RAPID COMMUNICATION

CONSTANT ABSOLUTE ETHANOL INTAKE BY SARDINIAN ALCOHOL-PREFERRING RATS INDEPENDENT OF ETHANOL CONCENTRATIONS

CARLA LOBINA, ROBERTA AGABIO, GIACOMO DIAZ, MAURO FA, FABIO FADDA, GIAN LUIGI GESSA, ROBERTA REAU and GIANCARLO COLOMBO*  

'Bernard B. Brodie' Department of Neuroscience, 1Department of Cytomorphology and 2Institute of Human Physiology, University of Cagliari, Via Porcell 4, I-09124 Cagliari and 3C.N.R. Centre for Neuropharmacology, Via Porcell 4, I-09124 Cagliari, Italy

(Received 1 August 1996; in revised form 20 September 1996; accepted 22 September 1996)

Abstract — The present study was designed to evaluate ethanol drinking behaviour in Sardinian alcohol-preferring (sP) and Sardinian alcohol-non-preferring (sNP) rats in the presence of different ethanol concentrations. Ethanol intake was tested under the two-bottle, free-choice regimen and continuous access schedule. Ethanol-naive sP and sNP rats were initially given ethanol solution at the standard, constant concentration of 10% (v/v) for 8 consecutive days (Phase 1). As expected, daily ethanol intake in sP rats rose from 4 to ~6g/kg; in contrast sNP rats consumed <10g/kg/day ethanol. Subsequently, an ascending series of ethanol concentrations, ranging from 3 to 60% (v/v), was presented to sP and sNP rats over a 28-day period (Phase 2). At concentrations varying from 7 to 30%, sP rats consumed constant amounts of absolute ethanol per kg of body weight (~6.0 g/kg/day). Daily ethanol intake in sNP rats remained constantly lower than 1.0 g/kg, irrespective of the ethanol concentration. Data from Phase 2 demonstrate the ability of sP rats to precisely adjust daily ethanol intake and support the hypothesis that voluntary ethanol drinking in sP rats is sustained by specific pharmacological effects of ethanol.

INTRODUCTION

Sardinian alcohol-preferring (sP) and alcohol-non-preferring (sNP) rats constitute one of the five pairs of rat lines selectively bred worldwide for opposite ethanol preference and consumption (for recent reviews on these rat lines, see Crabbe et al., 1994; Li et al., 1994). Under the conventionally used two-bottle free-choice regimen between 10% (v/v) ethanol solution and water, sP rats consume daily >4 g of ethanol/kg body weight and avoid water almost completely (Colombo et al., 1995). Daily ethanol intake occurs in distinct binges, rather than regularly distributed over the nocturnal phase, and pharmacologically relevant blood ethanol levels (BELs) are achieved at each drinking binge (Agabio et al., 1996). In contrast, sNP rats consume negligible amounts of ethanol and greatly prefer water (Colombo et al., 1995).

The present study was designed to further characterize the ethanol-drinking behaviour of sP rats, by determining ethanol intake in the presence of an ascending series of ethanol concentrations (3–60%, v/v). Previous studies have shown that voluntary ethanol intake in selectively bred, ethanol-preferring P and HAD rats was 1–2-fold higher when the ethanol solution was presented at concentrations of 20–25% (v/v), rather than at the standard 10% (v/v) concentration (Lankford et al., 1991; Lankford and Myers, 1994; Long et al., 1996), indicating the presence, in these rat lines, of a quite unexpected preference for ethanol concentrations >10%.

MATERIALS AND METHODS

Animals

Male sP (n = 10) and sNP (n = 9) rats, from the 35th generation and 75 days old at the start of the study, were used. The animal facility was maintained on a 12h:12h artificial light/dark cycle
Fig. 1. Daily ethanol intake of Sardinian alcohol-preferring and -non-preferring rats with ascending concentrations of ethanol. Sardinian alcohol-preferring (sP; filled circle; n = 10) and -non-preferring (sNP; open circle; n = 9) rats were kept under ethanol vs water free-choice regimen for 24 h/day. During Phases 1 and 3, ethanol was offered at the constant concentration of 10% (v/v); during Phase 2, ethanol concentration was changed every 2 days from 3 to 60% (v/v) as described under Materials and methods. Three-day wash-out periods (with replacement of ethanol by water) were interposed between the different phases. Each point is the mean ± SEM.

Procedure

Voluntary ethanol intake occurred under the two-bottle, free-choice regimen, as previously described (Agabio et al., 1996). The two bottles were presented for 24 h/day and their placing interchanged daily to prevent development of position preference. Ethanol and water consumptions were monitored daily at 07:00.

Phase 1. Ethanol was presented to sP and sNP rats at the stable concentration of 10% (v/v) for 8 consecutive days. After this initial phase, ethanol was replaced by water for 3 consecutive days (wash-out period).

Phase 2. Over the subsequent 28 days, the ethanol concentration was increased every 2 days according to the following sequence: 3, 4, 5, 7, 9, 11, 13, 15, 20, 25, 30, 40, 50, and 60% (v/v). At the end of Phase 2, a 3-day wash-out period was interposed.

Phase 3. Finally, ethanol solution was re-offered for 4 consecutive days at the fixed concentration of 10% (v/v).

Data analyses

The hypothesis that sP rats maintain a constant ethanol intake in the presence of an ascending series of ethanol concentrations does not exclude a different response at extreme concentrations. No assumption is made on the variability of these boundary conditions, consequently the key issue was the identification of a sufficiently extended sequence of data with zero slope. Following a post-hoc criterion, Phase 2 was divided into three segments (early, mid and late), corresponding to days 9–15 (ethanol concentration varying from 3 to 7%), 15–30 (7 to 30%) and 30–36 (30 to 60%). Data from these three segments, as well as those from Phases 1 and 3, were independently evaluated by regression analysis. Significance of
regression parameters (intercept and slope) was assessed by the usual t-test ratio (parameter/standard error). However, the null hypothesis of constant ethanol intake for the mid-segment of Phase 2 was not proven merely on the basis of a non-significant slope. Zero slope was globally assessed considering the range of data and relevant test values on Phase 2 in full (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The results of the present study are illustrated in Fig. 1; Table 1 shows the values of regression parameters and statistical significance of data from sP and sNP rats.

During Phase 1, the mean amount of ethanol consumed daily by sP rats was steadily > 4 g/kg and significantly rose to 5–6 g/kg. During Phase 2, daily ethanol intake in sP rats significantly increased from 2.5 to 6 g/kg as ethanol concentration was increased from 3 to 7% (early Phase 2); in contrast, at ethanol concentrations ranging from 7 to 30% (mid-Phase 2), the mean ethanol intake remained constant, ~6.0 g/kg/day; finally, as the ethanol concentration was increased from 30 to 60% (late Phase 2), the mean ethanol intake significantly declined to ~3.0 g/kg/day. Over Phase 3, ethanol consumption in sP rats remained rather constant at ~6.8 g/kg/day.

Daily total fluid intake in sP rats remained moderately constant (averaging 60–80 ml/kg) throughout the entire study; the only exception occurred in early Phase 2, when total fluid intake was steadily >100 ml/kg/day (data not shown).

The mean amount of ethanol consumed by sNP rats was constantly < 1.0 g/kg/day throughout Phases 1, 2 and 3.

Data from Phase 1 of the present study indicate that exposure of ethanol-naïve sP rats to ethanol solution at the standard concentration of 10% (v/v) resulted in a daily ethanol consumption >4 g/kg from the very first day; furthermore, ethanol intake significantly increased to ~6 g/kg within a few days. These data duplicate previous results from this laboratory (Colombo et al., 1995) and confirm that the rapid achievement of substantial levels of ethanol intake, which may underlie an immediate disclosure and acquisition of ethanol-reinforcing properties, is a relevant feature of ethanol drinking behaviour in sP rats.

During Phase 2, ascending concentrations of ethanol (ranging from 3 to 60%, v/v) were presented to sP rats over a 28-day period. As the ethanol concentration was increased from 7 to 30%, sP rats showed a stable and regular daily absolute intake of ethanol (~6 g/kg), with a proportional decline of volumes of ethanol solution. steadiness in daily absolute ethanol intake is shown by a virtually zero (0.0026) slope with 95% confidence limits of -0.0043 and +0.0048 (mid-Phase 2 segment; Table 1). The high p probability (0.903) makes the risk of false-negative or type-II errors irrelevant. Further evidence that the mid-Phase 2 segment had a zero slope is provided by the positive and negative trends (both statistically significant) of early and late Phase 2 segments, respectively, since a zero change point must necessarily be present between two ascending and descending curve segments. In the present study, the zero point was a plateau extending over a 16-day interval, during which ethanol concentration was increased from 7 to 30%.

Thus, sP rats appeared to be able to control precisely, day by day, the amount of ethanol solution consumed in order to maintain a constant absolute intake. The adjustment of ethanol intake, irrespective of the concentration at which the ethanol was presented, supports the hypothesis.

<table>
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<th>Phase</th>
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<th>sNP</th>
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<td>Slope</td>
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Table 1. Parameters (slope and intercept) and statistical significance of regression analysis of data from Phases 1, 2 (divided into early, mid and late segments) and 3 in sP and sNP rats.

During Phase 2, ascending concentrations of ethanol (ranging from 3 to 60%, v/v) were presented to sP rats over a 28-day period. As the ethanol concentration was increased from 7 to 30%, sP rats showed a stable and regular daily absolute intake of ethanol (~6 g/kg), with a proportional decline of volumes of ethanol solution. Steadiness in daily absolute ethanol intake is shown by a virtually zero (0.0026) slope with 95% confidence limits of -0.0043 and +0.0048 (mid-Phase 2 segment; Table 1). The high p probability (0.903) makes the risk of false-negative or type-II errors irrelevant. Further evidence that the mid-Phase 2 segment had a zero slope is provided by the positive and negative trends (both statistically significant) of early and late Phase 2 segments, respectively, since a zero change point must necessarily be present between two ascending and descending curve segments. In the present study, the zero point was a plateau extending over a 16-day interval, during which ethanol concentration was increased from 7 to 30%.

Thus, sP rats appeared to be able to control precisely, day by day, the amount of ethanol solution consumed in order to maintain a constant absolute intake. The adjustment of ethanol intake, irrespective of the concentration at which the ethanol was presented, supports the hypothesis.
that ethanol drinking in sP rats is sustained by the search for specific BELs and, in turn, for specific psychotrophic effects of ethanol (Colombo et al., 1995; Agabio et al., 1996), and not by gustatory and olfactory factors.

The drinking profile shown by sP rats in the present study, at ethanol concentrations varying from 7 to 30%, closely resembles a classical feature of drug self-administration in laboratory animals. Indeed, it has been extensively demonstrated that lowering the drug dose results in an increased number of injections, and vice versa; in both cases, drug intake over each daily session remains rather stable (see Caine et al., 1993).

The results from Phase 2 of the present study also indicate that sP rats consumed smaller amounts of ethanol (in g/kg) at extreme concentrations. At concentrations varying from 3 to 5%, despite polydipsic-like drinking of ethanol solution (which determined mean total fluid intakes >100 ml/kg/day), daily ethanol intake (in g/kg) in sP rats was relatively low; volumes of ethanol solution needed to produce those pharmacological effects perceived by rats during Phase 1 were presumably too large to be consumed. At concentrations ≥40%, ethanol intake was reduced, possibly due to an aversive response to the taste of ethanol solution, which might have limited ethanol drinking.

Contrary to the results of Phase 2 (present study) with sP rats, earlier studies have shown a progressive increase in the daily amount of voluntarily consumed ethanol (in g/kg) by the selectively bred, ethanol-preferring P and HAD rats as the ethanol concentration was increased from 3 to 30% (v/v) (Lankford et al., 1991; Lankford and Myers, 1994; Long et al., 1996). A relatively similar pattern to that shown by P and HAD rats has also been observed in the ethanol-preferring AA rats when ethanol concentrations in the 5–40% (v/v) range were presented (Sinclair et al., 1992). Thus, although P, HAD, AA and sP rat lines have been selectively bred for high voluntary ethanol consumption under similar criteria, the different ethanol drinking patterns, when ascending concentrations of ethanol were presented, might imply the presence of some difference, among these rat lines, in the mechanisms regulating voluntary ethanol intake.

In sharp contrast to the profile of voluntary ethanol drinking in sP rats, daily ethanol intake in sNP rats was steadily <1.0 g/kg, irrespective of the ethanol concentration. These results are consistent with the notion that ethanol is not reinforcing in this rat line.

Acknowledgements — The authors are grateful to Mrs M. Elena Vincis and Mr Antonio Serrau for animal care and breeding. The authors wish to thank Mr Hugh Sugden for language editing of the manuscript. The present study was partially supported by CNR grant #91.04142.STT75 and grant #4173/11668 from Assessorato Igiene e Sanità, Regione Autonoma della Sardegna.

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