol consumption in adult rats. Isolation stress for 24 hr over a period of 6 days produced a significant increase in ethanol consumption, which persisted during the 6-day recovery period. Pretreating the animals with diazepam (5 mg/kg, ip), or progesterone (5 mg/kg, ip), blocked the isolation stress-induced increase in ethanol consumption. Bicuculline (2 mg/kg, ip), a GABA-A receptor antagonist significantly attenuated the effect of both diazepam and progesterone on stress-induced modulation of ethanol consumption. Isolation stress also caused an increase in total fluid consumption, which was antagonised by both diazepam and progesterone. Like ethanol consumption, this effect of diazepam and progesterone on isolation stress-induced increase in total fluid consumption was attenuated by bicuculline. Neither diazepam nor progesterone produced an increase in ethanol consumption in non-stressed rats. However, unlike diazepam, progesterone administration to non-stressed rats caused a significant increase in total fluid consumption. Results of the present study thus show that GABAergic mechanisms may be playing an important role in isolation stress-induced increase in ethanol consumption.

Benzodiazepine (BZD)–GABA-A receptor complex has been implicated in the physiological regulation of many stress responses. BZDs which act by modulating GABA-A receptor-mediated chloride ion conductance have been shown to exert anxiolytic and anti-stress effects. Progesterone which potentiates binding to GABA-A receptor, is metabolised to allopregnanolone and tetrhydrodeoxycorticosterone in the neuronal glia of the brain and both these compounds are among the most potent of the known neurosteroids active at GABA-A receptors with affinities greater than BZDs. Some of the important effects of progesterone, such as antidepressant, anxiolytic, analgesic, neuroprotective, locomotor suppressive activity, sexual receptivity, neural control of circulation, etc, are reported to be mediated via these neurosteroids. In the present study an attempt has been made to investigate the effect of diazepam, a BZD and progesterone, a pregnancy precursor of neurosteroids, on the isolation stress-induced free choice ethanol consumption in rats in order to delineate the role of GABA-A receptor chloride channel complex in this response.

Materials and Methods

Animals—The study was carried out in male Wistar rats 8-10 months old, weighing between 375 and

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400 g. The animals were housed 6/cage in polyvinyl cages (17x11x6 inches) in temperature (22±2°C) and humidity controlled conditions with 12 hr light—12 hr dark cycle. The rats were allowed to acclimatize for one week before commencing the experiment, so as to nullify any effect of stress due to experimental group housing. The food (Pellet diet, Golden Feeds, Delhi) and drinking solutions were available ad libitum.

Drinking solutions—Two solutions were presented to animals for drinking. One consisted of 0.2% saccharin solution in tap water and the other of 10% ethanol (v/v) in 0.2% saccharin solution. The solutions were freshly prepared every day.

Experimental groups—Sixty rats were randomly divided into 2 main groups, Group I and II. Each group was further divided into 5 subgroups consisting of 6 animals each. The subgroups of group I were subjected to the following treatment: group V—vehicle, Group D—diazepam (5 mg/kg, ip), group S—isoalcohol stressed rats injected with vehicle, Group SD—isoalcohol stressed rats injected with diazepam (5 mg/kg, ip). Group SDB—isoalcohol stressed rats injected with diazepam (5 mg/kg, ip) + bicuculline methiodide (2 mg/kg, ip). Similarly, animals of Group II were treated with: Group V—vehicle, Group P—progesterone (5 mg/kg, ip), Group S—isoalcohol stressed rats injected with vehicle, Group SP—isoalcohol stressed rats injected with progesterone (5 mg/kg, ip), Group SPB—stressed rats injected with progesterone (5 mg/kg, ip) + bicuculline methiodide (2 mg/kg, ip). Since the data of control (vehicle) and stressed rats were similar in the two groups, the values were pooled together for comparison with the drug-treated groups.

Stress procedure—Following a 6-day base line period of group housing (6 rats/cage), the animals in the stressed groups were subjected to 6 days of isolation stress (test period). The isolation stress was induced by placing the animals alone 1 rat/cage (17x11x6 inches) in a novel environment of separate room. Stress period ranged from 1 to 7 hr on any particular day following a random unpredictable schedule. The total duration of exposure of isolation stress to each animal was of 24 hr over a period of 6 days. The non-stressed rats were exposed to continuous group housing condition of the animal room during this period. The isolation stress period was followed by a recovery period of 6 days. Consumption of both 10% ethanol in saccharin solution and saccharinated water were measured separately for baseline period, test period and recovery period.

Measurement of alcohol consumption—Saccharinated water solution was presented in two bottles for one week of acclimatization period. Following this period one bottle of saccharinated water solution was replaced by the ethanol + saccharin solution for the entire duration of the study. The volume of solution remaining in each bottle was recorded daily between 9.00 and 10.00 AM. The bottles were then refilled and the positions (left- right) alternated to prevent the development of positional preference. Daily fluid consumption (both 10% ethanol and saccharinated water) and daily ethanol intake (in terms of absolute alcohol) in ml/kg/day were calculated.

Drugs—Diazepam (Ranbaxy, India), progesterone and bicuculline methiodide (Sigma Chemical Co., USA) were used for the study. Diazepam injections containing benzyl alcohol USP NF 1.5% (w/v) were used. Progesterone was dispersed in 1% Tween 80 and diluted with saline. Drugs/vehicle were injected daily at 10.00 AM in a volume of 5 ml/kg just before the initiation of test (stress) period.

Statistical analysis—Mean 24 hr total fluid and ethanol consumption for each group of animals was calculated in ml/kg body weight. Data were analysed by using Hierarchical ANOVA and multiple comparisons were made by Tukey's test at 5% level of significance.

The experimental protocol was approved by the Institutional Animal Ethics and Research Review Committee. The care of the animals was as per the "Guidelines for the Care and Use of Animals in Scientific Research" prepared by the Indian National Science Academy, New Delhi.

Results

Adult rats exposed to isolation stress showed significantly increased ethanol consumption as compared to non-stressed animals [3.88±0.49 (mean±SD) in vehicle-treated control Vs 7.31±0.99 ml/kg/day in stressed animals or in terms of 10% ethanol solution 38.8±4.9 Vs 73.1±9.9 ml/kg/day] (P<0.001; F=37.992). Further, stressed animals exhibited a greater ethanol consumption during the test (stress) period (7.31 ± 0.99 or 73.1 ± 9.9 ml/kg/day of 10% ethanol solution) when compared to baseline (non-stressed) period (3.89 ± 0.49 or 38.9 ± 4.9 ml/kg/day of 10% ethanol solution) (P<0.001; F=111.603). This increase in ethanol consumption was observed to persist during the post- stress recovery period (Table 1). Pretreating the animals with diazepam (5 mg/kg, ip) or progesterone (5 mg/kg, ip) significantly attenuated
the effect of isolation stress on ethanol consumption. These effects of both diazepam and progesterone were blocked by bicuculline (2 mg/kg, ip; Fig. 1). Neither diazepam nor progesterone when administered to non-stressed rats were observed to modulate the free choice ethanol consumption (Table 1).

Total fluid consumption was significantly increased in isolation stressed rats (102.68 ± 8.31 in vehicle control vs 131.28 ± 6.5 mg/kg/day in stressed animals) (P < 0.001; F = 30.506) (Table 1). This stress-induced increase in total fluid consumption was significantly blocked by diazepam as well as by progesterone treatment. Further, like in case of ethanol consumption, effects of both diazepam and progesterone were antagonized by bicuculline pretreatment (Fig. 2). However, unlike diazepam, progesterone administration increased total fluid consumption even in non-stressed rats (Table 1).

Discussion

The results of the present study show that isolation stress produced a significant increase in free ethanol consumption in adult rats. These results corroborate the findings of other workers who also observed an increase ethanol consumption in young and adult rats when exposed to different stressful situations. The increase in ethanol consumption was effectively blocked by diazepam, a BZD and progesterone which is metabolized to neurosteroids, like allopregnanolone and tetrahydrodeoxycorticosterone in the neuronal glia of the brain. Both BZDs and neurosteroids have been shown to exhibit antistress activities and to attenuate various effects of stress, including rise in plasma corticosterone levels. The adrenal corticosterone hypersecretion produced by ACTH was reported to modulate stress-induced increase in ethanol consumption. For example, adrenalectomized rats failed to show an increase in ethanol consumption as compared to sham operated rats during stress induced by food restriction. Further, treatment of rats subjected to food restriction stress with cyanoketone, an inhibitor of enzyme involved in the stress-induced hypersecretion of adrenal corticosterone blocked the effect of stress on ethanol consumption. Hence, diazepam and progesterone in the present study may be inhibiting isolation stress-induced increase in ethanol consumption by modulating corticosterone secretion. The observed increase in ethanol consumption seen during the stress (test) period was maintained during the post-stress (recovery) period when compared to non-stressed control animals. This could be due to a decrease in negative feed back sensitivity of hypothalamic-pituitary-adrenocortical (HPA) axis to glucocorticoids due to persistently high corticosterone levels during the stress period. Alternatively maintenance of increased ethanol consumption during recovery period could be due to development of dependence to alcohol intake. Both diazepam and progesterone, when administered to non-stressed rats failed to modulate ethanol consumption. This observation is in agreement with the findings of Wolfe et al., who also reported that progesterone when administered to Myers High Ethanol Preferring rats did not change ethanol consumption.

Table 1 — Effect of progesterone, diazepam and isolation stress on ethanol and total fluid consumption in rats

<table>
<thead>
<tr>
<th>Ethanol consumption</th>
<th>Treatment (mg/kg)</th>
<th>Baseline period</th>
<th>Test period</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Vehicle (Control)</td>
<td>3.79 ± 0.44</td>
<td>3.88 ± 0.49</td>
<td>3.88 ± 0.51</td>
</tr>
<tr>
<td>P</td>
<td>Progesterone (5)</td>
<td>3.66 ± 0.49</td>
<td>4.05 ± 0.75</td>
<td>3.66 ± 0.39</td>
</tr>
<tr>
<td>D</td>
<td>Diazepam (5)</td>
<td>4.21 ± 0.50</td>
<td>4.42 ± 0.56</td>
<td>4.48 ± 0.77</td>
</tr>
<tr>
<td>S</td>
<td>Stress</td>
<td>3.89 ± 0.49</td>
<td>7.31 ± 0.99</td>
<td>7.11 ± 0.72</td>
</tr>
</tbody>
</table>

Total fluid consumption

<table>
<thead>
<tr>
<th>Ethanol consumption</th>
<th>Treatment (mg/kg)</th>
<th>Baseline period</th>
<th>Test period</th>
<th>Recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>Vehicle (Control)</td>
<td>100.59 ± 7.86</td>
<td>102.68 ± 8.31</td>
<td>102.35 ± 6.39</td>
</tr>
<tr>
<td>P</td>
<td>Progesterone (5)</td>
<td>101.27 ± 7.71</td>
<td>123.16 ± 4.05</td>
<td>120.08 ± 4.01</td>
</tr>
<tr>
<td>D</td>
<td>Diazepam (5)</td>
<td>98.77 ± 10.16</td>
<td>101.65 ± 5.51</td>
<td>102.87 ± 6.74</td>
</tr>
<tr>
<td>S</td>
<td>Stress</td>
<td>102.71 ± 8.44</td>
<td>131.28 ± 6.55</td>
<td>130.11 ± 9.30</td>
</tr>
</tbody>
</table>

*P value * < 0.001 (Hierarchical ANOVA with Tukey’s test).

a. Compared to control (vehicle) group.
b. Compared to progesterone/diazepam treated group.
B. Compared to baseline data of the respective group.
Fig. 1 — Effect of progesterone and diazepam on ethanol consumption in stressed rats. (Values are mean ± SD) 24 hour consumption of 10% ethanol solution by 6 rats over a 6 day period represented in ml/kg/day)

S = Stress; SP = Stress + Progesterone (5 mg/kg); SD = Stress + Diazepam (5 mg/kg); SPB = Stress + Progesterone + Bicuculline (2 mg/kg);
SDB = Stress + Diazepam + Bicuculline

*P value * < 0.001 (Hierarchical ANOVA with Tukey's test)

c. Compared to stress group. d. Compared to stress + progesterone/stress + diazepam group. B. Compared to baseline data of the respective group.

Fig. 2 — Effect of progesterone and diazepam on total fluid consumption in stressed rats

(Values are mean ± SD) 24 hour total fluid consumption by 6 rats over a 6 day period represented in ml/kg/day)

S = Stress; SP = Stress + Progesterone (5 mg/kg); SD = Stress + Diazepam (5 mg/kg); SPB = Stress + Progesterone + Bicuculline (2 mg/kg);
SDB = Stress + Diazepam + Bicuculline

*P value * < 0.001 (Hierarchical ANOVA with Tukey's test)

c. Compared to stress group. d. Compared to stress + progesterone/stress + diazepam group. B. Compared to baseline data of the respective group.
BZDs are known to produce their effects via modulation of GABA-A chloride channel complex. Further, neurosteroids, like allopregnanolone and tetrahydrodeoxycorticosterone are among the most potent of the known neurosteroids active at GABA-A receptors and produce a positive allosteric modulation of GABA evoked chloride currents. Both BZDs and neurosteroids have been shown to attenuate stress-induced increase in corticosterone levels. Besides, exposure to corticosterone is reported to modulate rat hippocampal GABA system. Thus, diazepam, a BZD and progesterone, which is metabolised to allopregnanolone and tetrahydrodeoxycorticosterone may be producing their effect by modulating central BZD-GABA-A receptor chloride channel complex which may in turn regulate the release of neuroendocrine hormones. This suggestion gains further credence from the observations of present study that the inhibitory effect of diazepam and progesterone on isolation stress-induced increase in ethanol consumption was significantly antagonized by bicusculine, a GABA-A receptor antagonist, implicating thereby the involvement of GABA-A receptor in this stress response.

Besides ethanol, total fluid consumption was also increased in stressed animals. This change appears to be due to an associated increase in ethanol consumption since, like ethanol intake, this increase in fluid consumption was attenuated by both diazepam and progesterone. Further, like ethanol consumption, these effects of diazepam and progesterone were also blocked by bicusculine. However, unlike diazepam, progesterone per se has increased total fluid consumption in non-stressed animals. Although at this point it is difficult to exactly explain this increase in total fluid consumption produced by progesterone in non-stressed rats, it could be related to its reported antimineralocorticoid activity resulting in natriuresis leading to activation of renin-angiotensin system which in turn would modulate thirst response.

References


