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Effects of Food, Mild Stress, and Distributed Intake on the Absorption and Plasma Concentration-Time Profile of Orally Ingested Ethanol in Pigtailed Macaques

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KALHORN, T F , D M BOWDEN AND J T SLATTERY *Effects of food, mild stress, and distributed intake on the absorption and plasma concentration-time profile of orally ingested ethanol in pigtailed macaques* PHARMACOL BIOCHEM BEHAV **24**(3) 491–496, 1986 —To evaluate the possibility of estimating ethanol plasma level from a record of voluntary intake, the effects of food, mild stress, and distributed dosing on the fraction of ethanol absorbed and the relative exposure to circulating blood ethanol were determined in four adult male pigtailed macaques *(Macaca nemestrina)* The animals received 0 6 g/kg ethanol IV and orally after an 18-hour fast, after a small meal, and under mild stress, distributed dosing was evaluated for the oral route only The concentration-time profile for the oral/fasting condition was very similar to that following an IV dose The dose was completely absorbed and peak plasma level occurred about 30 min after ingestion Peak plasma concentration was reduced by 42% following a small meal, 29% following mild stress, and 18% following distribution of the dose over 60 min The time to peak concentration was more than doubled by distributed dosing Relative exposure was reduced by 18 to 27% by all conditions of oral administration except feeding, which caused a 52% reduction The fraction of ethanol absorbed was influenced only by feeding, which caused a reduction of 20% The magnitude of changes in concentration-time profile produced by such factors precludes accurate estimation of blood levels from records of voluntary intake Furthermore, the effects of social factors on voluntary ethanol consumption may be mediated by centrally controlled changes in gastrointestinal function that alter the rate and extent of absorption of the drug

Ethanol Pharmacokinetics Feeding Stress Monkeys

EVIDENCE is accumulating that social context and other environmental factors may influence the consumption of ethanol in nonhuman primates [1–4, 6, 8, 12] as they do in humans Although the amount of ethanol ingested is usually the only dependent variable measured in such studies, it is often assumed that the subject may be drinking to achieve an ethanol-induced effect that correlates with plasma concentration of ethanol To the investigator seeking to identify the physiological mechanisms that mediate changes in ethanol consumption under different environmental conditions, it is essential to know the degree to which those conditions interact with dose to determine plasma levels of the drug This information is not usually available, however, because it is technically difficult to obtain blood samples for measurement of plasma levels without disrupting the behavioral context whose influence is being studied

In a previous study, we demonstrated that, to the extent that the concentration-time profile depends on the pharmacokinetics of ethanol elimination from the circulation, one can estimate the concentration of ethanol in plasma with acceptable accuracy from a detailed record of consumption, body weight, and a distributed input model based on Michaelis-Menten kinetics [13] The purpose of the present study was to determine the degree to which several environmental factors, unavoidable in behavioral studies of spontaneous drinking in monkeys, may influence the ab-

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sorption and plasma concentration-time profiles The three factors studied were ingestion of a small amount of Monkey Chow, periodic mild stress, and distribution of the ethanol dose over a 60-min interval

METHOD

The subjects were four male pigtailed macaques (*Macaca nemestrina*) weighing 4 to 13 kg To allow drug administration and blood sampling without disturbing the monkey, a catheter was implanted chronically with its tip in the monkey's superior or inferior vena cava It was led out through the jugular or femoral vein, and thence outside the cage through a tether [13] This arrangement allowed the monkey to move freely about the cage without compromising IV ethanol administration and blood sampling procedures After the implantation, the animals recovered normal eating and behavior (2 to 4 days) before studies were begun

The monkeys were taught to drink approximately 20 ml of ethanol solution (20% v/v in normal saline or apple juice) within 3 min from a plastic syringe The vehicle, saline or apple juice, had no effect on the area under the plasma concentration-time curve (AUC) An oral dose of ethanol (0 6 g/kg) was administered to each monkey in separate series of studies under the following conditions fasting, fed, mild stress, and as a series of distributed doses (six 0 1-g/kg doses at 10-min intervals) The 0 6 g/kg dose was previously determined to be suitable for pharmacokinetic studies [13], while the distributed dose regimen was designed to simulate distributed drinking that would produce a peak concentration of ethanol later than oral administration in a bolus An IV dose (0 6 mg/kg) was administered under all except the distributed dose condition to test for potential effects of the environmental interventions on elimination of the drug Animals were fasted for 18 hr before each trial except in the fed condition, and were not handled or agitated except in the stress condition

In the fed condition, the monkeys ingested 1 5 biscuits/kg of Purina Monkey Chow (average biscuit weight, 5 8 g) under observation 15 min before receiving ethanol (In a regular meal, monkeys consume 5 to 10 biscuits per kg) Stress was induced by an unfamiliar animal handler, wearing catching gloves, who rattled and opened the cage and approached the monkey as if to catch it, but did not touch the animal The procedure lasted 10 sec and was repeated every 10 min for the first 3 hr after administration of ethanol The stress condition was always the last condition studied, so that the monkey would not associate ethanol administration with a stressful situation The animals were rested for 3 days between studies

After each dose, blood samples were collected through the tether every 5 min for the first 20 min, every 10 min for the next 40 min, and every 15 min for the next 3 hr Plasma was separated and ethanol concentration was determined by gas chromatography [13]

The data were analyzed to assess effects on four parameters peak time, peak concentration, fraction of dose absorbed, and relative exposure based on AUC Ordinarily, fraction of dose absorbed would suffice as a measure of an animal's relative exposure to ethanol if the drug were eliminated linearly, but the concentration-dependent clearance of ethanol causes the AUC to vary not only as a function of the fraction of dose absorbed but also of the rate at which it is absorbed [24] Relative exposure was therefore used as one means of comparing the actual plasma concentrations ob-

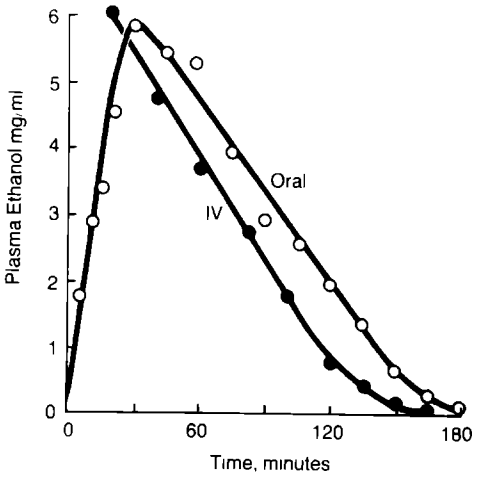

FIG. 1 Representative concentration-time profiles of plasma ethanol following 0 6 g/ml doses administered IV and orally to subject 78329

served in a given experimental state with those observed in a standard state (IV/fasting), it is affected by both the rate and the extent of absorption

Pharmacokinetic parameter values were determined from the IV studies using the MKMODEL program [11] of the PROPHET computer system (Bolt, Beranek and Newman, Inc , Cambridge, MA) Values of C_0, V_{max} and K_m were determined by fitting the data obtained in the IV/fasting condition to the equation

$$dC/dt = C_0 - (V_{max} * C/(K_m + C))$$

where C is ethanol concentration at time t, V_{max} is the maximum velocity of elimination, and K_m is the apparent *in vivo* Michaelis constant C_0 is the concentration at time zero extrapolated from the linear portion of the plot of concentration versus time Although ethanol is eliminated by enzymes in addition to alcohol dehydrogenase [7, 14, 16–18], the equation above was adequate to describe the data [13, 21–23] AUC was determined by the trapezoidal rule [9] The fraction of dose absorbed was determined by the method of Martis and Levy [15] According to the method, instantaneous values of clearance were calculated from V_{max} and K_m values obtained from the IV dose and the concentration values from the data being evaluated, and were used to determine the amount of ethanol that would have had to reach the systemic circulation to produce the observed concentration-time profile Relative exposure for a given condition was determined as the ratio of AUC observed under that condition to the AUC in the IV/fasting condition

RESULTS AND DISCUSSION

The ethanol elimination curve following administration of 0 6 g/kg in the oral/fasting condition was very similar to that following the same dose in the IV/fasting condition (Fig 1) Concentration-time profiles in the IV/fed and IV/stress conditions were indistinguishable from those in the IV/fasting condition, and are not shown in the figure The peak plasma level with oral administration under fasting conditions occurred about 30 min after dosing (Table 1) The amount ab-

TABLE 1

MAGNITUDE AND TIME OF PEAK PLASMA ETHANOL CONCENTRATION FOLLOWING 0 6 g/kg ETHANOL ADMINISTERED ORALLY UNDER FOUR EXPERIMENTAL CONDITIONS

	Peak Concentration, μg/ml				Peak Time, min*			
Monkey	Fasting	Fed	Stress	Distributed Dose	Fasting	Fed	Stress	Distributed Dose
71038	799	368	ND	542	20	38	ND	75
70066	585	447	515	650	38	30	50	60
78329	587	322	395	459	30	50	60	75
80369	536	310	431	414	30	30	75	90
Mean	627	366†	447†	516‡	29	37‡	62‡	75†
SD	117	63	62	104	7	9	13	12

*Determined from highest observed concentration
†Significantly different from fasting condition, $p<0.05$, paired t-test
‡Not significantly different from fasting condition
ND, not determined because of loss of catheter patency

TABLE 2

FRACTION OF ETHANOL DOSE (0 6 g/kg) ABSORBED UNDER FOUR EXPERIMENTAL CONDITIONS

	IV Route			Oral Route			
Monkey	Fasting*	Fed	Stress	Fasting	Fed	Stress	Distributed Dose
71038	1 0	ND	ND	1 0	0 78	ND	0 99
70066	1 0	0 94	1 07	0 88	0 77	0 86	0 96
78329	1 0	1 03	0 94	1 02	0 81	1 03	0 96
80369	1 0	0 99	ND	0 99	0 85	0 93	1 0
Mean	1 0	0 99	1 01	0 97	0 80†	0 94	0 98
SD		0 05	0.09	0 06	0 04	0 09	0 02

*Value is 1 0 by definition
†95% Confidence limits exclude 1 0
ND, not determined because of loss of catheter patency

TABLE 3

RELATIVE EXPOSURE* TO ETHANOL FOLLOWING ADMINISTRATION (0 6 g/kg) UNDER FOUR EXPERIMENTAL CONDITIONS

	IV Route			Oral Route			
Monkey	Fasting†	Fed	Stress	Fasting	Fed	Stress	Distributed Dose
71038	1 0	ND	ND	0 91	0 42	ND	0 68
70066	1 0	0 96	1 09	0 67	0 57	0 75	0 85
78329	1 0	0 96	0 87	0 85	0 44	0 72	0 68
80369	1 0	1 0	ND	0 83	0 49	0 87	0 69
Mean	1 0	0 97	0 90	0 82‡	0 48‡§	0 78‡	0 73‡
SD		0 02	0 15	0 10	0 07	0 08	0 08

*Ratio of AUC under the given route/condition to AUC under IV/fasting condition
†Value is 1 0 by definition
‡95% Confidence limits exclude 1 0 (comparison with IV/fasting condition)
§Significantly different from oral/fasting condition ($p<0.05$ by paired t-test)
ND, not determined because of loss of catheter patency

TABLE 4

SIMULATED EFFECT OF ABSORPTION RATE CONSTANT ON ETHANOL PEAK CONCENTRATION, TIME OF PEAK CONCENTRATION, AND AUC

Absorption Half-life, min	Peak Concentration		Peak Time		AUC	
	μg/ml	% change*	min	% change*	(μg/ml) hr	% change*
6 9	540	0	27	0	872	0
8 3	516	−4	27	0	856	−2
10 4	490	−9	36	+33	831	−5
13 9	445	−18	45	+67	790	−9
20 8	375	−31	54	+100	710	−19
41 6	239	−56	63	+133	512	−41

*Percent difference from value with absorption half-life of 6 9 min

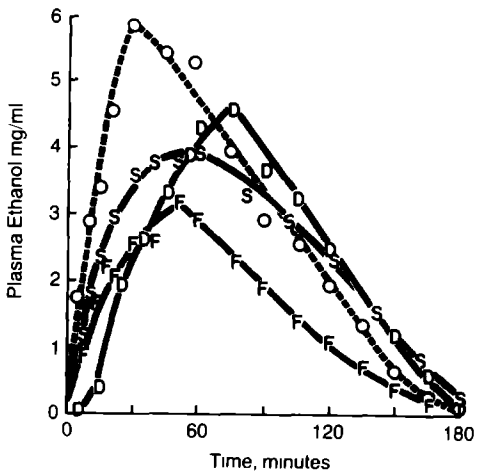

FIG 2 Representative concentration-time profiles of plasma ethanol following 0 6 g/kg doses administered orally, under conditions of fasting (O), prefeeding (F), stress (S), and distributed dosing (D) to subject 78329

sorbed into the circulation in the oral/fasting condition was not significantly different from the amount administered (Table 2), and while the relative exposure, as measured by the AUC, was 18% less than that following IV injection (Table 3), the rate of decline in plasma concentration was almost identical (Fig 1)

Feeding, mild stress, and distributed dosing, however, produced major distortions of the concentration-time profile (Fig. 2) The changes were most pronounced in the magnitude of the peak plasma ethanol concentration (Table 1) The mean peak concentration after feeding 1.5 biscuit/kg was 366 μg/ml, 42% less than in the fasting condition In separate experiments in which two monkeys were tested once each, an effect of similar magnitude was produced by prior feeding of as little as 2 g of glucose. Minor stress reduced the mean peak concentration by 29% Distributed dosing appeared to reduce it by 18%, but that effect was not statistically significant The time to peak concentration was significantly delayed only in the distributed dosing condition (Table 1) The time to peak concentration appeared to be delayed by stress, but because technical difficulties allowed only three monkeys to be included in the pairwise comparison, the difference relative to oral/fasting was not significant by paired comparison t-test (By unpaired t-test, the difference was significant at the $p=0$ 02 level)

Comparing the oral/fasting condition with other conditions of oral administration revealed that the fraction of the dose absorbed into the circulation was not influenced by mild stress or distributed dosing, but was significantly reduced (18%) if the animal had eaten (Table 2) Likewise, relative exposure under the conditions of oral administration was unaffected by mild stress and distributed dosing, but was significantly reduced (41%) by feeding (Table 3) The fact that feeding and stress did not influence the extent of absorption (Table 2) or relative exposure (Table 3) when ethanol was administered IV suggests that feeding and stress affect the absorption of ethanol rather than its elimination

Because of the apparent contradiction in the peak time, peak concentration, and relative exposure results with regard to the effect of the various conditions on ethanol absorption rate, a simulation was done to determine the relative sensitivity of those three parameters to absorption rate The simulation was based on the mean values of V_{max}, K_m, and volume of distribution determined in the IV/fasting condition Different rates of absorption (Table 4) were simulated on the basis of a first-order absorption rate constant that was varied from values of 1 0 hr^{-1} to 6 0 hr^{-1} These values correspond to absorption half-lives of 41 6 min to 6 93 min Fastest absorption, produced by an absorption half-life of 6 93 min (absorption rate constant of 6 0 hr^{-1}), gave the highest peak concentration, earliest peak time, and greatest AUC The relative sensitivity of the three parameters to changes in absorption rate constant can be determined by comparing the percent change as a function of the absorption rate constant Peak time was the most sensitive to changes in the value of the absorption rate constant, followed by peak concentration and AUC Thus, slower absorption might cause a change in peak time with less effect on peak concentration and little effect on relative exposure The apparent absence of a strong effect on peak concentration in the monkeys is attributed to the fact that the interval between blood samples was 10 to 15 min during the time of interest Thus, the resolution of measurement of peak time was not adequate to detect variation as a function of absorption rate

Integrating the results obtained from the monkeys and the

simulations, one finds that oral administration substantially diminishes relative exposure to ethanol Under conditions of oral administration, feeding and stress cause reductions in peak concentration and a trend to later peak times, probably by reducing the rate of absorption into the circulation In addition, food reduces the extent to which ethanol is absorbed following oral administration, which leads to reduced relative exposure and a further reduction of peak concentration Mild stress and distributed dosing do not greatly influence the fraction of the dose that is absorbed

Theoretically, the decrease in the apparent fraction of ethanol absorbed in the fed condition, as judged by plasma levels, could have been due either to a decrease in the fraction of dose absorbed from the gastrointestinal tract or to an enhanced first-pass effect At concentrations close to the value of K_m, ethanol has a rather high (0 75) hepatic extraction ratio in several species, but the value drops to less than 0 05 as concentration increases [5] The value of K_m in our monkeys was about 60 μg/ml [13] When ethanol was administered in the oral/fed condition, the plasma concentration quickly rose well above K_m (Fig 1) The first-pass effect under these conditions would be quite small Also, there was no first-pass effect apparent when ethanol was administered in the oral/fasting condition Thus, the decreased relative exposure observed under the oral/fed condition in this study most likely resulted from a decrease in the fraction of the dose absorbed from the gastrointestinal tract This finding coincides with results of other studies that have shown that eating can diminish the AUC of orally administered ethanol in humans [19, 20, 23] It also supports the view of Henningfield and Meisch [10] that feeding increases the amount of alcohol that must be ingested to achieve a plasma level associated with a given effect In their study, rhesus macaques that had been drinking ethanol almost daily for about a year showed a preference for ethanol over water, and drank to intoxication when ethanol was available Presession feeding was associated with, on average, 64% greater intake of ethanol than post-session feeding Consistent with our findings, the authors proposed that the increase in ethanol consumption seen in macaques following a meal resulted primarily from decreases in the rate and extent of ethanol absorption

The results of this study show that consumption of a minimal amount of food, exposure to mild stress, and distributed dosing alter the time course of ethanol concentration in plasma after oral ingestion to a degree that is doubtless sufficient to affect the relation between behavior and dose The findings have two major implications for the behavioral scientist interested in investigating the influence of environmental factors on ethanol intake First, one cannot accurately estimate plasma ethanol concentration or exposure simply on the basis of a record of the schedule of intake and pharmacokinetic parameters such as V_{max} and K_m for ethanol in the circulation [13] Unscheduled eating is difficult to control in macaques on a Monkey Chow diet, for they can sequester several biscuits in their cheek pouches and swallow them at any time for many hours after ingestion In most behavioral studies, this difficulty can be avoided only by withholding food and ethanol for a relatively long period, e g , overnight [10] Even if feeding, schedule of ethanol intake, and exposure to handlers were totally controlled or recorded, one could not assume that threatening behavior by other animals in the social group did not have the same kind of effect on peak concentration as the approach of an unfamiliar person Ironically, to eliminate the social factor would be to eliminate the possibility of answering the questions of greatest interest from a neurobehavioral standpoint

The second implication is of considerable theoretical interest, namely, environmental stimuli may influence ethanol intake more by influencing the rate at which ethanol is absorbed into the circulation than by resetting a cerebral threshold mechanism that regulates the blood levels of ethanol at which ethanol ingestion commences and ceases On the basis of the findings reported here, it is quite conceivable that the variations in ethanol intake seen in animals and people under different social circumstances are greatly, if not predominantly, determined by centrally mediated changes in absorption, i e , the rate and extent to which ethanol is transferred from the gastrointestinal tract into the blood stream Changes in the rate of gastric emptying, gut peristalsis, and mesenteric blood flow are among the peripheral mechanisms subject to central neural control that could mediate such changes An important question for future research on the social determinants of voluntary ethanol consumption in the nonhuman primate model will be whether the effects of environmental stimuli are mediated directly through the central nervous system, e g , by action on neural circuits that mediate taste preferences, threshold of the blood level necessary for a reinforcing effect and the like, or indirectly through changes in gastrointestinal function that enhance or impede absorption of the drug

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