Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1167–1174, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Voluntary Alcohol Intake in the Hypertension Prone Dahl Rat

L. A. GRUPP, E. PERLANSKI, I. R. WANLESS* AND R. B. STEWART

Department of Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8
Neurobiology Department, Addiction Research Foundation of Ontario, Toronto, Ontario, Canada M5S 2S1
*and *Department of Pathology, Toronto Western Hospital, Toronto, Ontario, Canada M5T 2S8*

Received 6 May 1985

GRUPP, L. A., E. PERLANSKI, I. R. WANLESS AND R. B. STEWART. *Voluntary alcohol intake in the hypertension prone Dahl rat.* PHARMACOL BIOCHEM BEHAV **24**(5) 1167–1174, 1986.—Previous work in our laboratory has shown that alterations in the sodium content of the diet which alter salt appetite, can modify ethanol self-selection and intoxication. The present experiment investigates the relationship between the intakes of sodium and ethanol on the one hand and the development of hypertension, by measuring voluntary ethanol consumption and blood pressure in two rat lines, the salt sensitive (SS) and salt resistant (SR) Dahl rats, specially bred to show differential sensitivity to dietary sodium supplements. All rats were given 24 hr access to 6% (v/v) ethanol and water and first offered a control diet (0.5% Na) followed by a 4% and then an 8% sodium supplemented diet. While on the control diet there were significant between strain differences in ethanol consumption, suggesting that the SS animals came genetically prepared to consume more ethanol. Blood pressure measured at regular intervals indicated significant changes only in the SS rats even though both lines as well as a group of Wistar rats, added for purposes of comparison, all increased their ethanol intake to the salt supplemented diets. A second experiment suggested that the initial difference in consumption between the SS and SR lines may be related to central nervous system sensitivity since differences were found in salt appetite but not in taste sensitivity or in the absorption, distribution or metabolism of the drug. These findings demonstrate that the Dahl SS rat is predisposed to consume more ethanol than the SR rat even before exposure to the hypertension-inducing diet, and that this predisposition is probably central in origin. Since ethanol consumption and hypertension were correlated only in the SS rats who were already predisposed to hypertension for other reasons, the notion of a direct link between the two could not be supported.

Alcohol drinking Dahl rat Hypertension Renin angiotensin system Salt appetite

THE experimental study of alcohol abuse has been hampered by the fact that most animals will not voluntarily consume sufficient amounts of alcohol to produce tolerance, physical dependence or significant organ damage. Investigators in this field have attempted to deal with this problem: (a) by developing specific training procedures such as schedule-induced polydypsia [7] and other operant conditioning techniques [18,20], designed to increase alcohol intake; (b) by developing genetically selected lines of rats or mice which are differentially sensitive to alcohol either with respect to intoxication or consumption. For example, the most affected (MA) and least affected (LA) rats [24] or the long sleep and short sleep mice [19] differ with respect to intoxication, while the alcohol accepting (AA) and alcohol-non-accepting (ANA) [6], the preferring (P) and nonpreferring (NP) rats [17] or the C57BL and DBA mice [14] differ with respect to intake [14]. It is interesting to note that all but the latter two strains were selected and bred on the basis of their specific responses to alcohol. The C57BL and DBA mice were originally bred for use in cancer research and only later found to respond differentially to alcohol [26]. The experiments described below pertain to solution b above and are the first to present data on a line of rats which, like the C57BL, were initially developed for study in another (but related) field of research, but which we now report are differentially sensitive to alcohol.

In the early 1960s Dahl and his associates described two lines of rats which, by selective breeding from the original Sprague Dawley stock, differed in their cardiovascular response to salt ingestion [2,3]. Rats of the salt-sensitive (SS) line developed sustained increases in blood pressure when fed a high salt diet (8% NaCl), while the salt-resistant (SR) line remained normotensive when fed the same high salt diet.

Our interest in the Dahl lines of rat stems from their differential response to a dietary salt supplement and from the fact that the diet-naive SS rat has a lower renin-angiotensin activity than the SR rat [12,22]. Over the past few years, studies in our laboratory have shown that alcohol intake and intoxication can be modulated either by changing the sodium content of the diet or by pharmacologically manipulating the system controlling sodium balance, i.e., the renin-angiotensin system. For example, we have shown that a low sodium diet plus a brief diuretic regimen, can produce a rapid and sustained decrease in alcohol intake in either a two bottle 24 hr choice paradigm [8] or an operant conditioning paradigm [10]. Conversely, a high salt diet can produce a selective and significant increase in alcohol intake using the two bottle choice paradigm [9]. Chronic injections of the synthetic mineralocorticoid DOCA which increases renin release were found to increase alcohol-induced intoxication and decrease alcohol intake, while chronic administration of indomethacin, which decreases renin release, decreased

alcohol-induced intoxication [9,11]. Thus, the investigation of the voluntary alcohol intake in the Dahl rats affords yet another examination, but from a rather different perspective, of the possible role of sodium and the renin angiotensin system in the control of alcohol intake. Finally, the use of Dahl rats as subjects in experiments on alcohol consumption provides an opportunity to further assess the relationship between hypertension and alcohol consumption.

EXPERIMENT 1

This experiment describes the changes in both alcohol and water intake of the SS and SR rats when their diet is supplemented with NaCl. For purposes of reference and comparison, a group of Wistar rats, a strain frequently used in alcohol research, is also tested.

SUBJECTS

The subjects were 30 naive male rats: 10 Dahl SS rats, 10 Dahl SR rats (Brookhaven Laboratory) and 10 Wistar rats (C-Charles River, Montréal). While Dahl rats are derived from Sprague-Dawley stock, our previous studies (as well as many others in the literature) involved the use of Wistar rats. For this reason, the latter group of animals was added for purpose of reference and comparison.

The animals began the experiment in the 300–350 g weight range and were individually housed in cages equipped with a glass feeder cup located between two Richter tubes which were spaced 15 cm apart. A 12 hr/12 hr light/dark cycle was in effect throughout the experiment with lights on at 7 a.m.

PROCEDURE

Ethanol and Water Consumption

All animals were allowed continuous access to two tubes, one containing a 6% ethanol (v/v) solution made up in distilled water and the other containing distilled water. The positions of the water and ethanol tubes were alternated daily and consumption was measured over consecutive 24 hr periods. The experiment consisted of six phases each lasting approximately 25 days. During phases 1, 3, and 6 all animals were fed powdered Purina Rat Chow which contains 0.5% sodium (control diet). During phases 2, 4, and 5 all rats were offered a diet of Purina Rat Chow supplemented with either 4% NaCl (phase 2) or 8% NaCl (phases 4 and 5).

Blood Pressure

At the end of phase 1, four animals from each group who drank the most ethanol during the final 5 days were chosen to assess changes in blood pressure. Blood pressures were measured by the tail-cuff method and were taken at the end of each of the six phases. The reported values are the average of four determinations per animal.

Histological Methods

For up to 120 days following phase 6, all rats were switched to the 8% salt supplemented diet and offered only a 10% (v/v) ethanol solution to drink. The animals were then sacrificed by decapitation and one hematoxylin and eosin stained section (approximately 0.5 cm^2) of liver and kidney from each animal was examined by light microscopy. The slides were graded blindly on two separate occasions for large and small droplet steatosis, hepatocellular eosinophilia, and hyaline inclusions on a scale of − to +++. Mitotic activity and acidophil bodies were graded − or +. Foci of necrosis were noted. Electron microscopy was performed on selected livers.

RESULTS AND DISCUSSION

Three animals from the SS group died before the end of the experiment, including one animal assigned to the blood pressure subgroup. Their data are not presented.

Ethanol Intake

Figure 1 presents the mean ethanol intake for each group of animals across the six phases of the experiment. A repeated measures ANOVA revealed a significant effect of Group, $F(2,24)=7.04$, $p<0.003$, a significant effect of Phase, $F(5,120)=102.96$, $p<0.000$, and a significant Group × Phase interaction, $F(10,120)=2.93$, $p<0.003$. This indicates that ethanol intake did vary significantly among the different groups of animals, that the diet significantly altered the amount of ethanol consumed and that differences among the groups depended on the diets offered.

Between group effects. Throughout all but the final phase of the experiment the SS rats tended to drink the most ethanol, followed by the SR rats and then the C rats. A simple effects analysis ($p<0.01$) confirmed significant differences among the groups during the first five phases (phase 1—$F(2,24)=11.01$; phase 2—$F(2,24)=4.49$; phase 3—$F(2,24)=3.91$; phase 4—$F(2,24)=5.37$; phase 5—$F(2,24)=8.51$; phase 6—$F(2,24)=0.40$). In order to examine the group differences more closely, pairwise post hoc group comparisons for each separate phase were performed using Duncan's test with $\alpha=0.05$.

During phase 1, when the animals had not yet been exposed to any salt supplement, the SS animals drank significantly more ethanol than both the SR and the C animals, while the SR animals also drank significantly more than the C animals. The introduction of a 4% NaCl supplement in phase 2 maintained the same rank order as in phase 1 except that there was no longer a significant difference in consumption between the SS and SR groups of animals. In phase 3, the salt supplement was removed, and the only significant difference in intake occurred between the SS and the C groups. An 8% NaCl supplement was introduced in phases 4 and 5. In both, a significant difference in consumption was seen between the SS and the C groups. The SS and SR rats continued to drink statistically similar amounts of ethanol. In addition, during phase 5, the SR and the C groups also differed significantly in intake. With the return to control diet in phase 6 all three groups drank similar amounts of ethanol.

Within group effects. A second set of post hoc comparisons using Duncan's test ($\alpha=0.05$) examined the effect of the dietary manipulations on the ethanol consumption of each individual group of animals.

For the SS group, the introduction of the 4% NaCl diet in phase 2 did not significantly alter ethanol intake, nor was there any change when the control diet was reintroduced in phase 3. However, the 8% NaCl supplement in phase 4 significantly elevated intake and its continued presence in phase 5 resulted in an even greater and significant elevation in intake. A return to control diet in phase 6 resulted in a significant drop in intake back to control diet levels.

The SR rats, on the other hand, significantly increased ethanol intake in response to the 4% NaCl diet (phase 2) to a level which did not change upon return to the control diet in phase 3. However, the 8% NaCl supplements in phases 4 and

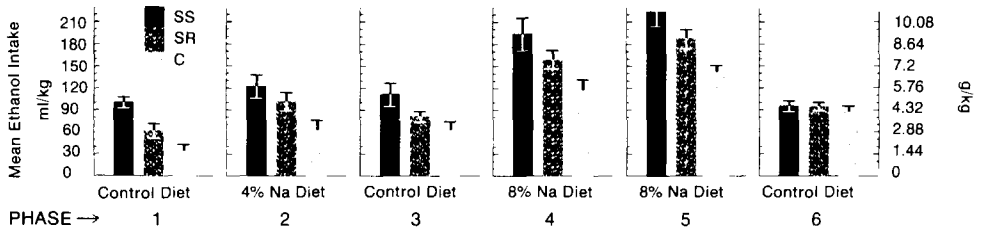

FIG. 1. Mean ethanol intake (in volume and grams per kg body weight) across the twenty-five sessions of each phase. SS—Dahl salt sensitive: SR—Dahl salt resistant; C—Wistar (Charles River). Bars represent ± standard error of the mean.

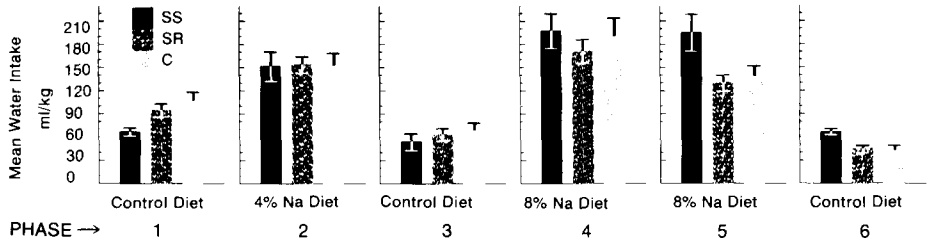

FIG. 2. Mean water intake in ml per kg body weight across the twenty-five sessions of each phase. Conventions as per Fig. 1.

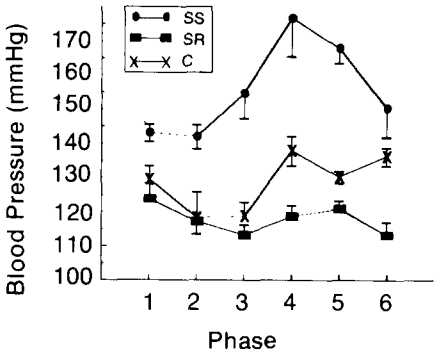

FIG. 3. Mean blood pressure in the three subgroups from each strain of rats measured at the end of each phase. Bars represent ± standard error of the mean.

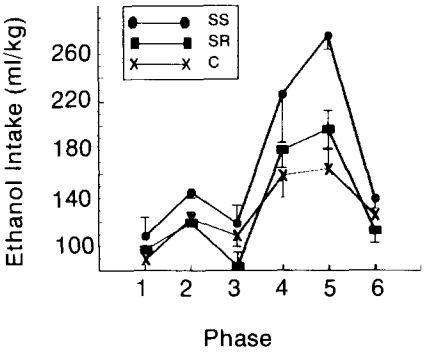

FIG. 4. Corresponding ethanol intake of the three subgroups from each rat strain in which blood pressure was measured as illustrated in Fig. 3. Bars represent ± standard error of the mean.

5 resulted in significant increases in intake during both phases followed by a return to levels approximately but significantly above their initial intake in phase 1.

The C rats showed a similar pattern of changes with the 4% NaCl supplement significantly increasing intake to a level which did not change after removal of the supplement in phase 3. As was the case with the two other groups, the 8% NaCl supplement significantly elevated intake in both phases 4 and 5. Upon its removal in phase 6, intake decreased, yet also remained significantly elevated with respect to the level originally attained in phase 1.

Water Intake

Figure 2 presents the mean water intake for each group across the six phases. A repeated measures ANOVA indicated that there was no significant effect of Group, $F(2,24)=0.306$, N.S., but a significant Phase effect, $F(5,120)=99.03$, $p<0.000$, and a significant Group × Phase interaction, $F(10,120)=3,54$ $p<0.001$. This indicates while the intake of water varied in response to the different dietary manipulations, there was no overall tendency for the groups to differ in the amounts they consumed. This stands in marked contrast to the effects on ethanol intake where group differences were in evidence. The significant interaction, however, suggests that differences in consumption among the groups did occur at some of the phases (e.g., phases 1, 5, 6).

Post hoc comparisons using Duncan's test ($\alpha=0.05$) showed that the groups significantly increased water intake after the 4% NaCl supplement was introduced and reduced

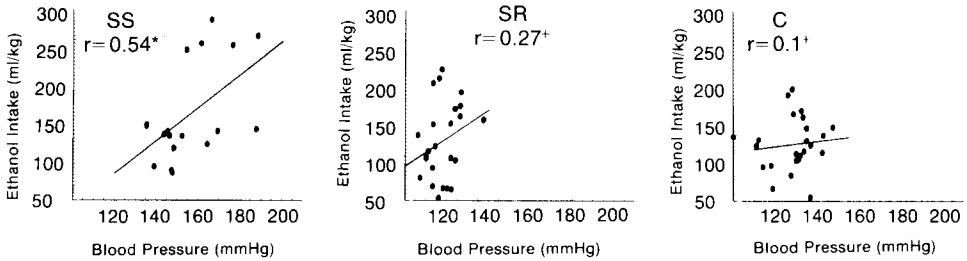

FIG. 5. Correlation between blood pressure and ethanol intake in the three subgroups from each strain throughout the six phases of the experiment. *Signifies a significant correlation coefficient, $F(1,16)=6.7$, $p<0.05$; +signifies a non significant correlation coefficient, F(1,22)1.7, n.s.—SR; $F(1,22)=0.27$, n.s.—C.

intake in response to its removal in phase 3. The introduction of the 8% NaCl supplement in phase 4 significantly increased intake again and to a point significantly higher than that achieved by the 4% NaCl supplement. However, the continued presence of the supplement (phase 5) resulted in a significant overall drop in intake (not in the SS group) to levels above control diet but equivalent to those achieved under the 4% NaCl supplement. The return to control diet in phase 6 resulted in a significant drop in intake to levels equivalent to those of phase 3, but lower than those seen at the beginning of the experiment (phase 1).

Post hoc Duncan's tests ($\alpha=0.05$) also examined the change in water intake for each group of animals across the six phases of the experiment. As can be seen in Fig. 2, SS rats increased their intake in response to the 4% NaCl supplement and significantly reduced their intake to phase 1 levels with the reinstatement of the control diet in phase 3. The introduction of the 8% NaCl supplement resulted in a significant increase in intake which exceeded that with the 4% NaCl diet and these animals continued to drink similar amounts during phase 5. The return of the control diet in phase 6 led to a reduction in intake to levels equivalent to those achieved during the other two control phases.

SR and C rats altered the water intake in essentially the same way as did the SS rats with the following two exceptions. Intake decreased significantly after prolonged exposure to the 8% diet (phase 5) and consumption in phases 3 and 6 was less than at the beginning of the experiment.

Blood Pressure

Figure 3 gives the mean blood pressures for each subgroup across the six phases of the experiment. In the SS subgroup, blood pressure was noticeably elevated after phases 4 and 5 where the 8% supplement was introduced but less so after the 4% supplement in phase 2. Both the SR and C subgroups displayed considerably smaller variations in pressure across the six phases. A two-way repeated measures ANOVA revealed a significant effect of Group, $F(2,8)=40.5$, $p<0.001$, of Phase, $F(5,40)=8.3$, $p<0.001$, and a significant Group × Phase interaction, $F(10,40)=4.0$, $p<0.001$. Post hoc analysis using Duncan's test ($\alpha=0.05$) confirmed that the 8% diet but not the 4% diet did indeed significantly increase the pressure in the SS subgroup but not the other two subgroups. Furthermore, blood pressure was significantly higher in the SS subgroup compared to the SR subgroup across all six phases of the experiment and significantly higher than the C group in all but the first and last phases. The SR and C subgroups had statistically equivalent blood pressures in all but the final phase.

The blood pressure in the SS subgroup after phase 6 was found to be statistically equal to its level after phase 1. This indicates that the intervening experience with the high salt diet did not result in a permanent change in blood pressure. This finding differs from that of Dahl and coworkers [2,4] who noted that as little as 42 days on an 8% NaCl regimen is sufficient to produce a permanent or self-sustained elevation in the blood pressure of SS rats. The SS animals in the present experiment received a 4% supplement for 25 days and an 8% supplement for 50 days. An intriguing possibility that might resolve this difference relates to the fact that the animals in the present experiment but not in the Dahl studies were consuming ethanol throughout the experiment and in particularly large quantities during the three salt supplemented phases. Thus it is possible that the consumption of ethanol reduced the impact of the salty diet on blood pressure. Indeed, this possibility is strengthened by the findings of Sanderson and coworkers [25] who showed that chronic ethanol ingestion can reduce the blood pressure in the spontaneously hypertensive strain of rats.

Figure 4 gives the ethanol intake for the same subgroups of rats. A two-way ANOVA confirmed what is clearly seen in this figure, i.e., that there was a significant difference in consumption among the three Groups, $F(2,8)=5.86$, $p<0.03$, and that more drinking occurred in some Phases than in others, $F(5,40)=26.7$, $p<0.001$. There was no significant Group × Phase interaction. Post hoc analysis (Duncan's test $\alpha=0.05$) indicated a consistent pattern for all three subgroups, i.e., that consumption during phases 4 and 5, the 8% salt supplemented phases, was significantly elevated over the phases where control diet was given. This finding stands in contrast to the effect of the diets on blood pressure, where only the SS subgroup showed a change to the 8% supplemented diet.

Figure 5 examines the relationship between blood pressure and ethanol intake in the three subgroups of animals. A significant correlation between these two measures was found only in the SS rats, $F(1,16)=6.7$, $p<0.05$, the only subgroup that responded to the 8% diet with an increase in blood pressure. Thus while all three subgroups increased their ethanol intake significantly to the 8% NaCl supplement, only one subgroup, the SS subgroup, genetically predisposed to develop hypertension, also increased its blood pressure concomitantly. Taken together, these findings indicate that in the presence of a known hypertensive agent, ethanol intake and blood pressure do not necessarily covary. This

suggests that chronic ethanol consumption may be correlated with an increase in blood pressure only when there is an independent (e.g., in the present instance, genetically determined) predisposition to develop hypertension. In other words, changes in both blood pressure and ethanol consumption may operate through a common factor (e.g., sodium chloride), which, in some susceptible subjects leads to both hypertension and a tendency to consume more ethanol while in others produces only a propensity to increase ethanol intake.

Morphological Changes

Most livers had hepatocellular globular inclusions which often filled the cytoplasm of cells adjacent to central veins. By electron microscopy these inclusions were shown to be giant mitochondria 0.5 to 5 μm in diameter. The inclusions were slightly more prominent in the SS group. There was minimal evidence of steatosis, necrosis and mitotic activity and fibrosis was absent. The kidneys of SS rats showed scarring of the cortex, tubular dilatation and intimal fibrosis of interlobular arteries with compromise of the lumina.

The hepatic mitochondrial changes have been well described both in humans and in experimental animals but the severity of involvement in the SS rats was greater than has been illustrated in the literature with various alcoholic regimens. Steatosis was minimal in this study and Mallory's hyaline, alcoholic hepatitis and fibrosis were not seen. The renal changes may be secondary to hypertension.

EXPERIMENT 2

In Experiment 1 we found that the salty diet-naive SS rat came biologically prepared to voluntarily consume more ethanol than the SR rat. Both lines in turn tended to consume more ethanol than the C rat even after the introduction of the 8% diet. The following experiment assesses three possible factors through which basic genetic differences may eventually give rise to functional differences in voluntary ethanol intake—taste, rate of ethanol metabolism and salt appetite.

There is a body of evidence indicating that ethanol intake is regulated not only by its post-absorptive consequences, but also by its gustatory and oro-sensory properties [21,23]. Rats typically prefer ethanol over water at low concentrations (up to 7%) and tend to avoid ethanol at higher concentrations. This preference-aversion function can be shifted towards greater acceptability simply by rendering animals anosmic [13]. The observed differences in consumption among the SS, SR and C rats may therefore in part reflect genetically determined differences in their gustatory and/or oro-sensory responses to the drug.

It is possible that differences in genetic make-up of the Dahl and C rats may lead to differences in how the drug is handled. For example, the AA line of rats which prefers ethanol is known to metabolize it faster than the ANA line which does not prefer ethanol [5]. Differences in ethanol consumption among the Dahl and C rats may be related to differences in the absorption, distribution or metabolism of the drug.

Previous work in our laboratory has indicated that those manipulations which induce a salt appetite can significantly decrease the voluntary intake of ethanol [8, 9, 10]. It is possible, then, that differences in genetic make-up may lead to differences in salt appetite and hence to differences in ethanol intake. This possibility is strengthened by the finding that naive SS rats are known to have a lower activity in the renin-angiotensin system than the SR rats [12,22].

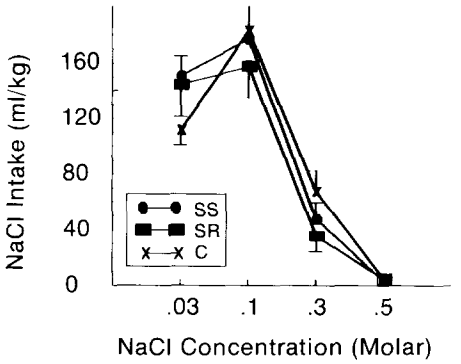

FIG. 6. Mean intake of four different NaCl concentrations for the three strains of rats. Bars represent ± standard error of the mean.

METHOD

Subjects

The subjects were 10 naive male SS rats, 10 naive male SR rats and 10 naive male C rats weighing 250–400 g. During the course of the experiment two of the SS rats and one SR rat died.

Apparatus

Animals were housed individually in hanging wire cages equipped with a glass feeder cup and two Richter tubes spaced 15 cm apart. A 12 hr/12 hr light/dark cycle was in effect with lights on at 7 a.m.

Procedure

Taste. All animals were offered a choice between water and four different NaCl concentrations—0.03, 0.1, 0.3 and 0.5 Molar (M). The NaCl solutions were offered in ascending order. Each concentration was tested according to a three day cycle during which NaCl and water were available on the first two days (with positions alternated) and only water was available on the third day. Purina rat chow was available throughout the testing.

Rate of metabolism. Approximately one week following the taste testing all animals were injected with a 2.5 g/kg dose of ethanol (IP) made as a 12.5% w/v concentration. Blood samples were obtained from the cut tip of the tail at 15 min intervals for the first hour and at 1 hr intervals thereafter. The last sample was taken at 5 hr post injection. These samples were prepared and analyzed according to the methods outlined in LeBlanc [16].

Salt appetite. All animals were offered a choice between water and five different NaCl solutions presented in ascending order—0.03, 0.06, 0.12, 0.3 and 0.5 M. Testing was carried out in a similar manner to the taste testing. For two weeks prior to the beginning of this experiment and throughout its duration, daily subcutaneous injections of desoxycorticosterone acetate (DOCA), 6 mg/animal were administered. The drug was suspended in sesame seed oil in a concentration of 0.3 ml/animal and was freshly prepared every 3–5 days.

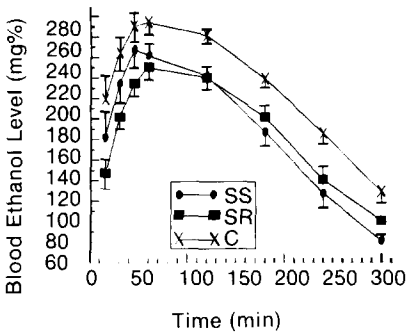

FIG. 7. Mean blood ethanol levels (mg/decalitre) for all three rat strains at various times after a 2.5 g/kg IP injection of ethanol (12.5% w/v). Bars represent ± standard error of the mean.

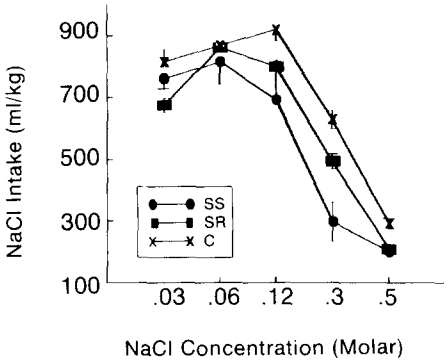

FIG. 8. Mean intake of five NaCl concentrations during chronic treatment with daily injections of the salt appetite inducing agent-DOCA (6 mg/animal/day). Bars represent ± standard error of the mean.

RESULTS

Taste

Figure 6 illustrates the relative intakes across the four concentrations. All groups tended to drink substantial amounts of the two lower NaCl concentrations and lesser amounts of the two higher concentrations. A two-way repeated measures ANOVA did not reveal any significant Group or Group × NaCl concentration differences, $F(2,24)=0.179$, N.S.; $F(6,72)=1.52$, N.S. The dramatic shifts in intake with changes in concentration was reflected in a significant NaCl concentration effect, $F(3,72)=102.5$, $p<0.001$. These findings indicate that at least with respect to a salty taste, the SS, SR and C rats do not differ in their gustatory function. This, in turn suggests that the observed differences in ethanol intake in the diet naive animals (phase 1, Experiment 1) may not be due to functional differences in taste sensitivity.

Rate of Metabolism

Figure 7 gives the mean blood ethanol levels for the three groups of animals at the eight sampling times. On the basis of this data the rates of metabolism, the concentrations at time zero (C_0) and the volumes of distribution (VD) were calculated. A one-way ANOVA performed on the VD and C_0 measures yielded a significant effect of Group, $F(2,24)=7.25$, $p<0.003$; $F(2,24)=7.64$, $p<0.003$, and the post hoc tests (Duncan's $\alpha=0.05$) indicated no significant difference in C_0 or VD between the SR and SS animals. Both of these groups, however, did have a significantly lower C_0 and VD than the C rats. A one-way ANOVA performed on the rate of metabolism again yielded a significant difference among the three Groups, $F(2,24)=6.89$, $p<0.004$, and the post hoc tests again revealed that both the SS and SR rats did not differ significantly from each other in metabolic rate. The only significant difference in metabolic rate was between the SS and C rats.

A two-way repeated measures ANOVA performed on the data from the four samples taken on the rising phase of the blood ethanol curve showed no significant effect of Group, $F(2,24)=3.08$, N.S., and a non-significant Group × Interval interaction, $F(6,72)=0.66$, N.S. The significant effect of Interval, $F(3,72)=52.5$, $p<0.001$, simply reflects the rising blood ethanol levels throughout the first hour post injection. Taken together these findings indicate that naive SS and SR Dahl rats are not different in their absorption, distribution or metabolism of ethanol. This suggests that the significant differences between these two groups in ethanol self-administration observed in the initial phase of Experiment 1 which persists as a rank order difference throughout the remainder of that experiment is not likely to be due to pharmacokinetic variables and may have its basis in altered sensitivity of the central nervous system.

Salt Appetite

A two-way repeated measures ANOVA revealed a significant effect of Group, $F(2,24)=6.27$, $p<0.006$, a significant effect of NaCl concentration, $F(4,96)=110.46$, $p<0.001$, and a significant Group × Concentration interaction, $F(8,96)=2.63$, $p<0.01$. This indicates that the groups differed significantly with respect to their NaCl consumption, that the amount of NaCl consumed depended on its concentration and that the differences among groups occurred at some NaCl concentrations and not others.

Figure 8 shows that across all the NaCl concentrations, the C rats tended to drink the most, followed by the SR and then the SS rats. Thus, there is a tendency for the SS rats to show less of a salt appetite than the SR rats. Post hoc analysis (Duncan's test $\alpha=0.05$) revealed that the SS and SR rats differed significantly at the 0.3 M concentration. The Wistar rats drank significantly more at all but the 0.06 M concentration. These data indicate that a significant difference between the two Dahl lines of rats does exist with respect to salt appetite elicited by chronic DOCA administration. This difference, however, is subtle and a function of the test concentration of NaCl offered. Wolf *et al.* [27] also tested DOCA-elicited salt appetite in Dahl rats but they used a smaller DOCA dose and a shorter period of treatment. Using only one NaCl concentration, and that one similar to the 0.12 M used in the present experiment (i.e., 0.15 M), they also reported no significant difference in sodium appetite. The subtlety in demonstrating differences in sodium appetite may reflect the contribution of processes other than salt appetite in determining the net amount of a particular concentration of NaCl that an animal will consume. Since most animals actually prefer to consume low concentrations of NaCl and avoid high ones, taste is an important variable and therefore the concentration of NaCl used to test for a salt appetite is clearly important. The fact that we found a clear cut differ-

ence between all three groups with respect to sodium appetite but only at one (intermediate) concentration, is an indication of the interplay between these various factors. Taken together these results show that the SS animal, who drank significantly more ethanol in the diet-naive phase 1 of Experiment 1 than did the SR animals, also displays a significantly lesser salt appetite than the SR animal. This further corroborates our previous work pointing to a relationship between salt appetite and voluntary ethanol consumption.

GENERAL DISCUSSION

The results of Experiment 1 demonstrate that the salt sensitive Dahl rat, genetically predisposed to develop hypertension, also comes predisposed to drink significantly more ethanol than either the salt resistant Dahl rat not predisposed to hypertension or a rat from an entirely different strain (Wistar). This initial difference in ethanol intake was especially evident and most robust in phase 1, *before* the introduction of the hypertension-inducing salt supplement. It persisted in the form of a rank order difference, SS SR C, in the subsequent salt-supplement phases even though all groups increased their intake and drank statistically equivalent amounts of ethanol.

The results of Experiment 2 indicate that the initial difference in ethanol intake between the two Dahl lines is not the result of differences in taste sensitivity since there was no differential response by the two lines in terms of the acceptibility of a number of different NaCl concentrations. Differences between the SS and SR rats due to pharmacokinetic variations in absorption, distribution or metabolism were also ruled out because the ethanol disappearance curves showed no significant differences in either the concentrations at time zero, volumes of distribution or rates of metabolism. However, differences between the Dahl lines on the one hand, and the C rats on the other, were seen in all these pharmacokinetic variables and could account for the overall difference in intake seen between the C and Dahl rats.

A difference between the two Dahl lines, however, was noted with respect to salt appetite with the SS line showing a lesser degree of salt appetite than the SR line. This finding is congruent with the work of Iwai [12] and Rapp [22] showing that SS rats have a lower renin-angiotensin activity than the SR rats. Our previous work has shown that changes in salt appetite are associated with changes in ethanol intake and intoxication [8–11]. The present finding that the two Dahl lines differ not with respect to taste or pharmacokinetics but do show differences in salt appetite lends further credence to the hypothesis that the underlying physiological state accompanying the changes in salt appetite can modify the sensitivity of the central nervous system to ethanol.

In recent years, controversy has surrounded the question of whether chronic ethanol consumption causes hypertension. Epidemiological data [1,15] have established a clear association; however, an association *per se* does not prove a cause and effect relationship. An alternative to this direct effect position is the notion that environmental factors act on genetically predisposed individuals to cause both hypertension and a propensity to consume more ethanol. Here the link between increased ethanol consumption and hypertension is indirect since both would stem from the presence of a third common factor (e.g., a chronically elevated salt content in the diet) which is permissive in the sense that its presence is a necessary but not sufficient condition for the change in both ethanol consumption and blood pressure. In the present study we found that while the salty diet was capable of increasing ethanol consumption in all three groups of animals to a point where they were chronically consuming large and statistically equivalent amounts of ethanol, only the genetically disposed SS line also showed a significant increase in blood pressure and a significant correlation between blood pressure and ethanol intake. Taken together these data do not support a direct link between ethanol and hypertension (although its use could be a contributory factor) but rather support the contention that the two are linked by indirect association.

In light of previous work suggesting an influence of a salty diet on ethanol intake, we were interested in monitoring ethanol intake and blood pressure in the Dahl lines of rat, one of which is genetically selected to show an enhanced response to a dietary sodium supplement. We found that the hypertension prone SS rat comes prepared to consume significantly more alcohol than the non-hypertension prone SR rat even before the development of hypertension by the introduction of a salt supplemented diet. This enhanced predisposition to alcohol did not appear to be related to either taste sensitivity or pharmacokinetic factors, but rather to be centrally mediated, perhaps through the renin angiotensin system since it was associated with significant differences in salt appetite. Lower activity in the renin-angiotensin system of the SS rat tends to support this notion. The development of hypertension only in the SS line after the introduction of the salty diet, despite the consumption of equivalent amounts of alcohol in all three groups of animals, lends support to the viewpoint that alcohol and hypertension may be related but in an indirect fashion.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Reinhard Schuller of the Computer Services Department, Addiction Research Foundation for the statistical analysis, and Mrs. Val Cabral for preparing the manuscript. This research was supported by the Addiction Research Foundation of Ontario.

REFERENCES

1. Ashley, M. J. and J. G. Rankin. Alcohol consumption and hypertension: the evidence from hazardous drinking and alcoholic populations. *Aust NZ J Med* **9:** 201–206, 1979.
2. Dahl, L. K. Effects of chronic excess salt feeding. Induction of self sustaining hypertension in rats. *J Exp Med* **114:** 231–236, 1961.
3. Dahl, L. K. M. Heine and L. Tassinari. Effects of chronic salt ingestion. Evidence that genetic factors play an important role in susceptibility to experimental hypertension. *J Exp Med* **115:** 1173–1190, 1962.
4. Dahl, L. K., K. D. Knudsen, M. A. Heine and G. A. Leith. Effects of chronic excess salt ingestion. Modification of experimental hypertension in the rat by variations in the diet. *Circ Res* **22:** 11–18, 1968.
5. Eriksson, C. J. P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol* **22:** 2283–2292, 1973.
6. Eriksson, K. Rat strains specially selected for their voluntary alcohol consumption. *Ann Med Exp Fenn* **49:** 67–72, 1971.

7. Falk, J. L., H. H. Samson and G. Winger. Behavioral maintenance of high concentrations of blood ethanol and physical dependence in the rat. *Science* **177:** 811–813, 1972.
8. Grupp, L. A., R. B. Stewart and E. Perlanski. Salt restriction and the voluntary intake of ethanol in rats. *Physiol Psychol* **12:** 242–246, 1984.
9. Grupp, L. A., E. Perlanski and R. B. Stewart. Dietary salt and DOCA-salt treatments modify ethanol self-selection in rats. *Behav Neural Biol* **40:** 239–250, 1984.
10. Grupp, L. A., R. B. Stewart and E. Perlanski. Diet and diuretics in the reduction of voluntary alcohol drinking in rats. *Alcohol*, in press, 1986.
11. Grupp, L. A., J. Elias, E. Perlanski and R. B. Stewart. Modification of ethanol-induced motor impairment by diet, diuretic, mineralocorticoid or prostaglandin synthetase inhibitor. *Psychopharmacology (Berlin)* **87:** 20–24, 1985.
12. Iwai, J., L. K. Dahl and K. D. Knudsen. Genetic influences on the renin-angiotensin system. Low renin activities in hypertension-prone rats. *Circ Res* **32:** 678–684, 1973.
13. Kahn, M. and E. Stellar. Alcohol preference in normal and anosmic rats. *J Comp Physiol Psychol* **53:** 571–575, 1960.
14. Kakihana, R., D. R. Brown, G. E. McClearn and I. R. Tabershaw. Brain sensitivity to alcohol in inbred mouse strains. *Science* **154:** 1574–1575, 1966.
15. Klatsky, A. L., G. D. Freedman, A. B. Seigelaub and M. J. Gerard. Alcohol consumption and blood pressure. *N Engl J Med* **296:** 1194–1200, 1977.
16. LeBlanc, A. E. Microdetermination of alcohol in blood by gas-liquid chromatography. *Can J Physiol Pharmacol* **46:** 665–667, 1968.
17. Lumeng, L., T. D. Hawkins and T.-K. Li. New strains of rats with alcohol preference and non-preference. In: *Alcohol and Aldehyde Metabolizing Systems—3*, edited by R. G. Thurman, J. R. Williamson, H. R. Drott and B. Chance. New York: Academic Press, 1977, pp. 537–544.
18. MacDonall, J. S. and M. Marcucella. Increasing rate of ethanol consumption of food and water satiated rats. *Pharmacol Biochem Behav* **10:** 211–216, 1979.
19. McClearn, G. E. and D. A. Rodgers. Differences in alcohol preference among inbred strains of mice. *Q J Stud Alcohol* **20:** 691–695, 1959.
20. Meisch, R. A. and T. Thompson. Ethanol as a reinforcer: Effects of fixed ratio size and food deprivation. *Psychopharmacologia* **28:** 171–183, 1973.
21. Nachman, M., C. Larue and J. LeMagnen. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. *Pharmacol Biochem Behav* **6:** 53–59, 1971.
22. Rapp, J. P., S. Y. Tan and H. S. Margolius. Plasma mineralocorticoids, plasma renin, and urinary kallikrein in salt-sensitive and salt-resistant rats. *Endocrinol Res Commun* **5:** 35–41, 1978.
23. Richter, C. P. and K. Campbell. Alcohol taste thresholds and concentration of solutions preferred by rats. *Science* **91:** 507–508, 1940.
24. Riley, E. P., E. X. Freed and D. Lester. Selective breeding of rats for an animal model of alcoholism: I. General procedures. *J Stud Alcohol* **37:** 1535–1547, 1976.
25. Sanderson, J. E., J. V. Jones and D. I. Graham. Effect of chronic alcohol ingestion on the heart and blood pressure of spontaneously hypertensive rats. *Clin Exp Hypertens [A]* **5:** 673–689, 1983.
26. Staats, J. The inbred mouse. In: *Biology of the Laboratory Mouse*, edited by E. L. Green. New York: McGraw Hill, 1966, pp. 1–11.
27. Wolf, G., L. K. Dahl and N. E. Miller. Voluntary sodium chloride intake of two strains of rats with opposite genetic susceptibility to experimental hypertension. *Proc Soc Exp Biol Med* **120:** 301–305, 1965.