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ORIGINAL PAPER

Enhanced dopamine D_2 receptor function in hypothalamus and corpus striatum: their role in liver, plasma and in vitro hepatocyte ALDH regulation in ethahol treated rats

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Abstract Dopamine D_2 receptors are involved in ethanol self- administration behavior and also suggested to mediate the onset and offset of ethanol drinking. In the present study, we investigated dopamine (DA) content and Dopamine D_2 (DA D_2) receptors in the hypothalamus and corpus striatum of ethanol treated rats and aldehyde dehydrogenase (ALDH) activity in the liver and plasma of ethanol treated rats and in vitro hepatocyte cultures. Hypothalamic and corpus striatal DA content decreased significantly ($P < 0.05$, $P < 0.001$ respectively) and homovanillic acid/dopamine (HVA/DA) ratio increased significantly ($P < 0.001$) in ethanol treated rats when compared to control. Scatchard analysis of [^{3}H] YM-09151-2 binding to DA D_2 receptors in hypothalamus showed a significant increase ($P < 0.001$) in B_{max} without any change in K_d in ethanol treated rats compared to control. The K_d of DA D_2 receptors significantly decreased ($P < 0.05$) in the corpus striatum of ethanol treated rats when compared to control. DA D_2 receptor affinity in the hypothalamus and corpus striatum of control and ethanol treated rats fitted to a single site model with unity as Hill slope value. The in vitro studies on hepatocyte cultures showed that 10^{-5} M and 10^{-7} M DA can reverse the increased ALDH activity in 10% ethanol treated cells to near control level. Sulpiride, an antagonist of DA D_2, reversed the effect of dopamine on 10% ethanol induced ALDH activity in hepatocytes. Our results showed a decreased dopamine concentration with enhanced DA D_2 receptors in the hypothalamus and corpus striatum of ethanol treated rats. Also, increased ALDH was observed in the plasma and liver of ethanol treated rats and in vitro hepatocyte cultures with 10% ethanol as a compensatory mechanism for increased aldehyde production due to increased dopamine metabolism. A decrease in dopamine concentration in major brain regions is coupled with an increase in ALDH activity in liver and plasma, which contributes to the tendency for alcoholism. Since the administration of 10^{-5} M and 10^{-7} M DA can reverse the increased ALDH activity in ethanol treated cells to near control level, this has therapeutic application to correct ethanol addicts from addiction due to allergic reaction observed in aldehyde accumulation.

Keywords ALDH · [^{3}H] YM-09151-2 · Dopamine · Hypothalamus · Corpus striatum · Hepatocyte culture

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Introduction

Ethanol affects brain function by interacting with multiple neurotransmitter systems, thereby disrupting the delicate balance of neurotransmitters [1]. Apart from GABAergic and glutamatergic systems, there are strong evidences highlighting the importance of the dopaminergic system's involvement in mediating the craving effects of ethanol [2–6]. The rewarding effect of ethanol seems to be related to the activation of dopamine neurons, especially with an increased release of dopamine in the nucleus accumbens [7, 8]. It is assumed that in ethanol dependence, dopamine neurons are sensitized through cues related to drinking. A number of neurochemical pathways have been implicated in ethanol addiction, including the dopamine (DA), serotonin (5-HT), norepinephrine (NE), gamma-aminobutyric acid (GABA), opioid and cannabinoid neurotransmitter systems. The dopaminergic pathway, in particular, is associated with

cognitive, endocrine, and motor functions and reinforcement of addictive substances or behaviors [9]. This contributes to the central nervous system (CNS) depression that occurs with prolonged drinking. The brain of ethanol addicts seems to contain abnormalities that reduce the effectiveness of the dopaminergic system. Serotoninergic system appears to be involved in ethanol consumption and reinforcement by activating dopaminergic system [10]. The dopamine D_2 receptor is one of the five physiologically distinct dopamine receptors (D_1, D_2, D_3, D_4 and D_5) found on the synaptic membranes of neurons in the brain [11]. High levels of DA D_2 receptors could protect against ethanol addiction [9] and over expression of DA D_2 reduces ethanol self-administration [12]. Since it is reported [13] that DA D_2 receptor has an association with ethanol addiction, the possible role of DA D_2 receptor locus in the etiology of ethanol addiction has been the focus of considerable attention [13]. Dopamine D_3 receptor genes (DA D_3), also in the same class as DA D_2 but with different pharmacological properties [14], are involved in ethanol-seeking behavior [15]. Hypothalamic function is seriously disrupted by ethanol exposure during development [16]. Ethanol is a potent stimulator of hypothalamic β-endorphin, and hence hypothalamus is an important region affected by ethanol consumption [17]. There are evidences that suggest the role of central and autonomic nervous systems in the regulation of hepatic function [18, 19, 20]. However, little is known about neurotransmitters that mediate the effects of the central nervous system on hepatic ALDH activity. ALDH has been advocated as a marker of ethanol intake. The absence or low levels of ALDH may be associated with ethanol-induced flushing or other reactions to ethanol and therefore, with reduced ethanol use [21]. The acetaldehyde produced from ethanol is metabolized quickly to acetate by ALDH [22]. It is reported that ethanol metabolism is impaired by a nonfunctional form of the enzyme aldehyde dehydrogenase [23]. The precise mechanism by which ALDH regulates voluntary ethanol intake is yet to be elucidated. In this study, we investigated the hypothalamic and corpus striatal DA content, DA D_2 receptors and ALDH activity in the liver and plasma of ethanol treated rats and in vitro hepatocyte cultures.

Materials and methods

Chemicals used for the study

Dopamine, Sodium octyl sulphonate, Tris–buffer, homovanillic acid (HVA), NAD^+, Propionaldehyde, ascorbic acid, pargyline, calcium chloride, Tris–HCl, Perchloric acid, Sodium dihydrogen phosphate, acetonitrile, Sulpiride, rat tail collagen, William's media E, Dithiothreitol (DTT), collagenase type IV, Earle's balanced salts (EBSS) and Fetal Calf Serum (FCS) used in the present study were purchased from SIGMA Chemical Co., St. Louis, U.S.A. HPLC solvents of HPLC grade were purchased from SRL and Merck India.

Ethylenediamine tetra acetic acid (EDTA), (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES, Bovine serum albumin (BSA), Sodium Carbonate (Na_2CO_3), NaOH, Potassium sodium tartrate ($NaKC_4H_4O_6$), $MgCl_2$, NaCl, KCl, $CuSO_4$ and pyrazole were purchased from SRL, Merck and Himedia, India.

Animals

Wistar adult rats of 180–200 g body weight purchased from Amrita Institute of Medical Sciences, Cochin were used for this experiment. All animals were housed in separate cages in 12-h light and 12-h dark periods with free access to water/ethanol and food. All animal care and procedures were in accordance with the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) and National Institute of Health (NIH) guidelines.

Administration of ethanol and tissue preparation

Rats were given free access to 15% (v/v) (approx.7.5 g/Kg body wt./day) ethanol for 15 days as per the modified procedure of Pediconi et al. [24]. Controls were given free access to water for 15 days. All control and ethanol treated rats have the access to dry food pellets during these period. The rats were sacrificed by decapitation on the 15th day of the experiment. The body and brain parts were dissected out according to the procedure of Glowinski and Iversen [25]. The tissues were stored at −70°C for various experiments.

Quantification of dopamine by HPLC

Dopamine and the respective metabolite content were assayed according to Paulose et al. [26], using high-performance liquid chromatography (HPLC) integrated with an electrochemical detector (HPLC-ECD) (Shimadzu, Japan) fitted with C18-CLC-ODS reverse-phase column. The tissue from the brain regions were homogenized in 0.4 N perchloric acid. The homogenate was centrifuged at 5,000 × *g* for 10 min at 4°C (Biofuge stratos Refrigerated Centrifuge, Japan). The clear supernatant filtered through 0.22 μm HPLC grade filters were used for HPLC analysis. Mobile phase was 75 mM sodium dihydrogen orthophosphate, 1 mM sodium octyl sulphonate, 50 mM EDTA and 7% acetonitrile (pH 3.25), filtered through 0.22 μm filter (Millipore) and degassed. A Shimadzu pump (model 10 AS) was used to deliver the solvent at a rate of 1.0 ml/min.

Dopamine and the respective metabolite content was identified by using an electrochemical detector (Model 6A, Shimadzu, Japan), with a retention potential of +0.80 V. The peaks were identified by their retention time compared with those of standards and quantitatively estimated using an integrator (Shimadzu, C-R6A-Chromatopac) interfaced with the detector.

Radio receptor assay (Dopamine D_2 receptor binding studies)

Dopamine D_2 receptor binding assay was done according to the modified procedure of Unis et al. [27]. The dissected hypothalamus and corpus striatum were homogenized in 10 volumes of ice cold 50 mM Tris–HCl buffer, along with 1 mM EDTA, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 120 mM NaCl, and 5 mM KCl pH.7.4. The homogenate was centrifuged at 48,000 × *g* for 30 min. The pellet was washed and recentrifuged with 50 volumes of the buffer at 48,000 × *g* for 30 min. The pellet was suspended in an appropriate volume of buffer. Binding assays in the hypothalamus and corpus striatum were done using different concentrations i.e., 0.25 nM to 2.5 nM of [^{3}H] YM-09151-2 in 50 mM Tris–HCl buffer, 1 mM EDTA, 5 mM $MgCl_2$, 1.5 mM $CaCl_2$, 120 mM NaCl, 5 mM KCl with 10 μM pargyline and 0.1% ascorbic acid in a total incubation volume of 300 μl containing 200–300 μg of protein. Specific binding was determined using 5.0 μM unlabelled sulpiride. Competition studies were carried out with 1.0 nM [^{3}H] YM-09151-2 in each tube with unlabelled sulpiride concentrations varying from 10^{-10} to 10^{-3} M. Tubes were incubated at 25°C for 1 h and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly three times with 5.0 ml of ice cold 50 mM Tris buffer, pH 7.4. Bound radioactivity was counted with cocktail-T in a Wallac 1409 liquid scintillation counter. The receptor binding parameters were determined using Scatchard analysis [28].

Analysis of the receptor binding data

Receptor binding parameters analysis

The receptor binding parameters determined using Scatchard analysis [28]. The maximal binding (B_{max}) and equilibrium dissociation constant (K_d) were derived by linear regression analysis by plotting the specific binding of the radioligand on *x*-axis and bound/free on *y*-axis using Sigma plot computer software. The B_{max} is a measure of the total number of receptors present in the tissue and the K_d represents affinity of the receptors for the radioligand. K_d is inversely related to receptor affinity or the "strength" of binding. Competitive binding data were analysed using non-linear regression curve–fitting procedure (GraphPad PRISM^{TM}, San Diego, USA).

Displacement curve analysis

The data of the competitive binding assays are represented graphically with the log of concentration of the competing drug on *x*-axis and percentage of the radioligand bound on the *y*-axis. The steepness of the binding curve can be quantified with a slope factor, often called a Hill slope. A one-site competitive binding curve that follows the law of mass action has a slope of −1.0. The concentration of competitor that competes for half the specific binding was defined as EC_{50}. It is same as IC_{50}. The affinity of the receptor for the competing drug is designated as K_i and is defined as the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors.

Collagen-coating of culture dishes and hepatocyte culture

Sterile rat-tail collagen solution (100 mg/ml in 0.1% acetic acid) was added to each 35 mm culture dish and spread uniformly. After 2 h, the unattached collagen is aspirated out and the dishes are washed thrice with sterile phosphate buffered saline. Finally, sterile Earl's balanced salt solution (EBSS, Sigma) was added and the dishes were left in the sterile hood till the seeding of cells.

Hepatocytes were isolated from adult male Wistar rats by collagenase perfusion [29]. The liver was perfused in situ with Ca^{2+}-free perfusion buffer (pH 7.4) containing 142 mM NaCl, 6.7 mM KCl, 10 mM HEPES, and 5.5 mM NaOH, and then with Ca^{2+} containing collagenase buffer (pH 7.6) containing 67 mM NaCl, 6.7 mM KCl, 100 mM HEPES, 4.76 mM $CaCl_2.2H_2O$, 66 mM NaOH and Collagenase type IV (0.05%). After perfusion the hepatocytes were dispersed from the perfused liver, filtered through nylon mesh and washed by three centrifugations in EBSS. The final cell pellet was resuspended in William's Medium E. Cell viability was tested by tryphan blue exclusion. The hepatocyte preparation having a viability of >90% as assessed by tryphan blue exclusion were chosen for culture. Hepatocytes were plated on rat tail collagen coated dishes at a density of 7 lakhs cells/35 mm culture dish in 1 ml of William's medium E containing 10% FCS. Cells were allowed to settle and adhere for three hours in medium. After that the plating media was replaced by serum- free William's medium E containing 10% ethanol, dopamine and sulpiride with concentrations 10^{-7}, 10^{-5} and 10^{-3} M. The cultures were incubated for 24 h at 37°C in 5% CO_2.

Table 1 DA content and its metabolite in the hypothalamus and corpus striatum of control and experimental rats (nmoles/gm wet weight of the tissue)

Region	Animal status	DA	HVA	HVA/DA
Hypothalamus	Control	0.48 ± 0.03	0.04 ± 0.22	0.07 ± 0.01
	Ethanol treated	0.27 ± 0.04*	0.04 ± 0.01	0.17 ± 0.04***
Corpus striatum	Control	4.22 ± 0.62	1.37 ± 0.21	0.33 ± 0.04
	Ethanol treated	0.35 ± 0.07***	2.43 ± 0.17	5.21 ± 2.51***

Values are mean ± S.E.M of 4–6 separate experiments

* $P < 0.05$

*** $P < 0.001$ when compared with control

The contents were determined using HPLC connected with EC detector and the values were integrated Shimadzu chromatopac with standard values

Kinetic studies of aldehyde dehydrogenase from hepatocyte culture cells

Hepatocyte pellets were washed with 0.1 M sodium pyrophosphate buffer, pH 8.4 and suspended in 60 µl of sodium pyrophosphate buffer and homogenised in an ultrasound sonicator for 15 s. The tubes were centrifuged at 5,000 × *g* at 4°C for 10 min and the supernatant used for ALDH enzyme assay.

Aldehyde dehydrogenase activity was assayed in homogenate of cerebral cortex, liver and hepatocyte culture by the modified procedure of Gill et al. [30]. ALDH activity was measured using a Shimadzu UV1201 Spectrophotometer at 25°C at 340 nm. 1 ml of assay mixture contained 0.1 M sodium pyrophosphate buffer pH 8.4, 1.0 mM EDTA, and 5.0 mM dithiothreitol, tissue homogenate containing ALDH enzyme, 10 mM pyrazole and 10–200 µM propionaldehyde. 1.0 mM NAD^+ was added to initiate the reaction. One Unit of ALDH activity was calculated as 1 µmole of NADH formed/min. The results were expressed as Units/mg protein. Protein concentrations were estimated [31] using bovine serum albumin as standard.

Statistics

Statistical evaluations were performed by Student's *t*-test and ANOVA using InStat (Ver.2.04) computer programme. Linear regression Scatchard plots were made using SIGMA PLOT (Ver 2.03).

Results

Dopamine content of hypothalamus and corpus striatum was decreased in ethanol treated rats

The hypothalamic dopamine concentration of ethanol treated rats was significantly lower ($P < 0.05$) than that of control (Table 1). The homovanillic acid/dopamine (HVA/DA) ratio in hypothalamus showed a significant increase ($P < 0.001$) in ethanol treated rats when compared to control (Table 1). The striatal dopamine concentration showed a significant decrease and HVA/DA ratio showed a significant increase ($P < 0.001$) in ethanol treated rats when compared to control (Table 1).

Dopamine D_2 receptor function in hypothalamus and corpus striatum were increased in ethanol treated rats

Scatchard analysis of [^{3}H] YM-09151-2 binding in the hypothalamus of ethanol treated rats showed a significant increase in B_{max} ($P < 0.001$), without any change in K_d when compared to control (Fig. 1; Table 2). This reflected an increased receptor number without any change in affinity. In the corpus stiatum of ethanol treated rats, scatchard analysis showed a significant decrease in K_d ($P < 0.05$) without any change in B_{max} compared to

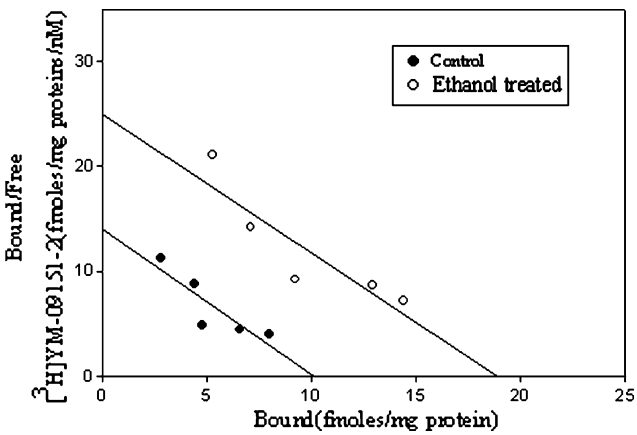

Fig. 1 Scatchard analysis of [^{3}H] YM-09151-2 against sulpiride in hypothalamus of control and ethanol treated rats. Incubation was done with different concentration, i. e. 0.25 nM to 2.5 nM of [^{3}H] YM-09151-2 along with 200–300 µg of protein. 5.0 µM sulpiride was used to determine the specific binding. Reaction was stopped by rapid filtration through GF/B (Whatman) Filters with ice cold Tris Buffer pH 7.4. Values are representation of 4–6 separate experiments

Table 2 [^{3}H] YM-019151-2 binding parameters in the hypothalamus of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	10.40 ± 0.32	0.76 ± 0.01
Ethanol treated	19.07 ± 0.43***	0.79 ± 0.06

Values are mean ± S.E.M of 4-6 separate experiments

*** $P < 0.001$ when compared with control

Membrane binding assays were done using different concentrations [^{3}H] YM-019151-2 i.e., 0.25 nM–2.5 nM for hypothalamus along with appropriate protein concentrations. Non-specific binding was determined using 50 μM unlabelled sulpiride

control (Fig. 2; Table 3). This showed an increased affinity of D_2 receptors for dopamine.

The binding data were confirmed by competition binding assay with [^{3}H] YM-09151-2 against sulpiride. Dopamine D_2 receptor affinity in the hypothalamus and corpus striatum of control and ethanol treated rats fitted to a single site model with unity as Hill slope value (Figs. 3 and 4; Tables 4 and 5).

ALDH activity of plasma and liver increased in ethanol treated rats

The ALDH activity showed a significant increase in V_{max} ($P < 0.05$) with a decreased Km ($P < 0.01$) in both plasma and liver of ethanol treated rats when compared to the control (Tables 6 and 7). This showed the increased activity of ALDH with greater affinity in ethanol treated rats.

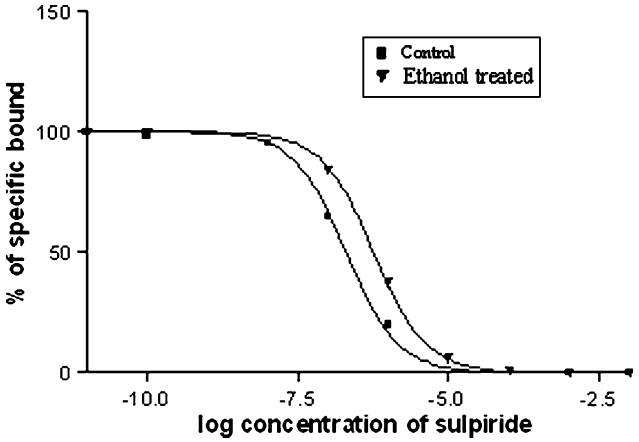

Fig. 2 Scatchard analysis of [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and ethanol treated rats. Incubation was done with different concentration, i. e. 0.25 nM to 2.5 nM of [^{3}H] YM-09151-2 along with 200-300 μg of protein. 5.0 μM sulpiride was used to determine the specific binding. Reaction was stopped by rapid filtration through GF/B (Whatman) Filters with ice cold Tris–Buffer pH 7.4. Values are representation of 4–6 separate experiments

Table 3 [^{3}H] YM-019151-2 binding parameters in the corpus striatum of control and ethanol treated rats

Experimental status	B_{max} (fmoles/mg protein)	K_d (nM)
Control	218.33 ± 20.65	1.32 ± 0.20
Ethanol treated	180.04 ± 21.63	0.92 ± 0.09*

Values are mean ± SEM of 4–6 separate experiments

* $P < 0.05$ when compared with control

Membrane binding assays were done using different concentrations [^{3}H] YM-019151-2 i.e., 0.25 nM to 2.5 nM for corpus striatum along with appropriate protein concentrations

Non-specific binding was determined using 50 μM unlabelled sulpiride

Increased ALDH activity of hepatocytes in ethanol treated rats reversed to near control level in presence of 10^{-5} M and 10^{-7} M dopamine

The 24 h culture studies in hepatocytes showed a significant increase in ALDH activity ($P < 0.001$) in 10% ethanol treated cells compared to control cells. This increase in activity in ethanol treated cells was reversed to near control level in presence of 10^{-5} M and 10^{-7} M Dopamine. Sulpiride, an antagonist of DA D_2, reversed the effect of dopamine on 10% ethanol induced ALDH activity in hepatocyte cells (Table 8). This reflected that the reversal of ALDH activity to near control level was mediated by dopamine D_2.

Discussion

The etiology of ethanol dependence is a complex interaction of psychosocial and biologic factors [32]. Dopamine is thought to be important for both the induction and the expression of behavioral sensitization [33, 34]. Dopaminergic activity in the brain has been known to play a pivotal role in mediating drug and ethanol addiction [35]. Studies report that sensitization to ethanol is associated with changes in the dopaminergic system [36]. The human DA D_2 receptor gene is an important candidate gene for ethanol addiction and/or for the modification of its severity [37–40]. Previous studies indicate that prolonged heavy drinking decreases DA transporter binding and disturbs synaptic DA transport [41]. Ethanol acts on dopaminergic neurons, producing long lasting changes on the systems. The decreased DA content with increased HVA/DA turnover rate in the hypothalamus and corpus striatum observed in our study is suggested to be the result of chronic treatment of ethanol as an attempt to stimulate DA receptor function.

Altered central DA function has been implicated as influencing the propensity for ethanol consumption in

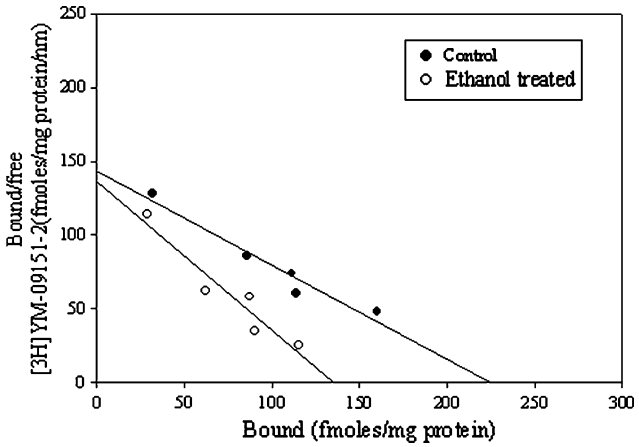

Fig. 3 Displacement of [^{3}H] YM-09151-2 against sulpiride in hypothalamus of control and ethanol treated rats. Competition studies were carried out with 1.0 nM [^{3}H] YM-09151-2 in each tube with the unlabelled sulpiride concentrations varying from 10^{-10} to 10^{-3} M. The tubes were incubated at 25°C for 1 h and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0 ml of ice cold buffer containing 50 mM Tris buffer, pH 7.4. Values are representation of 4–6 separate experiments

humans [42]. Enhanced 5-HT$_{2A}$ receptor status in the hypothalamus and corpus striatum of ethanol treated rats was reported [43]. Ethanol also acts directly through the production of neuroamines that interact with dopaminergic systems [13, 44]. DA receptor antagonists attenuate the locomotor-activating effects of acute ethanol, suggesting an important role of mesolimbic DA neurons in mediating the acute effects of ethanol [45–47]. The decreased brain

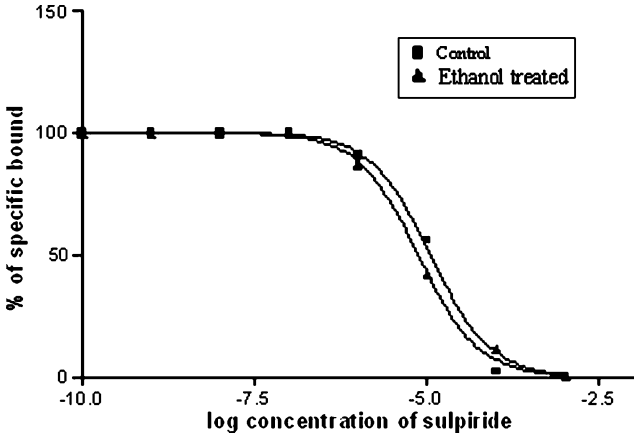

Fig. 4 Displacement of [^{3}H] YM-09151-2 against sulpiride in corpus striatum of control and ethanol treated rats. Competition studies were carried out with 1.0 nM [^{3}H] YM-09151-2 in each tube with the unlabelled sulpiride concentrations varying from 10^{-10} to 10^{-3} M. The tubes were incubated at 25°C for 1 h and filtered rapidly through GF/B filters (Whatman). The filters were washed quickly by three successive washing with 3.0 ml of ice cold buffer containing 50 mM Tris buffer, pH 7.4. Values are representation of 4–6 separate experiments

Table 4 Binding parameters of [^{3}H] YM-019151-2 with sulpiride in the hypothalamus of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One-site	−7.113	7.70 x 10^{-8}	−0.99
Ethanol treated	One-site	−7.434	8.7 x 10^{-8}	−0.97

Values are mean of 4–6 separate experiments

Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA)

K_i—The affinity of the receptor for the competing drug. EC_{50} is the concentration of the competitor that competes for half the specific binding competition studies were carried out with 1.0 nM [^{3}H] YM-019151-2 in each tube with the unlabelled sulpiride concentrations varying from 10^{-10} to 10^{-3} M for hypothalamus

5-HT content leads to an up regulation of 5-HT$_{2A}$ in brainstem and an increased affinity of these receptors in cerebral cortex [48]. In the present study, scatchard analysis of [^{3}H] YM-09151-2 binding showed increased DA D_2 receptors in the hypothalamus and increased affinity of DA D_2 receptors in the corpus striatum. The increase in DA D_2 receptors is a compensatory mechanism for the decreased DA content in the hypothalamus. In the corpus striatum no change in receptor number was observed which was compensated by the increased affinity of the DA D_2 receptors to its ligand. The differences between ethanol treated and control rats in disposition of DA D_2 receptors show presynaptic changes in dopamine synthesis and postsynaptic changes in receptor availability during ethanol treatment. It has been reported that development of tolerance seems to be accompanied by changes in neuronal membrane structure which, in turn, affects the function of membrane-bound dopaminergic receptors [49].

The liver is richly innervated and signaling occurs between the liver and brain [50] and the brain plays an important regulatory role in hepatic functions. Liver dysfunction is associated with more extensive brain dysfunction in liver cirrhosis patients [51]. Dysfunction in

Table 5 Binding parameters of [^{3}H] YM-019151-2 with sulpiride in the corpus striatum of control and ethanol treated rats

Experimental status	Best fit model	(Log EC_{50})	K_i	Hill slope
Control	One-site	−4.949	1.12 x 10^{-5}	−0.99
Ethanol treated	One-site	−5.126	0.75 x 10^{-5}	−0.99

Values are mean of 4–6 separate experiments

Data were fitted with iterative nonlinear regression software (Prism, GraphPad, San Diego, CA)

K_i—The affinity of the receptor for the competing drug

EC_{50} is the concentration of the competitor that competes for half the specific binding competition studies were carried out with 1.0 nM [^{3}H] YM-019151-2 in each tube with the unlabelled sulpiride concentrations varying from 10^{-10} to 10^{-3} M for corpus striatum

Table 6 Kinetic parameters—V_{max} and K_m of aldehyde dehydrogenase in the plasma of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.17 ± 0.04	12.50 ± 0.05
Ethanol treated	0.25 ± 0.05*	3.69 ± 0.65**

Values are mean ± S.E.M. of 4–6 separate experiments

* $P < 0.05$

** $P < 0.01$ when compared with control

Table 7 Kinetic parameters—V_{max} and K_m—of aldehyde dehydrogenase in the liver of control and ethanol treated rats

Experimental status	V_{max} (Units/mg protein)	K_m (μM)
Control	0.52 ± 0.04	21.06 ± 0.40
Ethanol treated	0.69 ± 0.04*	10.84 ± 0.05**

Values are mean ± S.E.M. of 4–6 separate experiments

* $P < 0.05$

** $P < 0.01$ when compared with control

dopaminergic transmission has been associated with craving for ethanol and influencing withdrawal symptoms [52]. It is reported that the DA neuron dysfunction is correlated with change in levels of ALDH expression in human conditions of Parkinsonism and schizophrenia [53]. It is reported that aldehyde dehydrogenase activity is higher in the animals preferring ethanol than in those ones rejecting ethanol [54]. George et al. [55] reported the cerebral cortex and brainstem DA D_2 receptor mediated functional regulation on central and peripheral ALDH enzyme. The chronic ethanol treatment exerted a hypothalamic mediated stimulatory effect on hepatic ethanol metabolizing enzymes [56]. The alterations in the hypothalamic and striatal DA D_2 receptors are suggested to govern regulation of hepatic ALDH through sympathetic innervations. The decreased dopamine content in hypothalamus and striatum of ethanol treated rats might have resulted in its conversion to 3,4-dihydroxyphenylacetaldehyde (DOPAL), which can act as a substrate for ALDH. Thus the ALDH activity was enhanced to compensate the increased production of DOPAL. The increased ALDH activity in plasma and liver of ethanol treated rats is suggested to be a compensatory action to metabolize the excess DOPAL produced.

The regulation of sympathetic nervous system by various neurotransmitters is well studied. The involvement of the central GABA and 5-HT2 receptors in the liver regeneration is already reported from our lab [57, 58]. Pyroja et al. [59] reported that an increased 5-HT content and 5-HT_{2C} receptor in the brainstem and cerebral cortex facilitated the active hepatocyte proliferation. The stimulatory role of GABA receptors on insulin secretion in the brainstem through sympathetic system was reported [60]. It was also reported that a decrease in dopamine D_2 receptor function in hypothalamus, brainstem and pancreas differentially regulates pancreatic islets insulin secretion in streptozotocin induced diabetic condition [61]. There is evidence for regulation of brain dopaminergic system on liver Cytochrome P450 (CYP) isoenzymes functions [62]. Developmental alteration in adrenergic regulation on Hepatic Glycogen Phosphorylase using isolated hepatocytes is reported [63]. This supports the effect of catecholamine on hepatic cells. The specific antagonist for each neurotransmitter or its agonist can inhibit the neurotransmitter mediated pathways in cells. Acetylcholine agonist, carbachol stimulated insulin secretion and its inhibition by antagonist atropine in rat pancreatic islets in vitro was reported [64]. Baclofen, $GABA_B$ agonist, triggered DNA synthesis in primary cultures of rat hepatocytes, mediated through the $GABA_B$ receptors [65] were reported highlighting the effect of neurotransmitter

Table 8 Kinetic parameters—V_{max} and K_m—of aldehyde dehydrogenase in the 24 h hepatocyte culture of rats

Cell status	V_{max} (units/mg protein)	K_m (μM)
Control	6.1 ± 0.01	26.25 ± 0.12
10% Ethanol treated	14.5 ± 0.05@@@	23.5 ± 0.15
10^{-3} M Dopamine + 10% Ethanol treated	2.5 ± 0.88**	28.17 ± 2.20***
10^{-5} M Dopamine + 10% Ethanol treated	5. 7 ± 0.57***	26.67 ± 2.21
10^{-7} M Dopamine + 10% Ethanol treated	4. 0 ± 1.16***	23.33 ± 0.83
10^{-3} M Dopamine + Sulpiride +10% Ethanol treated	14.667 ± 0.67@@@	25.0 ± 0.0*
10^{-5} M Dopamine + Sulpiride +10% Ethanol treated	14.067 ± 0.88@@@	27.5 ± 2.50*
10^{-7} M Dopamine + Sulpiride +10% Ethanol treated	12.333 ± 0.88@@@	25.83 ± 0.44**

Values are mean ± S.E.M. of 4–6 separate experiments

Each experimental group contains 8 groups of rats

@@@ $P < 0.001$ when compared with control

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with ethanol treated

The ALDH enzyme activity was measured using a Shimadzu UV1201 Spectrophotometer at 25°C at 340 nm

agonist on hepatic functions. β-adrenergic receptors mediated positive regulation on islet cell proliferation and insulin secretion in vitro was also reported [66].The culture studies showed that at 10^{-5} M and 10^{-7} M dopamine concentrations, the ALDH enzyme activity was reduced to near normal level. Sulpiride, an antagonist of DA D_2, along with dopamine showed a reversal of the effect shown by dopamine on 10% ethanol treated cells. Changes in the ALDH activity levels in culture appear to be modulated by the DA mediated enzyme regulation. Our results showed that there is a decreased dopamine content with enhanced DA D_2 receptors in the hypothalamus and corpus striatum of ethanol treated rats compared to control. Also, increased ALDH was observed in the plasma and liver of ethanol treated rats and in vitro hepatocyte cultures treated with 10% ethanol. Since the administration of 10^{-5} M and 10^{-7} M DA can reverse the increased ALDH activity in ethanol treated cells to near control level, this has therapeutic application to correct ethanol addicts from addiction.

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