AN INVESTIGATION OF THE POSSIBLE ROLE OF ALDEHYDE DEHYDROGENASE AS A REGULATOR OF VOLUNTARY ETHANOL CONSUMPTION

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ABSTRACT

AN INVESTIGATION OF THE POSSIBLE ROLE OF ALDEHYDE DEHYDROGENASE AS A REGULATOR OF VOLUNTARY ETHANOL CONSUMPTION

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The relationship between hepatic and cerebral acetaldehyde oxidizing capacity and voluntary ethanol consumption was investigated in the laboratory rat. In the first experiment possible relationships were examined in three of the most commonly used rat strains in the alcohol self administration field. Although some weak relationships were observed between hepatic aldehyde dehydrogenase (ALDH) activity and voluntary intake, only brain ALDH activity was demonstrated to consistantly correlate well with ethanol self administration levels. It was further observed that animals exposed to ethanol on a chronic basis demonstrated higher levels of hepatic ALDH activity as compared to control. The significance of this phenomenon with respect to the possible metabolic regulation of intake has been disputed in the literature. To further investigate the dynamics of this apparent enzyme induction and to elucidate its relation to the regulation of intake, a second experiment examined this phenomenon employing a time course paradigm. Tissue

samples were collected from animals chronically exposed to ethanol after various periods of termination of alcohol availability. It was observed that exposed animals demonstrated higher hepatic ALDH activity than controls up to 72 hours after the removal of ethanol. However, it was observed that as seen in the first experimemt only brain ALDH consistanly correlated with voluntary ethanol intake. The sum of these findings would seem to indicate a possible regulatory role for brain ALDH in the voluntary intake of ethanol. Furthermore, the present findings added to those previously reported suggest that this phenomenon may occur in numerous rat strains, and does not appear to be strain specific.

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The use of beverages containing ethyl alcohol (ethanol) parallels much of human history (Murphree, 1971). The ancient Epicurians embodied the consumption of wine in their philosophy which was later borrowed by the Romans and established as a religious as well as hedonistic ritual in homage to Bacchus, god of wine (Robinson, Breastead & Smith, 1964). The distillation of whiskey, one of the major chemical advances in processing alcoholic beverages, has been ascribed to Paracelsus in the Middle Ages (Seixas, 1979). Ethanol is considered to be the most extensively used mood altering drug in almost every human society (Lieber, 1976), and recent studies indicate that approximately 5-15% of North American and European drinkers are considered to be problem drinkers (Cahalan & Room, 1972; Hagnel & Tunvig, 1972; Weisman, Myers & Harding, 1980). The human emotional and physical costs of ethanol abuse are staggering, precipitating family breakdown, crime and automobile accidents (le Dain, 1973; Bacon,1968). In purely economic terms the ethanol related costs to North American society in 1975 was estimated at nearly $43 billion in lost production, medical expenses, motor vehicle accidents, fire losses, and the maintenance of social programs to deal with these problems (Seixas, 1979).

Like many other behaviour disorders ethanol abuse is

a multifactorial problem, with numerous potential etiologies (Jellinek, 1960). The complexity of the problem has given rise to a great deal of research attempting to understand the social and biological factors involved in ethanol abuse (Madden, 1979). With particular respect to possible biological factors, extensive research has been devoted to the elucidation of the neuropharmacological basis of ethanol self administration. To pursue such studies on a practical level many animals models of human alcoholism have been proposed. As early as 1926, it was demonstrated that laboratory rats will readily consume 8% ethanol solutions in self-selection conditions with water (Richter, 1926). Later studies confirmed these findings, demonstrating that laboratory animals will consume large amounts of ethanol in spite of its aversive taste (Kahn & Stellar, 1960; Wilson, 1972). Animals will also self administer ethanol through (Deneau, Yanagita & Seever's, 1969; Winger & Woods, 1973) and intragastric routes (Amit & Stern, 1969; Yanagita & Takahashi, 1973).

As it would appear from these investigations that ethanol may act as a positive reinforcer, much attention in the alcohol field has been paid to the neurochemical mechanisms subserving ethanol reinforcement. It has variously been demonstrated that electrical stimulation of

the lateral hypothalamus resulted in increased ethanol intake (Amit, Stern & Wise, 1970; Amit & Stern, 1971; Amir & Stern, 1978), and that electrolytic lesions of the ventral lateral hypothalamus attenuated ethanol preference (Amit, Meade, Levitan & Singer, 1976). As the lateral hypothalamus has been shown to be traversed by some of the major catecholamine pathways (Lindvall & Bjorklund, 1974; Ungerstedt, 1971) and it has been suggested that the catecholamines are involved in the mediation of motivated behaviours (Fibiger, 1978; German & Bowden, 1974), ethanol may be acting on the CNS at this level. However, as ethanol is widely used in society and yet only a small proportion of imbibers become abusers, it becomes obvious that the identification of generalized drug effects does not completely fulfill the task of explaining and describing the distinction between users and abusers.

To this end it has been proposed that biological factors may predispose people to chronic abuse of ethanol (Eriksson, 1975; Schuckit, 1980). Such a contention is not without precedent with respect to other complex behaviour disorders. Schizophrenia, another complex, multifactorial disorder has been suggested to be tied in some fashion to a predispositional factor (Meehl, 1962). Meehl, 1962 has put forth the idea that the incidence of such disorders reflects not only the environmental

influences placed upon the individual but some synergistic effect of a predispositional factor and the environment.

A predispositional factor may reflect any number of possible mechanisms related to ethanol consumption, however, those previously suggested by Omenn (1975) and Schuckit (1980) seem most pertinant.

High risk individuals may display different acute responses to a dose of ethanol. These responses could increase an individual's liability toward alcoholism by resulting in a more pleasant or intense intoxicating effect. On the other hand some individuals may display acute responses of a less intense quality, theoretically implying the need for consumption at greater levels to acheive a given degree of reinforncement. This process may work in the opposite direction as well resutling in aversive responses to acute exposure, protecting the individual from further drug experiences. This may be characterized by the so called "oriental flushing response," often observed in Mongoloid peoples after ethanol consumption and which is associated with irritability, skin flushing, nausea, etc., (Wolff, 1972).

Predisposition toward alcoholism may reflect differences in more chronic reactions to the drug. This may be exemplified by a differential developement of tolerance to ethanol in high and low risk individuals,

resulting in those persons with rapid tolerance taking continually increasing amounts of the drug over time. This has been suggested to be linked to an alteration in vulnerability to possible dependance to the drug (Schuckit, 1980). In addition, such a mechanism may be involved in the mediation of the time course and severity of dependance (Schuckit, 1980).

As can be seen from the previous suggestions, a predispostion toward alcoholism appears to be potentially related to differnces in the metabolism of ethanol. Such a predisposition may be explained in terms of different types of ethanol metabolism, perhaps affected by altered forms of the enzymes alcohol or aldehyde dehydrogenase (Li, 1977; von Wartburg, 1971), influencing the level of intoxication, length of drug effects, the effect ethanol has on CNS neurotransmitters or the amount of acetaldehyde which develops after drug exposure. Such a predispositional factor may be the biochemical mediator of a genetic difference, may represent an acquired trait or may be some interaction of acquired and inherited traits.

The following sections of the introduction will review the areas of ethanol metabolism and potential predispositional factors in alcoholism and outline the present investigation.

Determinants of Ethanol Utilization Capacity

A. System Dynamics and Routes of Ethanol Metabolism

Ethanol is a unique drug in that its effect is not related to absorbtion factors which must be taken into account with other psychoactive compounds. It has an extremely high aqueous solubility and is not dependant on the rate of dissolution (Fingl & Woodbury, 1975). Absorbtion of ethanol into the body after oral administration in man and animals is generally dependant upon concentration and the amount of food present in the stomach (Erickson, 1979). High ethanol concentrations tend to reduce gastric motility and hence absorbtion, and high fat foods tend to diminish blood ethanol concentrations (Welling et al., 1977). As ethanol has a high aqueous solubility it is held that it will accumulate in tissues with the highest water content (Harger, Hulpieu & Lamb, 1937). After oral administration ethanol has been detected in virtually every tissue of the body, including brain (Erickson, 1979). The elimination of absorbed ethanol is extremely efficient with over 90% being metabolized in the body (Erickson, 1979).

It is generally agreed that alcohol dehydrogenase (ADH.alcohol:NAD oxidoreductase E.C.1.1.1.1) is the major

oxidative enzyme for ethanol in mammalian species. Upon absorbtion, ethanol is oxidized to acetaldehyde by ADH which is concentrated primarily in liver (Lundquist et al., 1962; Tygstrup, Winkler & Lundquist, 1965; Lundquist, 1975). Smaller amounts of ADH have been identified in extrahepatic tissues, most notably the kidney and gastric mucosa (Krebs, 1969; Lundquist, 1971; Raskin & Sokoloff, 1972) as well as in brain (Raskin & Sokoloff, 1968; 1970). It has previously been demonstrated that ADH can be found only in the cytosol of cells identified as containing this enzyme (Nyberg, Schuberth & Augard, 1953; Rognstad & Clark, 1974; Havre et al., 1976).

As ADH is the principal enzyme in ethanol metabolism, its activity may be directly related to an organism's ethanol utilization capacity. The oxidation reaction of ethanol by ADH in the presence of nicotinamide adenine dinucleotide (NAD+) is initiated by ethanol concentrations as low as 260uM (Feytmans & Leighton, 1973). Ethanol concentration is not the sole factor determining the rate of this reaction, however, and may not be rate limiting (Lindros et al., 1974). Although the metabolism of ethanol is seen to follow zero order kinetics to blood concentration as high as 4mM (Loomis, 1950; Lundquist, 1971), the dependance of this reaction on NAD+ as a cofactor necessitates an examination of the contribution

of this cofactor to the overall rate of reaction. Due to the cytosolic location of ADH, reducing equivalents from cytosolic reduced NAD+ (NADH) must be translocated into the mitochondria via shuttle systems for oxidation by the electron transport chain (Stryer, 1975). This system enables the regeneration of NAD+ from NADH (Stryer, 1975). The dependance of ADH on NAD+ for catalytic activity, thus makes the efficiency of NAD+ regeneration crucial to the normal procedure of the reaction. If the capacity of alcohol dehydrogenase is markedly higher than the flux through these regenerative steps, control of ethanol utilization could be exerted nearly exclusively by the respiratory chain (Rognstad & Grunnet, 1979).

A possible test of the importance of the respiratory chain in control of ethanol utilization has been proposed through the use of uncoupling agents. By addition of dinitrophenol it has been demonstrated that ethanol uptake by rat liver slices can be increased up to 100%, while increasing oxygen uptake (Videla & Isreal, 1970). Reports on the magnitude of a similar effect in perfused whole livers appear to be conflictual. Seiden et al., (1974), showed a 100% increase in ethanol uptake in livers of both fed and starved rats, while Eriksson et al., (1974), showed a 20% increase in fed rats only.

Meijer et al., (1975) using the compound FCCP

(carbonyl cyanide p-triflouromethoxyphenyl hydrozine) demonstrated a stimulatory effect on livers of fed rats only. They concluded that the NADH shuttle systems were rate limiting in the starved rats while the rate of the electron transport chain controlled metabolism in fed rats. It was further noted by these authors that fed rats had higher basal ethanol oxidation rates. The possible significance of this effect with respect to ethanol utilization capacity lies in two distinct areas: Principally, these studies show the relation between the dynamics of primary ethanol metabolism and nutrition. Secondarily, these reports raise the possiblity that differences in ethanol metabolism may be related to differential sensitivity to nutritional manipulations.

Another aspect which must be taken into account in evaluating ADH activity as a mediator of ethanol utilization capacity is the existance of multiple or multi-molecular forms of the enzyme (von Wartburg et al., 1965). It has been demonstrated that different types of multiple forms of ADH exist in many species, the structural differences are known in some cases and the enzymatic properties frequently vary (Jornvall, 1979). Human liver ADH is a dimer formed by random association of alpha, beta and gamma subunits by three structural gene loci, ADH1, ADH2, ADH3 (Smith et al., 1973). This implies

numerous combinations of pairs of polypeptides selected from the three polypeptides coded by the corresponding gene loci. A similar pattern of isozymes has been reported for laboratory mice by Holmes (1977;1978;1979). As these various forms of the enzyme may differ with repsect to their activity with ethanol as a substrate, it has been suggested that the measurement of gross ADH activity could be misleading (Jornvall, 1979). These multiple forms may display different Michealis constants (Km) for ethanol and hence a single concentration used in assay may only be useful in evaluating the relative activity of a single form. This is especially true when comparing differing activities between individuals in relation to consumption. Furthermore, as these multiple forms may have different affinities for ethanol they must be taken into account in evaluating different drinking patterns. Individuals who consume small amounts of ethanol spaced over considerable time would probably never tax the forms of the enzyme activated only at high ethanol concentrations. Conversly, individuals who consume large amounts rapidly would require a metabolizing system capable of acting efficiently at high substrate concentrations. In addition, this latter pattern of drinking as seen frequently in alcoholics (Mello & Medelsson, 1972), may result in sufficiently high ethanol

levels to inhibit low Km isozymes, thereby increasing the reliance on high Km forms for effective ethanol removal (Jornvall, 1979).

The capacity of ADH to act in the regultation of ethanol utilization capacity may be tied to adaptive responses observed after ethanol exposure. The total activity of hepatic ADH has been observed to increase after prolonged ethanol feeding (Hawkins, Kalant & Khanna, 1966; Videla, Bernstein & Isreal, 1973). These reports have been disputed by other studies indicating no change in ADH activity, subsequent to chronic ethanol exposure (Bartlett & Barnet, 1949; von Wartburg, 1971). It is of interest to note that all these studies relied on forced choice paradigms and no previous investigation has examined this phenomenon under self selection conditions. It is possible that these conflicting observations could be related to different patterns of ADH isozymes as previously described here. Different strains may display various isozyme patterns which may alter during ethanol exposure after the typical isozyme response of lactic dehydrogenase (Jornvall, 1979). Such an alteration in the differential formation of higher capacity isozymes may be reflected in an apparent increase in overall activity at ethanol concentrations typically used for ADH assays. Support for such a contention comes indirectly from the

observations of Berger and Weiner, (1977) who showed different isozymes of aldehyde dehydrogenase in alcohol prefering rats. Thus, animals within one strain may show different tendencies to have isozyme levels altered in the presence of an antagonist.

Although ADH is the principal enzyme for ethanol oxidation in vivo (Hawkins & Kalant, 1972), additional removal may occur through the action of the enzyme catalase (E.C.1.11.1.6, Keilin & Hartree, 1945) and the microsomal ethanol oxidizing system (MEOS, Lieber & DeCarli, 1968). Catalase has recently been identified in brain and may participate in ethanol oxidation there as well (Cohen et al., 1980). It appears however, that this enzyme is dependant on peroxide formation and hence will not react with ethanol without sufficient peroxide in the system (Rognstad & Grunnet, 1979). Isselbacher and Carter (1974) reported a 25% increase in liver catalase activity following ethanol exposure. The importance of this effect has been disputed by Rognstad and Grunnet, (1979) who claim that at normal rates of peroxide generation this would produce only a 3% increase in catalase mediated ethanol oxidation. It can be concluded from these reports that the catalase system may be important in ethanol metabolism, but only in the presence of sufficient peroxide. The contribution of the MEOS is less clear and

it has been suggested to be only an artifact due to catalase contamination (Erickson, 1973). Additional doubt as to the significance of this system is cast by the lack of an in vivo demonstration of involvement, even in a most recent study investigating highly purified liver microsomal cytochrome (Miwa et al., 1978).

B. Distribution and Metabolism of Ethanol Derived Acetaldehyde

The primary metabolite of ethanol oxidation is acetaldehyde, which has been shown to be highly toxic (Lindros, 1978). Among humans a relatively low blood level will cause individuals to exhibit extreme discomfort (Wolff, 1972). This response serves as the basis for the administration of drugs which interfere with normal acetaldehyde metabolism, to deter alcoholics from drinking (Hald & Jacobson, 1948; Jacobson, 1952; Raby, 1953; Walsh, 1971; Ferguson, 1956). As the rate of ethanol oxidation under normal conditions is approximately 100mg per kilogram per hour in man (Blomstrand & Holmstrom, 1969) and 300mg per kilogram per hour in rat (Owens & Marshal, 1955), the rate of acetaldehyde metabolism must approximate that rate so as to prevent a toxic build up. Although ethanol oxidation occurs primarily in liver

cytosol (Lundquist, 1975), it appears that acetaldehyde metabolism may occur to a greater extent in extrahepatic tissues. Unlike alcohol dehydrogenase, aldehyde dehydrogenase, the principal acetaldehyde metabolizing enzyme, is ubiquitous to the body (Deitrich, 1966).

The importance of extrahepatic acetaldehyde oxidative pathways appears to be related to the amounts of ethanol consumed. Consumption of moderate quantities of ethanol results in only small amounts of acetaldehyde detectable in blood (Eriksson, 1977; Eriksson & Sipple, 1977). When larger doses are administered (>2g/kg) acetaldehyde formation may proceed at a faster rate than hepatic acetaldehyde elimination capacity, resulting in peripheral acetaldehyde accumulation (Lindros, Vihma & Forsander, 1972; Raskin & Sokoloff, 1972). Furthermore, as ethanol is a highly soluble compound (Fingl & Woodbury, 1975), it may pass into extrahepatic tissues, some of which possess oxidative pathways for the conversion of ethanol to acetaldehyde. This is particularly true of the brain, as both ADH (Raskin & Sokoloff, 1972) and catalase (Cohen et al., 1980) have been reported to occur there. It may be assumed then, that acetaldehyde may arise in brain either from direct in situ ethanol oxidation or from hepatic formation, although this latter route has been disputed (Lindros & Hillbom, 1979; Sippel, 1974; Tabakoff et al.,

The major enzyme of acetaldehyde oxidation as previously stated is aldehyde dehydrogenase (ALDH.aldehyde:NAD oxidoreductase; E.C.1.2.1.3). ALDH may exist in cytosolic, microsomal and mitochondrial forms in all tissues reported bearing this enzyme (Weiner, 1979). Multimolecular forms of the enzyme (isozymes), possessing different physical and catalytic properties can be found in many of the subcellular organelles, such as the mitochondria (Weiner, 1979). The molecular weight of highly purified liver ALDH's is reported to be over 200,000 (Feldman & Weiner, 1972). The subunit molecular weight determined with SDS polyacrylamide gel electrophoreisis is between 50,000 and 60,000 (Feldman & Weiner, 1972), thus, the enzyme appears to be a tetramer or composed of four polypeptides.

The formation of acetaldehyde catalyzed by ADH may be reversed either by the action of this same enzyme (Rognstad & Grunnet, 1979) or by aldehyde reductase (alcohol:NADH oxidoreductase; Gershman, 1975). Consequently, the removal of acetaldehyde from the system can be seen to greatly influence the ethanol utilization capacity of the organism. The dependance of ALDH on NAD+ as a cofactor follows in the same fashion as the alcohol dehydrogenase pathway. Therefore, it becomes apparent that regeneration of NAD+ will govern the rate of

acetaldehyde oxidation by ALDH. In addition, the concentration of acetaldehyde in the system will greatly influence the dynamics of this metabolic pathway. It has previously been reported that high acetaldehyde levels in the body will inhibit the high speed liver ALDH (Lesback et al., 1981). The significance of this effect in ethanol utilization capacity lies in the great variability in activity observed in the differing subcellular forms of the enzyme.

It appears that in rat, the oxidation of acetaldehyde in liver takes place in the matrix space of the mitochondria (Deitrich & Siew, 1974). Thus, a unique situation arises such that acetaldehyde is produced in the cytosol but diffuses into the mitochondria before being oxidized, even though multimolecular forms or isozymes which possess low Kms (μM range) for acetaldehyde are located in the cytosol (Tank & Weiner, 1977). The universal nature of this sytem appears to be questionable, as various rodent strains display differing proportions of cytosolic and mitochondrial activity (Deitrich & Siew, 1974). This situation is further complicated by the fact that both high and low Km forms of the enzyme exist in all subcellular fractions (Deitrich et al., 1978). It can be taken then, that differing reports on the relationship between various ALDH forms and their respective oxidizing

capacities may function according to strain and substrate concentrations used in the assay technique.

It has been reported that at least two forms of the cytosolic enzyme are inducable by phenobarbitol and various other agents (Deitrich et al., 1978; Deitrich et al., 1977). The relevency of this finding to ethanol utilization capacity has been disputed by numerous authors. Eriksson et al., (1975), measured the rate of oxidation of acetaldehyde in animals that were capable of having their isozymes induced, compared to animals which were not. After administration of an intraperitoneal dose of ethanol, they reported no difference in the rate of ethanol oxidation and the rate of acetaldehyde clearance was virtually the same for all animals. More recently, Peterson et al., (1977) repeated these experiments and came to an opposite conclusion. In a paradigm where phenobarbitol was administered as an inducer to the inducable and non-inducable animals they found that the rate of ethanol oxidation was faster in the inducable rats (Peterson et al., 1977). These authors also reported lower acetaldehyde levels in the inducable strain. The increased rate of ethanol metabolism could therefore be related to the rapid removal of acetaldehyde, thereby preventing sufficient build up for it to be reduced back into ethanol.

Another factor affecting ALDH which may possibly influence its role in mediating ethanol utilization is the adaptive change observed to follow prolonged exposure. It has been documented that prolonged feeding of ethanol resulted in an increase in liver ALDH activity (Hasumura, Teschke and Lieber, 1976; Horton & Barrett, 1976). Specifically, the investigation of Hasumura, Teschke and Lieber (1976) revealed a significant increase in the high Km form of the enzyme. A most recent report provided evidence for a diminution of liver ALDH activity after prolonged ethanol exposure (Lesback et al., 1981). Other studies have disputed these findings by showing no change in enzymatic activity after ethanol exposure (Horton, 1971). With respect to brain ALDH, previous reports demonstrate an equal lack of consensus. Studies by Raskin & Sokoloff (1972) and Tabakoff & Buggan (1974) have shown no change in ALDH activity following ethanol exposure. Amir (1978a) showed an induction, arguing for an adaptive increase in brain ALDH as a function of ethanol exposure. It is difficult to reconcile these conflicting reports on brain ALDH alterations as Amir (1978a) employed electrical brain stimulation to induce drinking and this might have affected enzyme activity in itself.

From all of these studies employing different animals and various assay techniques we can only conclude that

each may reflect a different aspect of the system. Furthermore, as metabolic capacity appears to be a reflection of various forms of enzyme present, each report may represent varying contributions of multiple forms to overall activity across animals. Most importantly, all these studies looking at alterations of ALDH activity employed forced choice intake of alcohol or invasive techniques of ethanol administration and no reports appear to exist regarding a similar effect in unmanipulated animals, drinking alcohol freely under conditions of choice.

The Involvement of Predispostional Factors in the Mediation of Alcoholism

A. Human studies

The notion of alcoholism as a disorder governed at least in part by predispositional factors can be traced back to the earliest period of serious scientific investigation in the area. In the ninteenth century the American Association for the Study and Prevention of Inebriety proposed a hereditary predispostion towards alcoholism (Seixas, 1979). This contention gained a greater impetus in the alcohol field when Williams (1947)

proposed his genetotrophic theory of alcoholism. Since that time there has been much controversy over the importance of "nature vs. nurture," in the production of alcoholism. Unfortunately almost all of the argument has been generated by the popular misconception that if the existance of genetically determined metabolic and behavioural factors in the etiology of alcoholism is accepted, the environmental factors must be ruled out. Such an argument is logically untenable for most simple phenomena and even less tenable for a disorder as complex as alcoholism. The elementary rule of genetics is that the phenotype is determined by both the inherited genotype and environmental factors impinging upon the individual. Discussion of purely genetic factors although helpful in understanding certain aspects of the disorder, would be less than adequate as a real life discription. Conversly, to assume a completely environmentally influenced phenomenon would discount the current degree of understanding of genetics. From the foregoing, it can be taken that any viable experimental approach to understanding a predispositional influence in alcoholism must assume a dynamic interaction of genetic and environmental influences.

The complexity of alcoholism would seem to indicate a polygenetic system operating across the various

manifestations of the disorder (Schuckit, 1980). This means that a characteristic such as alcoholism is a function of a quantitatively variable phenotype which is dependant upon the interaction of numerous genes and the environment. Such a system is reflected by many other phenotypes such as intelligence, height, skin color, etc., (Eriksson, 1975). The general nature of polygenetic inheritance and the statistical methods for determining the degree of heritability was first proposed by Falconer (1960). Essentially this system is based on two distinct types of genes called "major genes," which govern qualitative differences and "minor genes," which control the degree of expression of a given trait. The typical feature of quantitive and polygenetically determined traits is that they follow a normal distribution (Eriksson, 1975). Interestingly, such a statistical distribution reflects the continuum on which behavioural scientists often judge a trait in a population and deduce deviancy from the norm. In other words alcohol consumption as a polygenetic trait would reflect the greatest percentage of individuals in the middle range of consumption and very few persons abstainers of abusers. This statistical distribution appears to have sufficient support in the literature from reports on the universal nature of alcohol consumption in society and the degree of

abuse (Lieber, 1976; Cahalan & Room, 1972).

It has been a long noted observation that alcoholism appears "to run in families" (Schuckit, 1980). The initial full scale study of this observation was conducted in Sweden (Amark, 1951). In this investigation using an extensive survey of parents and offspring it was found that the morbidity risk was 21% in brothers, 26% in fathers, 2% in sisters and 0.9% in mothers. It was futher reported that male children who had one alcoholic parent had a morbidity risk of 33% while being only 17% for sons of non-alcoholic parents. These findings were later supported and extended by Winoker et al., (1970) who in an exhaustive study examined the role of heritability in numerous psychiatric illnesses in addition to alcoholism. The findings of this later report suggest a morbidity risk of 20-30% for male children of alcoholics and twice as high a risk for children with one biological parent being alcoholic as compared to fostered controls. Later studies by Schuckit, (1972a; b; 1978) show significant correlations between deviant behaviours in adolescence including alcohol abuse and parental alcoholism. McKenna and Pickens, (1981) reported most recently that children of two alcoholics were more likely than children of one or non-alcoholics to be younger when first intoxicated, to have more pretreatment behavioural problems and to proceed

more rapidly from first intoxication to alcoholism treatment.

A further test of the heritability of alcoholism has been proposed through examinations of the concordance rate amongst twins. The initial landmark investigation employed 48 pairs of monozygotic and 128 pairs of dizygotic twins (Kaij, 1960). Kaij, (1960) concluded that genetics did indeed play a part in both the consumption pattern and developement of chronic alcoholism. A subsequent study by Partenan et al., (1966) showed that both the frequency of drinking and amount consumed on a given occasion were influenced by heritability factors. A larger sample of both monozygotic and dizygotic twins supported the findings of Partenan et al., (1960) by demonstrating a high concordance rate for the quantity of alcohol consumed (Jonsson & Nilsson, 1968).

Although the family and twin studies would seem to indicate an inherited predisposition towards alcoholism it has been argued that these reports fall short of a concrete demonstration. These studies have been specifically criticized with respect to an inability to ascertain whether the high frequency of alcoholism in family members is due to shared genetic material or to the environmental influences of having an alcoholic family. This question has been answered to some extent by the

various adoption studies investigating the contribution of biological and foster parent phenotypes to the developement of alcoholism in offspring. In a study conducted by Schuckit, (1972) it was shown that alcoholism occured in 10% of cases where the stepparent was alcoholic and the biological parent was not. Conversly, the rate was 64% for subjects who were raised by non alcoholic step parents but who had an alcoholic as their biological parent. Recently a better controlled adoption study with a larger sample was performed in Denmark by Goodwin et al., (1973). This study paid special attention to the age at which the children were adopted so as to reduce any possible confounding by environmental influences of the biological parents. These authors demonstrated that within the constraints of early age of adoption, children of biological parents who were alcoholic were twice as likely to develop alcoholism as compared to foster controls.

In the absence of an identifiable factor directly involved in the mediation of ethanol consumption any discussion of a predispositional propensity would be purely academic. One area which may hold the greatest promise in this respect is observed differences in alcohol metabolism. Studies by Vessel et al., (1971) and Vessel, (1972) have employed a genetic-environmental analysis

investigating ethanol elimination rates in 14 pairs of twins and six prisoners living in a common environment. These studies reported that ethanol elimination rates were almost completely determined by heritability factors. These findings were given further support by the results of Forsander and Eriksson, (1974) showing very high concordance rates for ethanol utilization capacity between monozygotic and dizygotic twins.

Additional validation of these observations may be taken from proported ethnic differences in ethanol metabolism. It was first observed by Fenna et al., (1971) the North American Caucasians had higher ethanol elimination rates than American Indians or Eskimos. Later Wolff, (1972; 1973) studied facial flushing in Caucasian and Oriental populations, suggesting that variations in the reactions of the autonomic nervous system were under genetic control. If it is true that ethanol metabolism is governed to some extent by genetic variations the paramount question would be which mechanism mediates these differences.

Recent reports on the identification of atypical forms of ADH and ALDH may represent part of the answer (Harada, Misawa & Agarwal, 1980; Goedde, Harada & Agarwal, 1979). These authors argue that a fast migrating isozyme of ADH may be responsible for high acetaldehyde levels

reported after ethanol consumption in orientals. They further contend that a low ALDH activity would result in persistant high levels of acetaldehyde in the body.

These findings appear to be highly significant in light of recent theories on the mediation of psychopharmacological responses to ethanol. During the past decade a great deal of evidence has emerged suggesting a role for acetaldehyde in the behavioural and pharmacological effects of ethanol (Amir et al., 1980; Lindros, 1980; Amit et al., 1980a). One author has gone so far as to suggest that alcoholism is in fact acetaldehydism (Raskin, 1975). Acetaldehyde has been implicated in the mediation of both the reinforcing and aversive effects of ethanol consumption (Amit et al., 1980b; Eriksson, 1980). As these reports suggest that the behavioural and pharmacological effects generated by acetaldehyde may be a function of physiological titres, metabolic processes involved in the elimination of this substance may play an important regulatory role in its putative psychopharmacological actions. Furthermore, reports in the literature indicate that alcoholics and children of alcholics display higher acetaldehyde levels in response to a dose of ethanol as compared to controls (Schuckit, 1980; Lindros et al., 1980).

Probably, these differences in acetaldehyde levels

amongst various ethnic groups and between alcholics and controls are not due to variation in ADH isozymes but are more closely related to the elimination capacity of ALDH. In both human and animal studies the presence of more active ADH isozymes have not been found to have much effect on the the overall ethanol elimination rate (Eriksson, 1975). Thus, it appears more likely that differences in ALDH will directly mediate the observed difference in acetaldehyde elimination and hence the overall rate of ethanol utilization.

B. Animal Studies

A method of choice in the examination of genetic influences over ethanol consumption levels has been the use of inbred lines, using special brother-sister matings. After approximately 20 generations of inbreeding, the strains are practically homozygous, which means that roughly 99% of all gene loci are identical (Eriksson, 1975). In the earliest of such selective breeding attempts, McClearn & Rogers, (1956) and Rogers, (1966) demonstrated that voluntary ethanol intake is very different between various inbred mice strains. It has further been reported that a great deal of variability exists amongst these inbred lines with respect to ethanol

concentrations consumed (Fuller, 1964). A biometrical analysis of Fuller's, (1964) and McClearn & Rogers', (1962) data showed that approximately 80% of the total variance in voluntary drinking behaviour is under genetic control (Brewster, 1968). This same author presented data on alcohol consumption of Maudsley reactive (MR) and Maudsley non-reactive (MNR) rat strains demonstrating that 72% of the total variance in ethanol intake was genetically controlled in these animals. The specialized rat strains developed for ethanol preferance at the Alko laboratories in Finnland have been well documented (Eriksson, 1968; 1969). The Alko, Alcohol (AA) rats voluntarily drank 5-10g of ethanol per kg., per day, taking an average of 25-40% of their energy needs from ethanol. The Alko, Non-Alcohol (ANA) strain almost completely avoids ethanol solutions, consuming less than 1g ethanol per kg., per day.

With regards to metabolic differences in these specialized animals there has been much controversy concerning difference in liver ADH activity (Bennett & Hebert, 1960; Eriksson & Pikkaranian, 1968; Schlesinger et al., 1966; Sheppard et al., 1968). However, it has been found that only a slight difference in total ADH activity exists between high and low drinking mouse strains (Erissson, 1975).

A more likely hypothesis is based on the findings that the high drinking mouse strains also have higher levels of ALDH activity (Schlesinger et al., 1966; Sheppard et al., 1968). If ethanol intake is regulated by the acetaldehyde levels produced, the higher ALDH activity might allow the drinker strains to consume more ethanol. A better validation of this mechanism is found in reports on the relationship between cerebral ALDH activity and voluntary ethanol intake (Amir, 1977; Amir & Stern, 1978; Amir, 1978a; Sinclair & Lindros, 1981). These studies all indicate a significant correlation between voluntary ethanol intake and rates of brain ALDH activity. As the CNS may be involved in the mediation of at least part of the responses observed in the presence of acetaldehyde in the body, this relationship may be the level at which a predispositional propensity is expressed. Such a system may be tied to the biogenic amines as ALDH has been shown to be a necessary pathway for de-amination (Duncan & Sourkes, 1974; Tabakoff & Gelpke, 1975; von Wartburg et al., 1976). The importance of this relationship has recently been disputed by Inoue et al., (1980) who observed no differences in cerebral ALDH activity in the preferring and non-preferring rat strains, although large differences in ethanol consumption were observed. These conflicting reports may be a function of different assay

methods employed, or due to differences in the relative importance of other factors which influence intake, which may differ across the various inbred strains investigated.

The Present Investigation

The lack of agreement over the possible relationship between cerebral ALDH activity and voluntary ethanol intake points up the problematic nature of the attempts to make generalizations from data collected on specialized strains (Amir, 1978a; Inoue et al., 1981). In each case (Amir, 1978a; Inoue et al., 1981), the observations may be valid with respect to factors which exert the most direct control over drinking in a particular group of animals. For this purpose the present investigation sought to reexamine this issue using the most commonly employed animals in the field of self selection. Experiment 1 compared drinking levels and its relation to metabolic capacity in three of the most widely used rat strains. The relationship between hepatic and cerebral acetaldehyde oxidizing capacity was compared in individual animals to voluntary consumption levels of ethanol. The second experiment investigated the significance of possible adaptive increases in ALDH activity following prolonged exposure, as a possible mediator of voluntary intake. The

relative importance of hepatic ALDH induction was examined in light of the apparent non-inducable nature of cerebral ALDH in order to elucidate at which physiological level the enzyme may be most closely tied to the mediation of voluntary consumption.

Experiment 1

A growing body of evidence has emerged in recent years suggesting a role for acetaldehyde, the primary metabolite of ethanol in the behavioural and pharmacological effects of alcohol (Amir et al., 1980; Lindros, 1980; Amit et al., 1980a). It has been reported that acetaldehyde may display both positively reinforcing and aversive properties in man (Amit et al., 1980b; Hald & Jacobson, 1948) and animals (Amit et al.,1980b; Eriksson, 1980).

As the behavioural and pharmacological effects generated by acetaldehyde appear to be a function of physiological titres (Amit et al., 1980b), metabolic processes involved in the elimination of this substance may play an important regulatory role in its putative psycholpharmacological actions. Although the presence of appreciable amounts of acetaldehyde in brain during ethanol intoxication have yet to conclusively demonstrated (Lindros & Hillbom, 1979; Sipple, 1974; Tabakoff et al., 1976; Wescott et al., 1980), the absence of such a demonstration does not entirely preclude the possibility of some quantities accumulating. Furthermore, the amount of acetaldehyde necessary to produce a reinforcing effect and its half life in brain have also not yet been determined. Central acetaldehyde accumulation could

result from direct ethanol oxidation in brain. In fact, it appears that the brain does possess the necessary oxidative enzymes for ethanol metabolism, for example, alcohol dehydrogenase (Tabakoff & Gelpke, 1975) and catalase (Cohen et al., 1980). If such a mechanism were indeed operating, then the probability of sufficient quantities of acetaldehyde accumulating to elicit any of its suggested effects might diminish with an increased rate of elimination. In addition, the efficiency of central acetaldehyde elimination is of particular importance since it is dependant on aldehyde dehydrogenase (ALDH), which is also involved in biogenic amine metabolism (Duncan & Sourkes, 1974; Tabakoff & Gelpke, 1975; von Wartburg et al., 1975). This common mechanism for biogenic amine deamination and acetaldehyde oxidation may result in substrate competition (Truitt & Walsh, 1976), giving rise to numerous effects in the CNS as has previously been suggested by Amir (1977).

It has been reported that a direct relationship exists between brain ALDH activity and ethanol consumption under a variety of manipulations and conditions (Amir, 1977; Amir & Stern, 1978; Amir, 1978a; Sinclair & Lindros, 1981). Although Amir (1978a) reported consistant high correlations within strain, as well as between strains differing in alcohol intake, this latter finding has been

disputed (Inoue et al., 1981). This discrepancy between the findings of Amir (1978a) and Inoue et al., (1981) may be attributed to the relative importance of other possible mediational factors which may differ in the various strains tested as a function of selective breeding programs.

Although a large proportion of previous studies investigating the relationship between ALDH activity and drinking employed specialized strains, the bulk of alcohol research relies on common outbred strains. For this reason the present study sought to investigate more systematically the relationship between ALDH activity and ethanol consumption in the most frequently used strains. Possible relationships were investigated in unmanipulated animals consuming ethanol on a voluntary choice basis.

Methods

Animals: Male rats of the Long Evans, Wistar and Sprague Dawley strains were obtained from Canadian Breeding Farm Laboratories (St. Constant, Quebec). All animals weighed approximately 150-175g., at the start of the experiment. They were housed singly in stainless steel cages, in a room regulated for constant temperature and humidity, in a 12h light cycle. Drinking fluids were

presented in glass Richter tubes and standard lab chow was freely available throughout the experiment. Animals were randomly distributed into ethanol drinking and control (water only) subgroups. The numbers of animals used is each group are presented in the figures and tables.

Ethanol Exposure Procedure: Animals were initially exposed to ethanol on an alternate-day, free-choice paradigm modified from the procedure of Amit et al., (1970). On alternate days animals were offered a free choice between water and increasing concentrations of ethanol, presented in Richter tubes mounted in front of the home cages. On intervening days only water was made available in both tubes. Ethanol solutions were prepared by mixing 95% ethanol with tap water. On the first day of exposure a 2% (v/v) ethanol solution was presented in a free choice with water. Concentrations were increased in increments of 2% with two alternate day presentations at each concentration. On the day after the second presentation of 10% ethanol, animals were switched to an every-day presentation achedule of a 10% ethanol solution, for 28 days. Fluid consumption and body weights were measured daily and ethanol consumption calculated in g/kg body wt/day. The position of the Richter tubes was changed daily throughout the exposure period. Ethanol was withdrawn 48h prior to the sacrifice of the animals for

tissue preparations.

Preparation of Brain and Liver Tissues: Animals were decapitated and their brains and livers were rapidly removed, washed with ice cold .25M sucrose, blotted dry and weighed. Tissue fractions were homogenized (Teflon on glass) in sufficient .25M sucrose containing 1% Triton-X100 to make 10% brain and liver homogenates. Homogenates were centrifuged for 1h at 100,000 x g, at 0 C, and then the clear supernatant was decanted and used as the enzyme source. All samples were frozen at -70 C until assayed.

Assay of Aldehyde Dehydrogenase: Total activity of ALDH was determined by measurement of the rate of enzyme catalyzed NAD+ dependant production of indole-3-acetaldehyde (modified from Duncan & Sourkes, 1978). A reaction mixture of 0.5 ml of sodium phosphate buffer (pH 7.4), 0.3 ml distilled water, 50µl of 0.01M NAD+ (final conc. 5×10^{-4}) and 50µl of the enzyme was pre-incubated for 10 minutes at 30 C. The reaction was initiated by the addition of 0.1 ml of 5 x 10 M indole-3-acetaldehyde (Sigma Co.) bringing the total volume to 1.0 ml. The reaction was terminated after 10 minutes by the addition of 0.2 ml of 1M semicarbazide HCl (final conc. 0.16M) and the unreacted aldehyde was extracted into dichloroethane. ALDH activity was

estimated from the flourescence (excitation 280 nanometers, emission 365 nanometers) of the indole-3-acetic acid formed and was expressed in nanograms/h/μg protein. Recovery of known amounts of indole-3-acetic acid carried through the incubation from solutions containing no NAD+ or enzyme was quantitative. Comparison of samples carried through the incubation and external standards revealed that total recovery of indole-3-acetic acid was 40%. The reaction was linear for at least 15 min, indicating complete saturation of the enzyme. Protein content was measured after the method of Lowry et al., (1951) and bovine serum albumin (Sigma Co.) was used as standard. All assays were carried through in duplicate for both enzyme activity and protein determination.

Results

Ethanol Intake: A two-way analysis of variance was performed on ethanol consumption over the 28 day baseline period. The total amounts of ethanol consumed by the three strains did not differ significantly $F(2,20)=2.08$, $p>.05$. As well there was no significant effect of day on group ethanol intake across the baseline period $F(12,120)=1.43$, $p>.05$. In other words, the ethanol intake

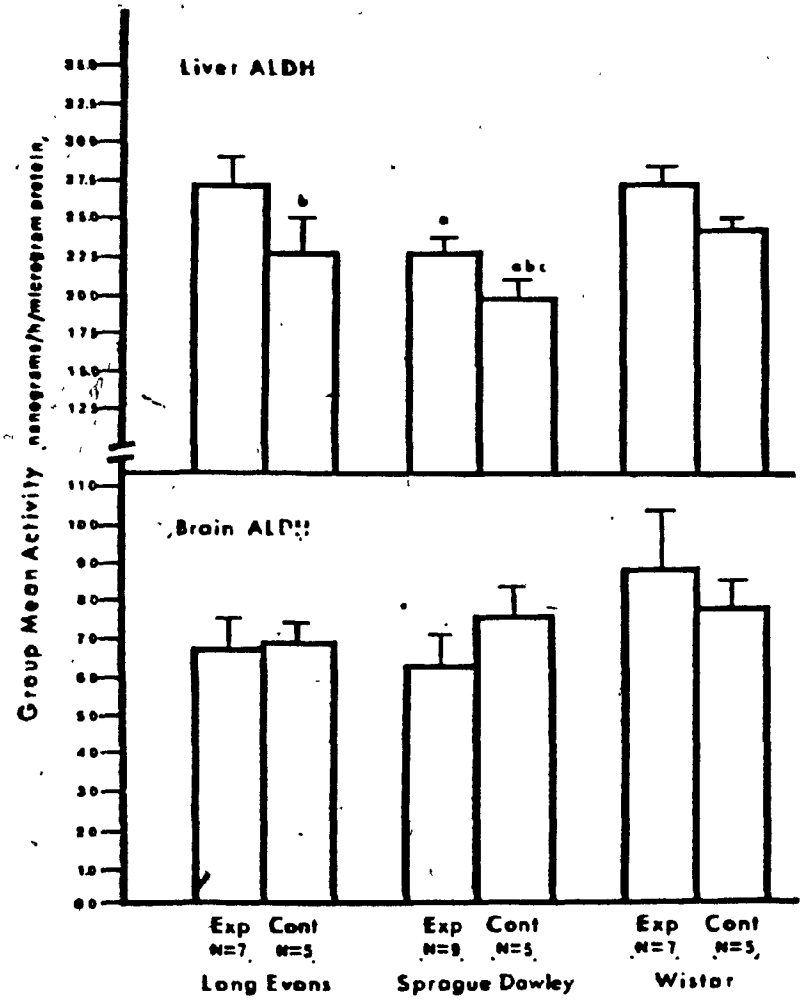
did not differ between groups or within groups throughout the experiment. The average daily ethanol intake of the three strains were as follows: Long Evans 3.29±.54 g/kg, Sprague Dawley 2.52±.51 g/kg, Wistar 2.47±.48 g/kg.

Brain and Liver ALDH Activity: The levels of brain ALDH activity did not differ significantly between ethanol drinking and control animals as presented in Figure 1. A comparison of brain ALDH activity across strain in ethanol drinking animals showed no significant difference $F(2,20)= 1.89$, $p>.05$.

A two-way analysis of variance for liver ALDH activity levels showed that unlike brain ALDH, levels in liver were significantly higher in ethanol consuming animals than in controls $F(1,32)= 19.61$, $p<.001$. A significant difference existed as well across strains for liver ALDH activity levels $F(2,32)= 15.38$, $p<.001$. A post hoc comparison of liver ALDH levels for control and experimental animals was performed using the Tukey HSD test (Kirk, 1968) and results are presented in Figure 1.

Analysis of the relationship between brain and liver ALDH activity and ethanol consumption within groups is presented in Table 1. In addtion, a test of significance between two correlations (Bruning & Kintz, 1978) was performed for the relation between brain and liver ALDH activity and ethanol intake for the three strains. Long

Figure 1. Total cerebral ALDH activity and liver ALDH activity in ethanol consuming and control animals of the Long Evans, Sprague-Dawley and Wistar strains.



Data represents values of enzyme activity of ethanol consuming and control (water-only) animals. Activity is expressed in nanograms Indole-3-acetic acid/h/µg protein. Subscript letters indicate significant difference ($p<.05$), from following group: a) Long Evans Experimental, b) Wistar Experimental, c) Wistar

Table 1. Correlations Computed Between Brain ALDH, Liver ALDH and Ethanol Consumption Within Long Evans, Sprague-Dawley and Wistar Strains.

	Long Evans (n=7)	Sprague-Dawley (n=9)	Wistar (n=7)
Brain ALDH: ethanol intake	0.837**	0.701**	0.710*
Liver ALDH: ethanol intake	0.480	0.670*	0.579

* $p<0.05$ (one tail)

** $p<0.02$ (one tail)

Evans $z=.322$, $p>.05$; Sprague Dawley $z=.098$, $p>.05$; Wistar $z=.322$, $p>.05$. An overall correlation was computed for the relation between brain and liver ALDH activity and ethanol intake across the three strains using a z-transformation for enzyme activity and ethanol intake measures. In general brain ALDH activity was found to be a better predictor of ethanol consumption than liver ALDH levels since a significant overall correlation was obtained between brain ALDH activity and ethanol consumption ($r=.631$, $n=23$, $p<.01$). On the other hand, the overall correlation between liver ALDH activity levels and ethanol consumption was not significant ($r=.372$, $n=23$, $p>.05$) for the same animals.

Discussion

The results of the present investigation confirm earlier findings suggesting a direct relationship between brain ALDH activity and ethanol consumption (Amir, 1977; Amir & Stern, 1978; Amir, 1978a; Sinclair & Lindros, 1981). The correlations presented show that within the same animals only the relationship between voluntary ethanol consumption and brain ALDH activity appears to be significant. In general these findings are in agreement with those previously reported by Amir (1978a). However,

it must be noted that while Amir (1978a) did not find differences in enzyme activity between experimental and control animals both in brain and liver, the present study found higher ALDH activity levels in livers of ethanol drinking animals than controls. By comparing both brain and liver ALDH activity in relation to ethanol consumption within the same animals, it is contended that one can obtain more conclusive evidence for suggesting a role specifically for cerebral ALDH in ethanol consumption.

Experiment 2

In trying to evaluate the functional relationship between ALDH activity levels and voluntary ethanol intake one factor which must be taken into account is the apparent change in metabolic capacity resulting from prolonged alcohol exposure. If the metabolic capacity of an organism determines in some fashion the rate and amounts voluntarily consumed of a particular substance, then changes in capacity could be seen to influence consumption patterns over time. With respect to the possible determinants of ethanol intake much controversy exists as to the occurance of drug induced metabolic changes and most importantly to the relevence of this phenomenon to the mediation of voluntary intake. It has been reported that prolonged exposure to ethanol may result in an increase in liver ALDH capacity (Hasumura, Teschke & Lieber, 1976; Horton & Barrett, 1976). Other authors have offered opposite findings demonstrating a diminution in liver ALDH activity in animals chronically exposed to ethanol (Amir, 1978b; Lesback et al., 1981). One report found no alteration whatsoever in liver ALDH activity in rats drinking ethanol on a chronic basis (Horton, 1971). The apparent lack of agreement in reports investigating changes in liver ALDH activity in ethanol consuming rats represents, however, only a portion of a

larger and much confused situation.

The findings of experiment 1 in the present thesis are in agreement with previous reports indicating a direct relationship between brain ALDH activity and ethanol consumption (Amir, 1977; Amir & Stern, 1978; Amir, 1978a). However, these studies also reported higher correlations between brain ALDH activity and ethanol consumption than between liver ALDH activity and drinking. These differences in the degree of relationship between peripheral and central aldehyde metabolizing capacity and ethanol consumption, taken together with the apparent lack of central acetaldehyde under conditions of high peripheral levels (Lindros & Hillbom, 1979), would seem to indicate a dissociation between the potential effects of peripheral and central acetaldehyde. In further support of this apparent dissociation of peripheral and central acetaldehyde effect is the lack of concordance between exposure and metabolic changes in the two regions of the organism. In his initial study Amir, (1977) reported no differences in brain and liver ALDH activity between ethanol exposed and control animals in free drinking conditions. A subsequent study by Amir, (1978b) demonstrated an increase in brain ALDH activity and a decrease in liver ALDH activity in a forced drinking paradigm. Such an effect of opposite direction occuring

in peripheral and central regions may not only indicate differential effects of ethanol or acetaldehyde in the two regions but differing metabolic regulatory mechanisms as well. As the exact nature of the relation between metabolic capacity and ethanol consumption remains unclear, changes in capacity resulting from exposure represents yet another unknown variable with respect to the metabolic regulation of alcohol intake.

The present experiment sought to further investigate the role of peripheral and central acetaldehyde oxidizing capacity in voluntary ethanol consumption and metabolic changes resulting from exposure.

Methods

Animals: Male rats of the Wistar strain were obtained from Canadian Breeding Farm Laboratories (St. Constant, Quebec). All animals weighed approximately 150-175g., at the start of the experiment. They were housed singly in stainless steel cages, in a room regulated for constant temperature and humidity, in a 12h light cycle. Drinking fluids were presented in glass Richter tubes and standard lab chow was freely available throughout the experiment. Animals were randomly assigned to ethanol drinking and control (water only) groups. The

number of animals used in each group is presented in the figures and tables.

Ethanol Exposure Procedure: Animals were initially exposed to ethanol on an alternate-day, free-choice paradigm modified from the procedure of Amit et al., (1970). On alternate days animals were offered a free choice between water and increasing concentrations of ethanol, presented in Richter tubes mounted in frount of the home cages. On intervening days only water was made available in both tubes. Ethanol solutions were prepared by mixing 95% ethanol with tap water. On the first day of exposure a 2% (v/v) ethanol solution was presented in a free choice with water. Concentrations were increased by increments of 2% with two alternate day presentations at each concentration. On the day after the second presentation of 10%, animals were switched to an every-day presentation schedule of a 10% ethanol solution for 28 days. Fluid consumption and body weights were measured daily and ethanol consumption calculated in g/kg body wt/day. The position of the Richter tubes was changed daily throughout the exposure period.

Ethanol-consuming animals were divided into four groups according to the number of hours ethanol had been withdrawn, prior to sacrificing for tissue preparations. The groups were as follows: Group A (0 hrs), Group B (24

hrs), Group C (48 hrs), Group D (72 hrs) and Group E (control).

Preparation of Brain and Liver Tissues: Animals were decapitated and their brains and livers were rapidly removed and weighed. Tissue fractions were homogenized (Teflon on glass) in sufficient 0.25M sucrose buffer containing 1% Triton-X100 and 1mM glutathione to make 10% brain and liver homogenates. Homogenates were centrifuged for 1h at 100,000 x g, at 0°C, and then the clear supernatant was decanted and used as the enzyme source. All samples were frozen at -70°C until assayed.

Assay of Aldehyde Dehydrogenase: Total activity of ALDH was determined by measurement of the rate of enzyme catalyzed, NAD+ dependant production of indole-3-acetic acid from indole-3-acetaldehyde (modified from Duncan & Sourkes, 1978). A reaction mixture of 0.5ml of sodium phosphate buffer (pH 7.4), 0.3ml distilled water, 50µl of 0.01M NAD sodium bisulfite (Sigma Co.; final conc., 5×10^{-4}) and 50µl of the enzyme supernatant was pre-incubated for 10 minutes at 30°C. The reaction was initiated by the addition of 0.1ml of 5×10^{-3} M indole-3-acetaldehyde (Sigma Co.) bringing the total volume to 1.0ml. The reation was terminated after 10 minutes by the addition of 0.2ml of 1M semicarbazide HCl (final conc. 0.16M) and the unreacted aldehyde was extracted into dichloroethane

(2,2-ethylenedichloride). ALDH activity was estimated from the flourescence (excitation 280 nanometers, emission 365 nanometers) of the indole-3-acetic acid formed and was expressed in nanograms/h/ug protein. Recovery of known amounts of indole-3-acetic acid carried through the incubation from solution containing no NAD+ or enzyme was quantitative. Comparison of samples carried through the incubation and external standards revealed that total recovery of indole-3-acetic acid was 40%. The reaction was linear for at least 15 minutes indicating complete saturation of the enzyme. Protein content was measured after the method of Lowry et al., (1951) and bovine serum albumin (Sigma Co.) was used as standard. All assays were carried through in duplicate for both enzyme acitivity measurements and protein determination.

Results

Ethanol Intake: A two-way analysis of variance was performed on ethanol consumption over the 28 baseline period. The total amounts of ethanol consumed by the four groups did not differ singificantly $F(3,24)=2.68$, $p>.05$. Mean consumption for the four groups over the baseline period was as follows: Group A=3.01$\pm$.37 g/kg, Group=B 2.89$\pm$.27 g/kg, Group C=2.87$\pm$.25 g/kg, Group D=2.98$\pm$.29 g/kg.

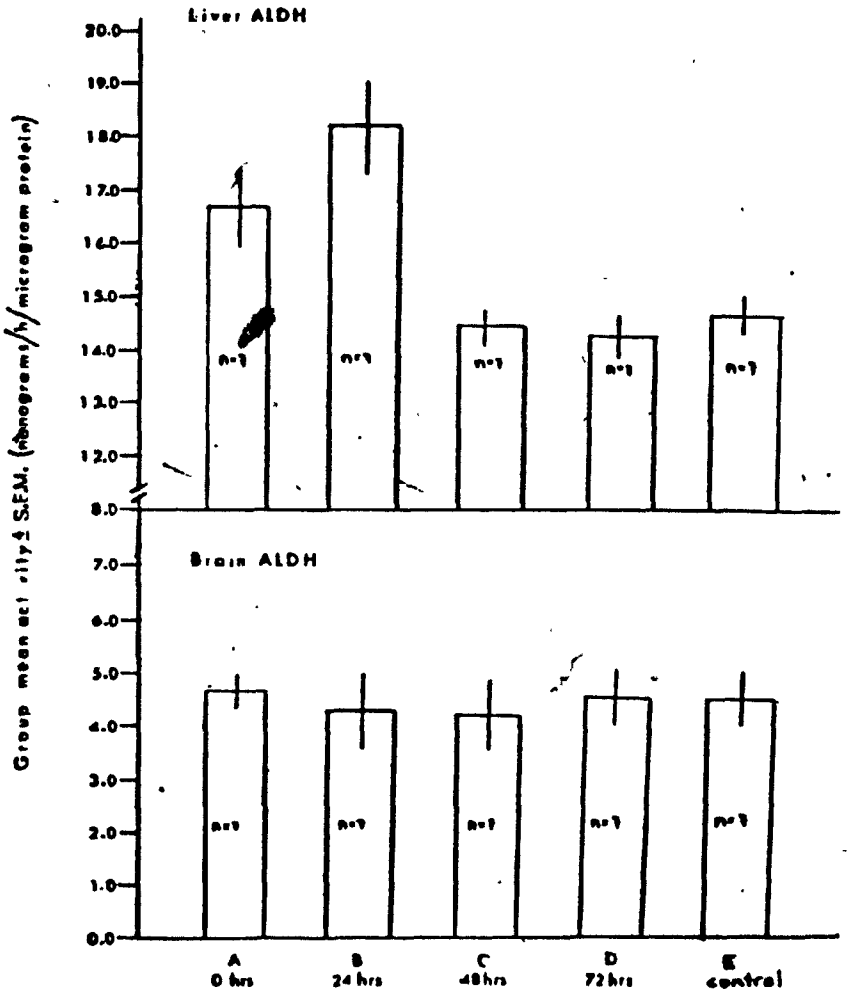
As well, there was no significant effect of days on group ethanol intake across the baseline period $F(81,548)=1.09$, $p>.05$. In other words, the ethanol intake did not differ between groups or within a group throughout the experiment.

Brain and Liver ALDH Activity: The levels of brain ALDH activity did not differ significantly between ethanol drinking and control groups across the time periods sampled $F(4,30)=1.69$, $p>.05$ (see Figure 2).

An analysis of variance on liver ALDH activity levels showed that unlike brain ALDH, levels in liver were significantly higher in ethanol consuming animals than in controls $F(4,30)=3.65$, $p<.05$. A post hoc analysis was performed using the Tukey HSD test (Kirk, 1968) and results are presented in Figure 2.

Analysis of the relationship between brain and liver ALDH activity and ethanol consumption within groups is presented in Table 2. In addition to the correlations comptuted, a test of significance between two correlations (Bruning & Kintz, 1978) was performed for the relation between ethanol intake and brain and liver ALDH actvity in each group: Group A $z=.444$, $p>.05$; Group B $z=.039$, $p>.05$; Group C $z=2.25$, $p>.05$; Group D $z=.731$, $p>.05$. In general, brain ALDH activity was found to be a better predictor of ethanol consumption than liver ALDH levels since a

Figure 2. Total cerebral ALDH activity and liver ALDH activity in ethanol consuming and control animals.



Data represents values of enzyme activity of ethanol consuming and control (water-only) animals. Activity is expressed in nanograms Indole-3-acetic acid /h/ µg protein.
* indicates significant difference ($p<0.05$, Tukey HSD test) from control.

Table 2: Correlations Computed Between Brain ALDH, Liver ALDH and Ethanol Consumption Within Ethanol Consuming Groups.

	Group A 0 hrs (n=7)	Group B 24 hrs (n=7)	Group C 48 hrs (n=7)	Group D 72 hrs (n=7)
Brain ALDH: ethanol intake	0.508	0.345	0.778*	0.821*
Liver ALDH: ethanol intake	0.708*	0.324	-0.506	0.566

* $p<0.05$

significant overall correlation was obtained between brain ALDH activity and ethanol intake ($r=.656$, $n=28$, $p<.01$). On the other hand, the overall correlation between liver ALDH activity levels and ethanol consumption was not significant ($r=.289$, $n=28$, $p<.05$) for the same animals.

Discussion

The results of the present experiment confirm earlier findings which suggest a direct relationship between brain ALDH activity and voluntary ethanol intake (Amir, 1977; Amir & Stern, 1978; Amir, 1978a). In addition, the observation that an apparent induction of liver ALDH activity occurs in free drinking animals is the first observation of this phenomenon employing a free choice paradigm. Although some previous reports exist on such a liver enzyme induction, by and large they rely on forced choice presentation paradigms with artificially high levels of drug consumed (Hasumura, Teschke & Lieber, 1976; Horton & Barrett, 1976). As the present experiment allowed for the occurance of an alteration in ALDH actvity only within levels of ethanol voluntarily consumed, perhaps the present findings can be taken to be more behaviourally relevent. Furthermore, the general lack of agreement over this phenomenon in the literature may be a

function of the confusion of behavioural (free-choice presentation) and pharmacological (forced choice presentation) procedures and differences in the effects generated by these varying methodologies.

General Discussion

The present findings confirm earlier reports suggesting a direct relationship between brain ALDH activity and ethanol consumption (Amir, 1977; Amir & Stern, 1978; Amir, 1978a; Sinclair & Lindros, 1981). The correlations presented show that within the same animals only the relationship between voluntary ethanol consumption and brain ALDH activity appears to be significant. In general, these findings are in agreement with those previously reported by Amir (1978a). However, while Amir, (1978a) did not find differences in enzyme activity between experimental and control animals in both brain and liver, the present study found higher ALDH levels in livers of ethanol drinking animals than controls. Furthermore, this earlier study employed seperate groups for the examination of brain and liver ALDH activity and its relation to ethanol consumption. As the study by Amir (1978a) employed commercially obtained Wistar rats, which are not genetically defined, variations in results using seperate brain and liver groups could have arisen from inconsistancies in these commercially raised animals. By comparing both brain and liver ALDH activity in relation to ethanol consumption within the same animals, it is therefore contended that one can

obtain more conclusive evidence for suggesting a role for cerebral ALDH in ethanol consumption.

Most importantly, the present findings show that the relationship between brain ALDH activity and ethanol consumption exists in three of the most frequently employed rat strains in the alcohol self administration field. This finding, taken together with those previously reported (Amir, 1977; Amir & Stern, 1978; Amir, 1978a; Sinclair & Lindros, 1981), brings to five the number of rat strains observed to bear the relationship between brain ALDH activity and ethanol consumption.

The observation of an apparent increase or induction of liver ALDH activity demonstrates the possibility of such an effect occuring under conditions of voluntary intake. Previous reports of this nature have relied on forced choice presentation, resulting in artificially high levels of ethanol entering the system (Hasumura, Teschke & Lieber, 1976; Horton & Barrett, 1976). Although such an induction effect has been disputed in the literature (Amir, 1978b; Lesback et al., 1981), these opposing findings were based on a forced choice presentation paradigm. An explanation of the apparent lack of agreement over this phenomenon may lie in the interpretations drawn from the various paradigms. The central issue in all of these studies has been the

elucidation of metabolic processes which may be involved in the mediation of ethanol consumption. However, many of these studies employed pharmacological rather than behavioural paradigms to answer an essentially motivationally oriented question. Some authors have gone to great lengths to describe and caution other investigators about the methodological orientation one takes in constructing animal models and the inferences that can be drawn (Mello, 1973, Lester & Freed, 1973). Perhaps, with regards to previous observations of liver ALDH increases in chronically exposed animals a misdirected approach was applied. Indeed, the present findings clearly show that an increase in liver ALDH activity does not bear a functional relationship to the amounts of ethanol an animal will voluntarily consume. This may suggest different effects of ethanol or acetaldehyde in brain and in the periphery, both behaviourally and physiologically. Such a contention may be supported by the finding that differential effects on conditioned taste aversion learning occur with peripherally and centrally administered acetaldehyde (Brown, Smith & Rockman, 1978). The findings of Brown, Smith and Rockman, (1978) demonstrated that only periperhally administered acetaldehyde appears to have an aversive effect as measured by the conditioned taste

aversion paradigm. In additon, only brain ALDH activity appears to consistently correlate well with voluntary ethanol intake. The sum of these findings would seem to support a notion of cerebral ALDH activity somehow being involved in the regulation of ethanol consumption.

The precise nature of the involvement of brain ALDH in the regulation of voluntary ethanol intake appears to be related to a number of possibilities. One that has received a great deal of attention is the possible role of acetaldehyde in the behavioural and pharmacological effects of ethanol. As acetaldehyde, the reactive metabolite of ethanol has been shown to display various actions in relation to alcohol consumption (for reviews see Amir et al., 1980; Lindros, 1978), its rate of elimination may be tied to many of its observed effects. In fact, the earliest observations of the aversive effects of acetaldehyde in ethanol consumption were based on inhibition of ALDH by disulfiram (Hald & Jacobson, 1948) and calcium carbamide (Schlesinger et al., 1966).

The behavioural data showing that cerebral infusions of acetaldehyde are actively sought by animals through operant responding (Brown et al., 1979), and that the rate of responding correlates with subsequent preference for ethanol (Brown et al., 1980), further support the possible relationship between brain ALDH and ethanol intake. These

behavioural reports coupled with the presence of central oxidative pathways for ethanol metabolism (Cohen et al., 1981; Tabakoff & Gelpke, 1975) would seem to make viable a notion which ascribes a regulatory role for brain ALDH in ethanol intake, via acetaldehyde regulation.

An alternate hypothesis of the role of ALDH in ethanol consumption, concerns the involvement of the enzyme in catecholamine metabolism. ALDH has been shown to be a major route of monoamine deamination (Duncan & Sourkes, 1975; Tabakoff & Gelpke, 1975; von Wartburg et al., 1975). As well, even subtle amounts of acetaldehyde in the cellular milieu may competitively inhibit brain ALDH (Deitrich & Erwin, 1975; Hunt & Majchrowicz, 1974; Lahti & Majchrowicz, 1976; Thadani & Truitt, 1977), giving rise to increases in the steady state levels of its endogenous substrates--biogenic aldehydes. These biogenic aldehydes, through their accumulation, may affect neuronal functioning through their inhibitory effects on ATPase activity (Tabakoff, 1974; Erwin et al., 1975).

With respect to the present data, as brain ALDH activity did not differ as a function of exposure to ethanol; a more likely possibility would be that the individual levels of ALDH may have correspondingly resulted

in a differential predispostion to ethanol intake. It is not the intention of the present discussion to conclude that a behaviour as complex as ethanol consumption is rooted solely in the activity of a single enzyme. However, the notion of a biologfcal marker for alcoholism has long been entertained (Omennn & Motulsky, 1972) and if viable may be based in part on brain ALDH activity. A greater understanding of the role of ALDH in ethanol consumption appears then to be an important step in futhering our general knowledge of alcohol drinking.

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