Pharmacology Biochemistry & Behavior, Vol 10, pp 557–560 Printed in the U S A

Endocrine Factors Contributing to the Ethanol Preferences of Rodents[1]

J. ALLEN GOAS, RUSSELL W PELHAM[2] AND ARNOLD S. LIPPA

Central Nervous System Disease Research Section
Medical Research Division, American Cyanamid Company
Pearl River, NY 10965

(Received 17 February 1979)

GOAS, J A , R W PELHAM AND A S LIPPA *Endocrine factors contributing to the ethanol preferences of rodents* PHARMAC BIOCHEM BEHAV **10**(4) 557–560, 1979 —Groups of C57 Bl/6j mice (alcohol preferring) and DBA/2j mice (alcohol avoiding) were fasted for 24 hours and administered glucose At 30, 120 and 300 minutes after glucose, the C57 Bl/6j mice had significantly higher levels of plasma glucose than the DBA/2j strain These differences were observed in comparable groups given either forced access or no access to alcohol In ad lib fed animals never exposed to alcohol, C57 Bl/6j mice had higher levels of plasma insulin than DBA/2j mice Plasma levels of glucose and corticosterone were not significantly different in ad lib or fasted animals The injection of insulin zinc protamine to DBA/2j mice produced 100% convulsions within one hour, but produced no convulsions in C57 Bl/6j mice for as long as 4 hours after administration These data demonstrate that an insulin resistancy exists in C57 Bl/6j mice which is not dependent upon any prior alcohol experience Evidence supporting a functional relationship between this diabetogenic disturbance and alcohol preference was obtained in C57 Bl/6j mice which were allowed to choose between water or a 10% alcohol solution (v/v) Insulin zinc protamine produced a selective dose-dependent reduction in alcohol intake Additional support is received from the discovery that Chinese hamsters, a species genetically predisposed to diabetes, display an impressive preference for 10% alcohol

Ethanol preference Glucose Insulin C57 Bl/6j mice DBA/2j mice Chinese hamsters

SEVERAL studies have suggested the existence of a genetic predisposition in the etiology of human alcohol addiction [1, 2, 3]. However, because of the limitations inherent in human experimentation, it has not been possible to determine whether certain biochemical [4, 5, 6] disturbances in human alcoholics are an antecedent condition or a consequence of chronic alcohol consumption For instance, that an unexpectedly high percentage of human alcoholics display abnormally high glucose tolerance [6,7] suggests the possibility that diabetogenic disturbances may function as predispositional factors, which, if combined with appropriate environmental conditions, would produce a compelling stimulus for alcohol consumption and addiction [8]. Conversely, that alcohol administration may impair glucose tolerance in normal human subjects [6] suggests that the diabetogenic disturbances observed in alcoholics may represent a consequence rather than a precursor of chronic alcohol consumption

In order to circumvent the problems inherent in human research and to investigate the possibility that certain endocrine disorders may predispose an organism to prefer alcohol [8], we measured various endocrine parameters in C57 Bl/6j and DBA/2j mice The C57 Bl/6j strain is well known for its alcohol preference, consuming more than two-thirds of its total fluid intake as an alcohol solution [9,10] Any endocrine disorder found in naive C57 Bl/6j mice may represent a predisposing factor which would produce alcohol preference were these animals given the opportunity to drink alcohol Endocrine parameters measured in C57 Bl/6j mice were compared with those obtained from DBA/2j mice, a strain known to avoid alcohol [9,10]

Our results do indeed indicate a diabetogenic disturbance in C57 Bl/6j mice which may be of importance in determining the alcohol preference of these animals

METHOD

Mice were males of the C57 Bl/6j (C57) and DBA/2j (DBA) strains obtained from the Jackson Laboratories (Bar Harbor, ME) and weighed 20–25 g at the time of testing Male Chinese hamsters were obtained from the Chick Line Company (Camden, NJ) and weighed 25–30 g at the time of testing Upon arrival all animals were individually housed with food (Purina Chow) and water available ad lib At least 2 weeks were allowed for acclimation to environmental conditions

[1]Portions of these data were presented at IV International Congress of the International Society of Psychoneuroendocrinology, August, 1975, and at the 62nd Annual Meeting of the Federation of American Societies for Experimental Biology, April, 1978

[2]Present address Department of Neurology, Tufts-New England Medical Center, Boston, MA

TABLE 1
FLUID INTAKE AND PREFERENCES OF C57 AND DBA MICE (MEAN ML/DAY ± SEM)

	Total Fluid Intake		Preference	
Species	H_2O vs H_2O	H_2O vs 10% ETOH	10% ETOH	H_2O
C57 (N=12)	4 3 ± 1 7	4 5 ± 0 8	3 7 ± 0 5	0 8 ± 0 3
DBA (N=12)	4 9 ± 1 6	4 8 ± 0 5	0 4 ± 0 4	4 4 ± 0 4

Preference Testing

In order to confirm previous reports [9,10] that C57 mice prefer ethanol (ETOH), while DBA mice do not, 12 DBA and 12 C57 mice were presented with a 2-bottle (Richter Tube) choice between water and ETOH in their home cages After one week of water baseline measurements, a 10% ETOH (v/v) solution was placed in one bottle, with tap water available in the other ETOH solutions were prepared from 95% ETOH (Alcohol, U S P , 190 proof) and mixed with tap water to achieve the desired concentration

Intake data were collected each morning between 0800 and 1100 hours for two weeks ETOH evaporation control tubes were placed on empty cages adjacent to occupied cages in order to determine daily volume loss due to evaporation, and to correct the data for such loss After the daily data were collected, drinking tubes were rinsed, refilled, and replaced on the cages with the left-right positions reversed from the previous day These procedures were used for all additional preference tests

Determination of Plasma Glucose, Insulin and Corticosterone

Twenty C57 and 20 DBA mice were used in this experiment Half of the mice in each strain had been deprived of food 24 hr prior to testing Following decapitation, trunk blood was obtained from all animals (between 0800 and 0900 hr) and immediately centrifuged in heparinized tubes Plasma glucose was determined colorimetrically (Auto Analyzer® [Technicon, Tarrytown, NY] Method N-9a [11]) from half of the animals, while plasma immunoreactive insulin (IRI) and plasma corticosterone were determined by radioimmunoassay and competitive protein binding assay (Schwartz-Mann) from the remaining animals

Glucose Tolerance Test

Two groups of animals were used in this experiment One group of C57 and DBA mice were maintained in their home cages with normal ad lib food and water. The second group of C57 and DBA mice were also maintained on ad lib food but were offered 10% ETOH as their sole source of fluid Daily fluid intake measurements confirmed equivalent volume intakes for both pairs of groups This procedure lasted 14 days On Day 14, food was withdrawn from all mice and ad lib water replaced ETOH for the mice on the forced ETOH regime Following 24 hr of food deprivation, all animals were injected with glucose (350 mg, IP) and randomly selected for sacrifice at 0, 1/2, 1, 2 and 5 hr after injection (N=4–6/group) Plasma was collected and plasma glucose determined by the methods described above

Insulin-Induced Convulsions

Twelve C57 and 12 DBA mice were injected at 1200 hours with insulin zinc protamine (100 I U , IP) and observed for 4 hours for tonic-clonic convulsions The data were collected in 3 separate replications consisting of 4 C57 and 4 DBA mice per replication

Effects of Insulin on ETOH Preference

Sixteen C57 mice were allowed 3 weeks to establish a preference for ETOH (65% of the total fluid intake consumed as 10% ETOH) On the day of testing, all animals were injected subcutaneously with either 1 0, 2 5, 5 0 or 10 0 I U of insulin zinc protamine, after which 24 hr fluid intakes were monitored Data were compared to results obtained a week earlier from each animal after injection of an equivalent amount of the saline vehicle (1 ml/100 g)

ETOH Preference of Chinese Hamsters

Since the Chinese hamster displays a natural diabetogenic condition [12, 13, 14], it was of interest to determine the ETOH preference of this species. After a minimum of 2 weeks acclimation, 12 male Chinese hamsters were given an ETOH preference test (see Preference Testing) for 6 days, during which daily fluid intakes were monitored. This study was carried out in 2 separate replications with 6 animals in each replication

RESULTS AND DISCUSSION

As can be seen in Table 1, the total fluid intakes for both the C57 and DBA mice were equivalent regardless of whether the animals had the opportunity to drink ETOH or not However, in confirmation of previous reports [9,10], the C57 mice demonstrated a strong ETOH preference, drinking an average of 82% of their total fluid intake as a 10% ETOH solution In contrast, the DBA mice demonstrated a strong ETOH aversion drinking only an average of 8% of their total fluid intake as ETOH

As can be seen in Table 2, no significant differences ($p>0$ 05, t test) in plasma corticosterone levels were observed between C57 and DBA mice regardless of whether the animals were fed or fasted Likewise, plasma glucose levels were also equivalent in both strains of mice Although plasma IRI levels were equivalent in fasted C57 and DBA

TABLE 2

PLASMA HORMONE LEVELS IN C57 AND DBA MICE AFTER FASTED OR FREE FOOD CONDITIONS

		DBA	C57
Plasma Immunoreceptive Insulin (μ U/ml ± SEM)	Free Food	28 4 ± 1 6	52 0 ± 6 5*
	Fasted	24 8 ± 2 2	28 4 ± 0 8
Plasma Glucose (mg% ± SEM)	Free Food	165.0 ± 16 0	179 0 ± 12 0
	Fasted	104 0 ± 12 0	130 0 ± 8 0
Plasma Corticosterone (mg% ± SEM)	Free Food	10 9 ± 3 0	6 0 ± 1 2
	Fasted	21 5 ± 3 5	22 0 ± 3 0

*$p<0$ 02 between strains, t test
N=5/Group

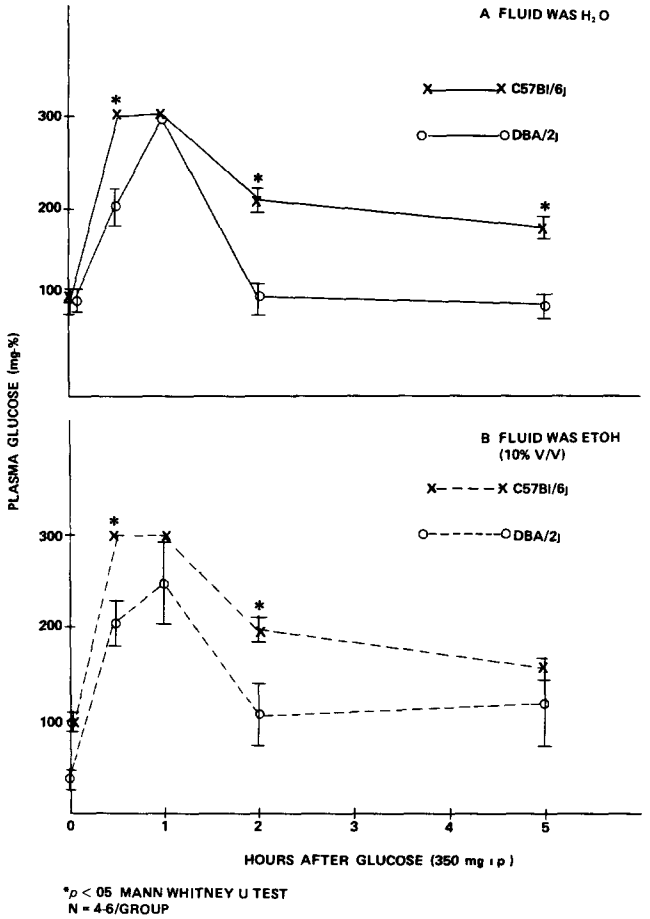

FIG 1 Results of the glucose tolerance test in C57 and DBA mice drinking with forced H_2O (A) or forced 10% ETOH (B)

mice, the IRI levels in non-fasted C57 mice were almost twice those of non-fasted DBA mice ($p<0$ 02, t-test).

That insulin levels twice those of the DBA were required to maintain equivalent glucose levels in the C57 mice suggests the possibility of a diabetogenic disturbance in the C57 mice

TABLE 3

INSULIN-INDUCED CONVULSIONS AFTER 100 I U (IP) INSULIN ZINC PROTAMINE

	%Mice Convulsing
DBA	100
C57	0

N=12/Group

TABLE 4

EFFECTS OF INSULIN ON ETOH PREFERENCE IN C57 MICE

	24-Hour Fluid Intake % Change from Control		
Dose of Insulin Zinc Protamine (I U , SC)	H_2O	10% ETOH	Total Fluid
1 0	+200	−21	− 7
2 5	− 33	−23	−25
5 0	+133	−46	−12
10 0	+100	−60	−50

N=4/Group

The data displayed in Fig. 1 are the glucose tolerance results obtained in fasted ETOH-experienced (Fig. 1b) and fasted ETOH-naive (Fig 1a) C57 and DBA mice In confirmation of the previous experiment, C57 and DBA mice displayed equivalent resting plasma glucose levels after a 24 hr fast. These results were obtained in either ETOH-naive animals or animals with forced ETOH exposure. After forced ETOH exposure, the C57 mice displayed a diabetogenic response to glucose when compared to the DBA mice, with significantly ($p<0.05$, Mann-Whitney U Test) higher glucose levels at 0.5 and 2 hours after glucose administration (Fig. 1b) The data in Fig 1a demontrate a similar diabetogenic pattern on the part of ETOH-naive C57, with significantly higher ($p<0.05$) plasma glucose levels occurring at 0 5, 2 and 5 hours after glucose administration These results demonstrate that the diabetogenic disturbance observed in the C57

TABLE 5
ETOH AND H_2O INTAKE ON CHINESE HAMSTERS

Days	Mean Daily 10% ETOH Intake (ml ± SEM)	Mean Daily H_2O Intake (ml ± SEM)
−3	—	5 0 ± 0 3
−2	—	5 4 ± 0 2
−1	—	5 2 ± 0 6
1	2 8 ± 0 4	1 4 ± 0 2
2	2 6 ± 0 5	1 8 ± 0 4
3	3 0 ± 0	1 6 ± 0 2
4	3 0 ± 0 4	0 4 ± 0 2
5	3 2 ± 0 6	1 4 ± 0 5
6	4 2 ± 0 6	1 2 ± 0 4

N=12

mice is not produced by chronic ETOH drinking, but rather exists in the absence of ETOH exposure

The diabetogenic disturbance observed in the glucose tolerance test may have been produced by either an insufficient amount of insulin released to the glucose challenge or by a resistance to whatever insulin is released That almost twice as much insulin was required by the C57 mice as the DBA mice to maintain similar glucose levels (Table 2) suggests an insulin resistance on the part of the C57 mice This hypotheses is supported by the fact that in the present experiment, none of the C57 mice were observed to convulse after an IP injection of insulin zinc protamine (100 I U), a dose which caused convulsions in 100% of the DBA mice (Table 3)

In order to determine if the diabetogenic disturbance displayed by the C57 mice played some functional role in their ETOH preference, an attempt was made to reverse the ETOH preference of these mice by exogenous administration of insulin zinc protamine (1–10 I U , SC) As can be seen in Table 4, insulin produced a dose-related decrement in ETOH intake and an increase in water intake A significant decrease ($p<0$ 05, paired t test) in total fluid intake only occurred after administration of the highest dose of insulin

As can be seen in Table 5, Chinese hamsters displayed a preference for 10% ETOH, which by the sixth day of exposure had increased to 78% of their total fluid intake

The data in this report identify a diabetogenic disturbance in the C57 mice, a strain which is well known for its ETOH preference The prolonged elevated plasma glucose levels in the glucose tolerance test clearly demonstrate that the C57 mice fail to adequately regulate plasma glucose levels after a glucose challenge

Since this effect was seen in ETOH-naive, as well as ETOH-experienced mice, genetic factors rather than prior ETOH experience must be responsible That the C57 mice required roughly twice as much insulin as the DBA mice to maintain similar plasma glucose levels suggests that the diabetogenic disturbance is due to a resistance of cellular glucose uptake mechanisms rather than insufficient insulin release The notion of insulin resistance is further supported by the observed absence of convulsions in the C57 mice given a dose of insulin which produced convulsions in 100% of the DBA mice tested

A functional role for insulin resistancy in determining ETOH preference in C57 mice is supported by the ability of exogenous insulin administration to selectively reduce ETOH intake Additional support is provided by the finding that Chinese hamsters, a species which displays an endogenous diabetogenic condition [12, 13, 14], also display an impressive preference for ETOH

REFERENCES

1 Goodwin, D W , F Schulsinger, N Moller, L Hermansen, G Winokur and S B Guze Drinking problems in adopted and non-adopted sons of alcoholics *Archs gen Psychiat Chicago* **31**: 164–169, 1974

2 Winokur, G , T Reich, J Rimmered and F N Pitts, Jr Alcoholism III Diagnosis and familial psychiatric illness in 259 alcoholic probands *Arch gen Psychiat Chicago* **23**: 104–111, 1970

3 Goodwin, D W , F Schulsinger, L Hermansen, S B Guze and G Winokur Alcohol problems in adoptees raised apart from alcoholic biological parents *Archs gen Psychiat Chicago* **28**: 238–243, 1973

4 Axelrod, D R Metabolic and endocrine aberrations in alcoholism In *The Biology of Alcoholism, Vol 3*, edited by B Kissin and H Begleiter New York Plenum Press, 1974, pp 291–302

5 Jenkins, D W , R E Eckel and J W Craig Alcoholic ketoacidosis *J Am med Ass* **217**: 177–183, 1971

6 Phillips, G B and H F Safrit Alcoholic diabetes *J Am med Ass* **217**: 1513–1519, 1971

7 Myrhed, M Alcohol consumption in relation to factors associated with ischemic heart disease VIII Blood glucose and intravenous glucose tolerance *Acta med scand Suppl* **567**: 58–62, 1975

8 Goas, J A , A S Lippa and R W Pelham Endocrine factors underlying the ethanol preference of C57 Bl/6j mice *Fedn Proc* **37**: 421, 1978

9 McClearn, G E and D A Rodgers Differences in alcohol preference among inbred strains of mice *Q Jl Stud Alcohol* **20**: 691–695, 1959

10 McClearn, G E and D A Rodgers Genetic factors in alcohol preference of laboratory mice *J comp physiol Psychol* **54**: 116–119, 1961

11 Hoffman, W S A rapid photoelectric method for the determination of glucose in blood and urine *J biol Chem* **120**: 51–55, 1937

12 Dulin, W E and G C Gerritsen Summary of biochemical, physiological and morphological changes associated with diabetes in the Chinese hamster *Proc , VIth Congr int Diabetes Fedn* , 1967, pp 806–812

13 Dulin, W E and G C Gerritsen Interaction of genetics and environment on diabetes in the Chinese hamster as compared with human and other diabetic animal species *Acta Diabet latinoam* **9**: *Suppl 1* 48–84, 1972

14 Dulin, W E , A Y Chang and G C Gerritsen Comparison of diabetes in the Chinese hamster, KK mouse and db mouse *Proc , VIIth Congr int Diabetes Fedn* , 1970, pp 868–880