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Mediation of Acute Ethanol-Induced Motor Disturbances by Cerebellar Adenosine in Rats[1]

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CLARK, M. AND M. S. DAR. *Mediation of acute ethanol-induced motor disturbances by cerebellar adenosine in rats.* PHARMACOL BIOCHEM BEHAV **30**(1) 155–161, 1988.—The possible involvement of brain adenosine in acute ethanol-induced motor incoordination (MI) and inhibition of spontaneous motor activity (SMA) was investigated in male Sprague-Dawley rats. Pretreatment with theophylline or 7-(2-chloroethyl)-theophylline, adenosine antagonists, markedly reduced ethanol-induced MI and inhibition of SMA during a 60 min test period compared with saline + ethanol group. On the contrary, pretreatment with (−)-N^6(R-phenylisopropyl)adenosine (R-PIA), an adenosine agonist, or dilazep, an adenosine uptake blocker, markedly potentiated the ethanol-induced MI as well as inhibition of SMA in a 60 min test period compared with saline + ethanol group. No effect on motor coordination was seen when the drug pretreatment was not followed by ethanol. However, the adenosine agonists and antagonists did alter SMA when the pretreatment with these drugs was not followed by ethanol. Ethanol clearance was not altered by the drug pretreatment as blood ethanol levels were similar in all groups except for lower ethanol levels in the R-PIA-treated group. Adenosine A_1 binding studies, using ^{3}H-R-PIA as the radioligand and crude membrane preparation from cerebellar cortex, revealed an increase in B_{max} with no significant change in K_d in ethanol-treated animals vs. saline control. Theophylline pretreatment prevented the increase in B_{max} elicited by ethanol. Collectively, the data suggest that endogenous cerebellar adenosine may be a participating factor in ethanol-induced motor dysfunctions.

Adenosine Ethanol Cerebellum Motor coordination Spontaneous motor activity Adenosine A_1 binding

THE proposed biological roles for adenosine and for precursor adenine nucleotides in the central nervous system (CNS) have increased considerably during the past years. Adenosine displays several receptor-mediated physiological actions and at present is generally accepted to act as a neuromodulator inhibiting neuronal firing [23] and synaptic transmission [16] as well as altering cyclic AMP concentrations in brain tissues [6,25]. The relative selective blockade of the effects of adenosine on neuronal firing and cyclic AMP formation by methylxanthines such as theophylline and caffeine suggests that pharmacological actions of these extensively used substances may be mediated by blockade of central adenosine receptors [27]. Such receptors have indeed been visualized by autoradiography on axon terminals of excitatory neurons [13]. The effects of adenosine such as inhibition of neuronal firing and synaptic transmission and their blockade by methylxanthines are mediated via a high affinity A_1 binding site which has nanomolar affinity and is associated with inhibition of adenylate cyclase activity [4, 5, 15, 23]. An adenosinergic nucleus in the magnocellular area of the ventral hypothalamus with extensively ramifying projections has been proposed on the basis of immunohistochemical staining for adenosine deaminase [18]. Moreover, the notion that a variety of CNS acting drugs may exert some of their effects by altering the levels and/or actions of adenosine was also proposed [20–22]. The possibility that the actions of some CNS stimulant and depressant agents involve adenosine was explored and reviewed recently [28].

Earlier we proposed [7] a possible role of adenosine in some of the CNS effects of ethanol. Pretreatment with theophylline, an adenosine antagonist, prior to acute ethanol administration markedly reduced the duration of ethanol-induced sleep and similarly decreased the intensity and duration of motor incoordination. In contrast, dipyridamole, an adenosine uptake blocker, prolonged the duration of hypnosis and potentiated the motor incoordination elicited by ethanol. These behavioral observations were made both in ethanol-naive and ethanol-dependent animals and in the latter case, a functional correlation was noted between the behavioral observation and the characteristics of brain adenosine binding sites. Proctor and Dunwiddie [24] later confirmed and extended our observations using "long-

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sleep" and "short-sleep" mice and suggested that ethanol can interact with purinergic systems in a complex fashion to affect behavior. Recently, we [8] obtained further evidence for the involvement of brain adenosine in acute ethanol-induced motor incoordination. Animals chronically fed caffeine and isobutylmethylxanthine, methylxanthine antagonists of adenosine, showed greater ethanol-induced motor incoordination which was associated with increased whole brain adenosine binding.

The present studies were carried out to further investigate the possible involvement of adenosine in acute ethanol-induced motor incoordination and inhibition of spontaneous motor activity in the rat. Cerebellum is one of the key brain areas involved in normal motor coordination and motor activity. Adenosine A_1 receptors, regarded to be mediating CNS depressive effects such as hypnosis, decreased spontaneous motor activity and ataxia of adenosine and its analogs [9], have been reported to be in high concentrations in the cortical region of the cerebellum [12]. Since ethanol is known to cause perturbation of plasma membranes [10,17] it is possible that ethanol may produce an alteration in neuronal membranes in the brain and therefore, possibly adenosine receptors located on the external aspects of these membranes. In view of this information we decided to study the binding characteristics of A_1 adenosine binding sites in the cortical region of cerebellum in order to have further insight regarding a possible neurochemical basis of acute ethanol-induced motor disturbances that we have observed. Behavioral studies (motor coordination and SMA) will also be carried out and an attempt will be made to establish a functional relationship between the cerebellar adenosine and ethanol-induced motor disturbances.

METHOD

Male Sprague-Dawley rats weighing 150–200 g were used in all experiments. These were bred and supplied by Animal Resource Center, located in the School of Medicine. The animals were housed three per cage, 1–2 days prior to actual use in the experiment in the animal housing quarters away from vivarium with a 12-hr light/12-hr dark, day-night cycle with food and water available ad lib. Temperature and humidity remained constant. To eliminate any possible diurnal variation in ethanol metabolism or in the levels of adenosine, all behavioral experiments were carried out between 7:00 a.m. and 12:00 noon. Throughout all behavioral experiments described in the present study, each animal was used for one experiment only.

Drugs

Theophylline and 7-(2-chloroethyl)theophylline (CET) were selected and used in the present investigation as the methylxanthine antagonists of adenosine. For agonistic action of adenosine, N^6-(R-2-phenylisopropyl)adenosine (R-PIA), a stable synthetic analog of adenosine, and dilazep, a reuptake blocker of adenosine, were employed. All drugs were administered by IP injection 10 min before ethanol administration. Drug/saline pretreatments were given as 0.5 ml/100 g body weight while ethanol (or saline) was given as 1.0 ml/100 g body weight. The doses of the drugs and ethanol used in the present study were: theophylline, 50 mg/kg; CET, 1.0 mg/kg; dilazep dihydrochloride, 50 mg/kg; R-PIA, 0.1 mg/kg; and ethanol, 1.5 g/kg. All drug solutions were prepared with 0.9% saline in which they were readily soluble, except R-PIA, which was dissolved with the aid of 2 N NaOH in saline and pH adjusted with HCl to 7.4. Except for dilazep dihydrochloride which was supplied as a gift by Degussa Pharma Gruppe (Frankfurt, FR Germany), all drugs were purchased from Research Biochemicals Inc. (Wayland, MA).

Motor Coordination

The degree of motor incoordination was determined by using a standard rat rota-rod treadmill (UGO Basil, Varese, Italy) which was operated at a speed of 12.5 rpm. Four rats could be placed and tested for motor incoordination on the rota-rod simultaneously. Rats were acclimatized to the treadmill 15–30 min prior to the actual experiment. Other experimental details including the time periods of motor coordination evaluation were similar to those previously reported for mice [7]. A separate dose response study enabled the selection of the test dose of ethanol, 1.5 g/kg IP, which produced no apparent sedation, yet produced significant motor incoordination. This dose of ethanol was used routinely to test for motor incoordination. All rats were administered the test dose of ethanol after pretreatment with drug (adenosine antagonist or agonist) or saline and then evaluated for motor coordination by rota-rod, each rat serving as his own control. Normal motor coordination was taken as the ability of each rat to remain on the rota-rod for an arbitrarily selected time of 180 sec and any animal which failed this test was not included in the study. The degree of motor incoordination is expressed as an activity ratio which is defined as the ratio of time the rat was able to stay on the rota-rod after one of the drugs/saline and/or ethanol administration compared to the time before drug/saline treatment (180 sec). The rota-rod evaluation of motor coordination started only after ethanol administration and lasted until 60 min post-ethanol. There was no rota-rod evaluation after the drug pretreatment and before the ethanol injection. Therefore, the time (sec) the animals stayed on the rota-rod after ethanol administration was recorded every 15 min for 60 min test period (i.e., 4 test periods/experiment) and when divided by 180 this yielded a ratio named in the present study as the activity ratio. Thus there was a fixed common denominator of 180 in all motor coordination experiments which permitted intergroup statistical comparison of activity ratio data. The activity ratio, therefore, would not exceed 1 and an activity ratio of 1 or close to 1 would indicate no alteration of motor coordination and a decreasing activity ratio would indicate increasing motor incoordination. For each drug pretreatment three separate rota-rod experiments (with a total of 12 rats) were conducted.

Spontaneous Motor Activity

Automex 2S animal activity monitors (Columbus Instruments, Columbus, OH) were used to measure spontaneous motor activity (SMA). The motor activity was measured on individual animals, i.e., there was one rat per activity monitor during recording sessions. There were five animal activity monitors and therefore, five rats were used simultaneously in a single experiment. For each drug or saline pretreatment, two separate motor activity experiments were conducted involving a total of ten animals. Fifteen to 30 min prior to actual experiment, the animals were acclimatized to the environment of the activity monitors for at least 10 min. This was followed by recording their baseline motor activity for a 60 min period. After appropriate drug or saline pretreatment followed by the test dose of 1.5 g/kg of ethanol, the motor activity was measured for another 60 min test period

in order to observe the effect of drug pretreatment on ethanol-induced inhibition of spontaneous motor activity. The motor activity data are presented as percent of saline control values (saline treated).

^{3}H-R-PIA Binding to Cerebellar Cortex

The binding studies were carried out on crude membranes prepared from the cerebellar cortex of rats from the following treatment groups: (1) saline + saline; (2) saline + ethanol; (3) theophylline + ethanol; and (4) theophylline + saline. Therefore, the binding study used membranes prepared from brains of animals pretreated with either saline or adenosine antagonist followed by the test dose of ethanol or saline. The main objective of the binding study was to observe the *in vivo* effect of ethanol, given either after saline or an adenosine antagonist pretreatment, on the cerebellar cortical ^{3}H-R-PIA binding. The procedure for the preparation of crude membranes would obviously wash out any residual ethanol given IP to the animals in the cerebellar cortical membranes. We previously reported [7] that ethanol *in vitro* exerts no effect on mouse brain ^{3}H-R-PIA binding. Thus, the binding studies were intended to see if acute ethanol administration alone or with theophylline pretreatment in the same dose used in motor coordination and spontaneous motor activity experiments can produce any *in vivo* changes in the neuronal membranes or adenosine A_1 binding characteristics during the 15 min (time of marked motor incoordination and inhibition of spontaneous motor activity) post-ethanol. Each animal was killed by decapitation 15 min after ethanol or saline treatment. The cerebellum was quickly removed and placed on dry ice to firm the tissue. The inner mass of fiber tracts and nuclei were excised which left the cerebellar cortex free for the binding assay. The tissue was homogenized with a Brinkmann polytron at a setting of 7 for 10 sec in an ice cold Hepes buffer (40 mM; pH 7.4) containing 10 mM $MgCl_2$ and centrifuged at 40,600 × g at 4°C for 15 min. The pellet was resuspended in 5 ml of cold Hepes buffer and the suspension was stored at −70°C until binding assays which were performed within 1–4 weeks.

Prior to binding assays, each crude membrane sample was incubated for 30 min at 37°C with adenosine deaminase (EC 3.5.4.4.; Sigma, St. Louis, MO) to deaminate endogenous adenosine. N^6-(2-phenylisopropyl)-(2, 8, ^{3}H)-adenosine (^{3}H-R-PIA) specific activity 49.9 Ci/mmol (NEN, Boston, MA) was used as the radioligand. Each incubation assay contained approximately 100 μg of the membrane protein and 0.25 to 10 nM of ^{3}H-R-PIA in a total volume of 300 μl of Hepes buffer. Nonspecific binding was measured in the presence of 100 μM R-PIA in addition to the above constituents in the same final volume and incubation conditions. At the end of incubation time, the binding assay was terminated by the addition of ice-cold buffer followed by suction filtration using Whatman GF/C glass microfiber filters and further washing with buffer. Each binding assay was performed using the crude membranes from only one rat. Each treatment group consisted of 4–6 rats. The binding data were subjected to Scatchard analysis to determine the characteristics of adenosine binding sites such as the equilibrium dissociation constant (K_d) and the maximum number of binding sites (B_{max}).

Blood Ethanol Measurement

Determination of blood ethanol followed the enzymatic method of Bonnichsen [3]. Mixed blood samples (50 μl) drawn from the tail were collected after cutting a 1–2 mm section off the tip of the tail. Tails were sectioned only once even though multiple samples were drawn at various times. Samples were collected for each treatment group 15, 30, 60, 90 and 120 min after 1.5 g/kg test dose of ethanol. The treatment groups consisted of (1) saline + ethanol; (2) R-PIA + ethanol; (3) dilazep + ethanol; (4) theophylline + ethanol; and (5) CET + ethanol. Each treatment group consisted of at least 5 rats.

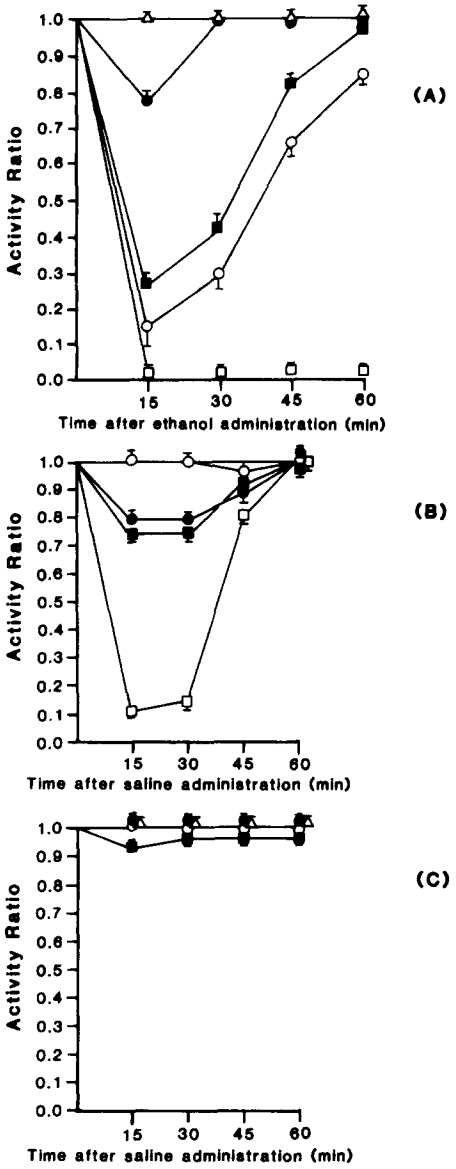

FIG. 1. Dose-response relationship between motor incoordination and concentrations of (A) ethanol; (B) R-PIA; (C) CET; and the test dose of theophylline and dilazep. Each point represents mean±SEM of 8 rats. (A) △: saline; ●: ethanol 0.5 g/kg; ■: ethanol 1.0 g/kg; ○: ethanol 1.5 g/kg; □: ethanol 2.0 g/kg. (B) ○: R-PIA 0.1 mg/kg + saline; ●: R-PIA 0.25 mg/kg + saline; ■: R-PIA 0.5 mg/kg + saline; □: R-PIA 1.0 mg/kg + saline. (C) ○: CET 0.5, 1.0, 2.5 and 4.0 mg/kg; ●: saline + saline; △: theophylline 50 mg/kg + saline; ■: dilazep 50 mg/kg + saline.

RESULTS

Figure 1A shows a separate dose-response relationship

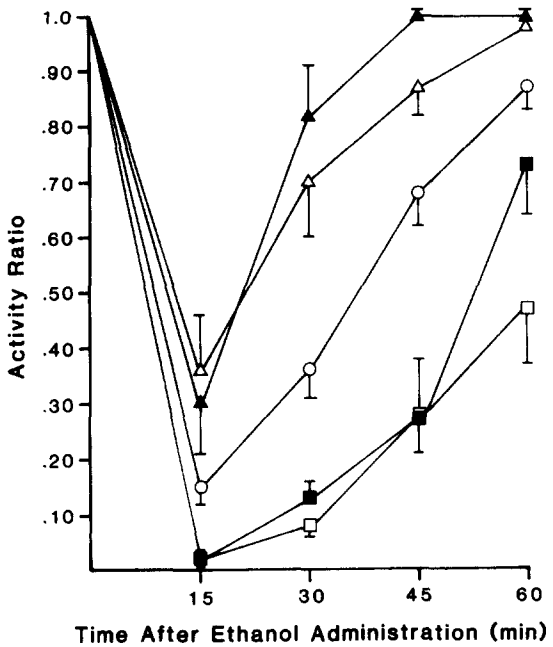

FIG. 2. Effect of theophylline, CET, R-PIA and dilazep on ethanol-induced motor incoordination. Each point represents mean±SEM of 12 rats. ○: saline + ethanol (1.5 g/kg); □: dilazep (50 mg/kg) + ethanol (1.5 g/kg); ■: R-PIA (0.10 mg/kg) + ethanol (1.5 g/kg); △: theophylline (50 mg/kg) + ethanol (1.5 g/kg); ▲: CET (1 mg/kg) + ethanol (1.5 g/kg).

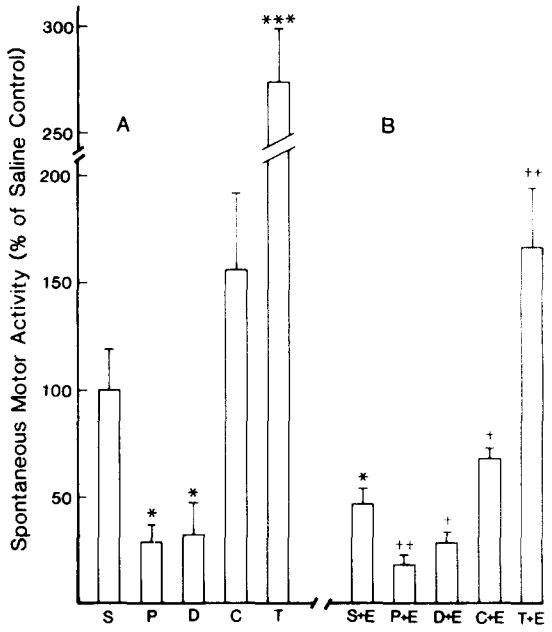

FIG. 3. Effect of theophylline, CET, R-PIA and dilazep administration on spontaneous motor activity (A) and on ethanol-induced inhibition of spontaneous motor activity (B) in a 60 min test period. Each bar represents mean±S.E.M. of 10 rats. *$p<0.05$; ***$p<0.001$ (compared to saline control); †$p<0.05$; ††$p<0.01$ (compared to saline + ethanol group). S=saline; P=R-PIA (0.1 mg/kg); D=dilazep-2HCl (50 mg/kg); C=7-(2-chloroethyl)theophylline (5 mg/kg); T=theophylline (50 mg/kg); E=ethanol (1.5 g/kg).

study between ethanol concentration and motor incoordination. Saline treatment did not produce any detectable motor incoordination. A significant motor incoordination was produced by 1.5 g/kg of ethanol but without any apparent sedation. The onset of motor incoordination by this dose of ethanol was quick and maximal within 15 min of its administration. By 30 min the motor incoordination due to ethanol started to decrease and by 60 min post-ethanol the animals exhibited 87% of normal motor coordination. The animals regained their normal motor coordination by 75 min after ethanol injection (data not shown in Fig. 1A). This apparently subsedative motor incoordinating dose of ethanol was adopted as a test dose in all motor incoordination studies.

In every motor coordination experiment, each animal served as his own control in that each animal reached the 180 sec criteria during the acclimatization period. The ethanol-induced motor incoordination was markedly potentiated (ANOVA followed by planned comparison of the means yielded, $p<0.05$) by R-PIA and dilazep pretreatment, 87% at 15 min in both cases and 64 and 78% at 30 min, respectively, of the normal coordination compared to saline + ethanol group (Fig. 2). The motor coordination was still markedly depressed at 45 min post-ethanol in these pretreated groups and by 60 min the animals pretreated with the R-PIA and dilazep were exhibiting 84 and 54%, respectively, of normal motor coordination (Fig. 2). These animals, however, regained their normal motor coordination by 90 min after ethanol injection (data not shown in Fig. 2). On the other hand, the pretreatment with methylxanthine antagonists of adenosine, theophylline and CET, markedly reduced (ANOVA, followed by planned comparison of the means yielded $p<0.05$) ethanol-induced motor incoordination at 15 and 30 min after ethanol injection compared to saline + ethanol group. Whereas the CET pretreated animals regained their normal motor coordination by 45 min, animals that received theophylline pretreatment reached normal motor coordination by 60 min (Fig. 2). Based on separate dose-response studies we did not observe any change in motor coordination when adenosine antagonists (Fig. 1C), agonist (Fig. 1B) or reuptake blocker (Fig. 1C) were administered alone at these doses.

We also investigated the possible involvement of adenosine in another motor behavior, namely the ethanol-induced inhibition of SMA. Saline was the vehicle for drug administration and, therefore, the saline-treated group served as control in all SMA experiments. The SMA data, after the administration of saline, adenosine agonist or antagonist in a 60 min recording period is presented in Fig. 3A. As expected, the dose of R-PIA and dilazep when injected alone inhibited control motor activity. On the other hand, both theophylline and CET, adenosine antagonists, produced increases in the control motor activity when each was given alone. When pretreatment with saline or one of these drugs is followed by the test dose (1.5 g/kg) of ethanol, marked inhibition in the motor activity was observed (Fig. 3B). Ethanol produced a 48% decrease, compared to saline control motor activity, in saline pretreated animals. The decrease in motor activity was 64% in R-PIA and 40% in dilazep pretreated groups when ethanol test dose of 1.5 g/kg was used when compared with the saline + ethanol group (Fig. 3B). However, the difference in SMA of the dilazep (D) group (Fig. 3A) and saline + ethanol (S+E) group (Fig. 3B)

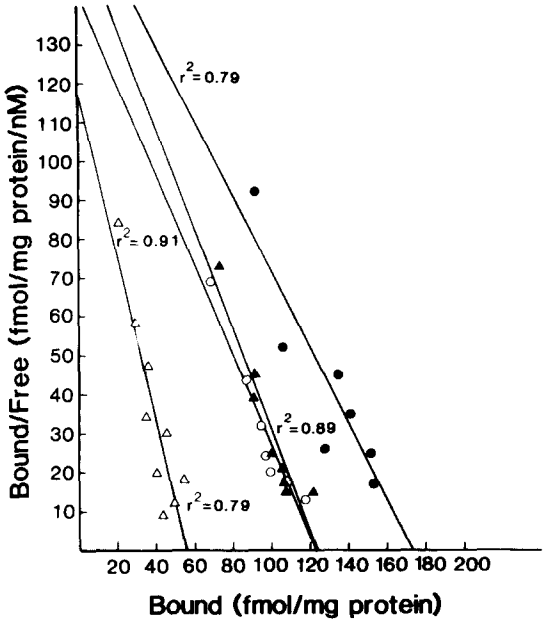

FIG. 4. Scatchard plots of ^{3}H-R-PIA binding in cerebellar cortex of rats pretreated with theophylline or saline (vehicle) followed by either ethanol or saline. Each point represents the average of data from 4–6 rats. ○: saline + saline; ●: saline + ethanol (1.5 g/kg); ▲: theophylline (50 mg/kg) + ethanol (1.5 g/kg); △: theophylline (50 mg/kg) + saline.

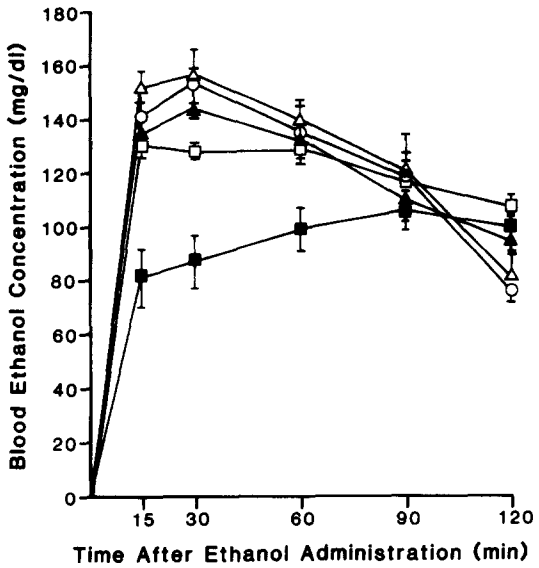

FIG. 5. Effect of theophylline, CET, R-PIA and dilazep on blood ethanol concentration. Each point represents mean±SEM of at least 5 rats. ○: saline + ethanol (1.5 g/kg); ■: R-PIA (0.1 mg/kg) + ethanol (1.5 g/kg); □: dilazep (50 mg/kg) + ethanol (1.5 g/kg); △: theophylline (50 mg/kg) + ethanol (1.5 g/kg); ▲: CET (1.0 mg/kg) + ethanol (1.5 g/kg).

was not statistically significant. The differences in SMA between R-PIA and dilazep groups (Fig. 3A) compared to R-PIA + ethanol (P+E) and dilazep + ethanol (D+E) groups (Fig. 3B), respectively, were not significant, perhaps because the SMA in these animals was nearly maximally inhibited within our detection range with little possibility of further inhibition. It is true that SMA could be inhibited up to 95% by ethanol as well as by adenosine agonists. However, the activity monitors we were using apparently were not sensitive enough to detect changes in SMA below 20–30%. This was the main reason that our SMA data on dilazep and R-PIA was not showing a clear further inhibition of ethanol-induced decrease in SMA compared to saline control group. However, when compared with S+E group, both D+E and P+E groups exhibited significant SMA inhibition (Fig. 3B).

CET pretreatment antagonized the ethanol-induced inhibition of motor activity 45% compared to saline + ethanol group (Fig. 3B). However, in the case of theophylline pretreatment, antagonism of ethanol-induced inhibition of SMA was not only total, but also produced a marked increase (255%) in SMA over and above the S+E group (Fig. 3B). It is readily apparent that theophylline and CET pretreatment attenuated the inhibitory effects of ethanol on SMA.

To test the possibility that ethanol-induced changes in motor coordination and motor activity might be due to the involvement of A_1 adenosine receptors in the brain, we studied the characteristics of the binding sites under the same experimental conditions in which we observed the motor disturbances in motor coordination and motor activity studies. Initially, we decided to conduct the binding studies in the cerebellar cortex, a specific brain area prominently involved in the modulation and control of motor coordination and motor activity. Scatchard analysis (Fig. 4) shows that the maximal number of binding sites (B_{max}) increased significantly ($p<0.01$) 15 min after the administration of a test dose of ethanol compared to saline control, 184.32±10.62 fmol/mg protein vs. 131.04±10.96 fmol/mg protein, respectively. Theophylline treatment alone resulted in a lower B_{max} compared to saline control group, 63.15±9.20 fmol/mg protein vs. 131.04±10.96 fmol/mg protein, respectively. Theophylline pretreatment prevented the rise of B_{max} due to ethanol when compared to saline control group (Fig. 4). The only change in dissociation constant (K_d) was observed in the theophylline + saline group which was significantly ($p<0.01$) lower than saline control, 0.59±0.07 nM vs. 1.22±0.12 nM, respectively.

Blood ethanol determinations were carried out in all groups used in motor incoordination and motor activity studies and results of these determinations have been presented in Fig. 5. There was no significant intergroup differences observed at any time period except in R-PIA + ethanol group in which blood ethanol levels were lower compared to all other groups, after 1.5 g/kg ethanol dose. Generally, the maximum rise of blood ethanol in all groups except R-PIA + ethanol was quick, within 15–30 min of ethanol injection, after which it began to decline. The blood ethanol levels in the case of R-PIA pretreated animals was significantly lower compared to saline + ethanol as well as all other groups at 15, 30 and 60 min after ethanol administration and the peak level in this group was attained relatively slower. The decline in blood ethanol concentration also appears to be relatively slower in R-PIA as well as dilazep pretreated groups (Fig. 5).

DISCUSSION

There was a marked potentiation of ethanol-induced motor incoordination by R-PIA and dilazep pretreatment while that with methylxanthines, theophylline and CET was significantly attenuated. Similarly, pretreatment with R-PIA

and dilazep significantly increased the ethanol-induced inhibition of SMA while theophylline and CET markedly attenuated it. The motor coordination and apparent behavior of animals were not altered when these agonists and antagonists of adenosine were administered alone. Dilazep and R-PIA have important peripheral effects, such as lowering of blood pressure, which could, by itself produce change in behavior and/or motor coordination. The lack of observation of any motor incoordination when R-PIA and dilazep were administered alone suggested that the CNS effect of these drugs was primarily responsible for potentiation of ethanol-induced motor incoordination, with little or no contribution of their peripheral cardiovascular effects (Fig. 1B and 1C). There was, however, a change in the SMA by these drugs when they were given alone. Thus, the data from motor coordination and SMA studies lend support to our earlier reports [7,8] and suggest that brain adenosine may be involved in these motor disturbing effects of ethanol. Results of blood ethanol determinations in R-PIA, dilazep, theophylline or CET-treated groups suggest that except perhaps R-PIA these drugs do not alter the clearance of ethanol. There was no significant difference in blood ethanol levels between saline + ethanol and all other groups except R-PIA + ethanol group which did exhibit a difference in the ethanol level as well as in the rate of rise and clearance of blood ethanol. The delay in the increase and elimination of ethanol due to R-PIA together with a lower peak compared to control group as well as other groups however, do not functionally correlate with the observed potentiation by R-PIA of ethanol-induced motor incoordination and inhibition of SMA. Thus the lower blood ethanol curve in R-PIA group suggests that the potentiation of ethanol-induced motor incoordination and inhibition of SMA by R-PIA is due to its pharmacodynamic effect rather than due to an apparent alteration in ethanol clearance. Overall, the blood ethanol data suggest a lack of involvement of an alteration of clearance of ethanol in the observed behavioral interactions between ethanol and adenosine agonists and antagonists.

We selected to study the possibility of changes induced by acute ethanol in the binding characteristics of adenosine A_1 receptors because the behavioral effects of adenosine appear to be linked to these (A_1) receptors. Synder *et al.* [27] have found that the relative CNS stimulatory effects of methylxanthines parallel their affinities for the adenosine receptors labeled by N^6-[^{3}H]cyclohexyladenosine which is believed to label A_1 adenosine binding sites. Using mice genetically selected for differential sensitivity to the hypnotic effects of ethanol, Fredholm *et al.* [11] found that long-sleep mice (high sensitivity to soporific effect of ethanol) were also more sensitive to the behavioral effects of R-PIA than short-sleep mice (low sensitivity to soporific effect of ethanol). They observed lower K_d values in the cortex and cerebellum of long-sleep mice and a higher B_{max} in the cortex only of the long-sleep mice. They found no differences in R-PIA-induced cAMP accumulations. These authors concluded that their findings could be "taken as circumstantial evidence that the behavioral effects of adenosine are mediated by A_1 receptors." The results of this binding study suggest a significant increase in the maximum number of binding sites (B_{max}) in ethanol-treated animals vs. saline controls. Although there was also an increase in the dissociation constant (K_d) of A_1 receptors in ethanol-treated group vs. saline control group, the difference was not statistically significant. Theophylline pretreatment prevented the increase in B_{max} due to ethanol. The decrease in B_{max} with theophylline alone might be due to adenosine A_1 binding site occupation by theophylline and by its competitive inhibition. Methylxanthines, caffeine and theophylline are competitive inhibitors for adenosine binding sites and block adenosine's effect on the CNS [20,27]. The observed decrease in B_{max} by theophylline alone is in good standing with the notion that theophylline's actions, as observed in the behavioral tests, to decrease ethanol's effect on motor disturbances is a result of this antagonism of adenosine A_1 binding sites. Thus, the Scatchard analysis of ^{3}H-R-PIA binding data bears a good functional relationship with the observed ethanol-induced motor incoordination and inhibition of SMA. Theophylline antagonized both of these ethanol-induced motor disturbances as well as ethanol-induced increase in B_{max} in cerebellar cortex. The apparent alteration in one of the two binding characteristics of the adenosine A_1 binding site, i.e., B_{max} and not K_d, by a single acute administration of ethanol is not without analogy. A single acute dose of ethanol given IP was recently reported to increase B_{max} and not K_d of high affinity GABA binding sites in the rat cerebellum [26].

It was not surprising that we observed significant changes in the binding characteristics (B_{max}) of adenosine A_1 binding sites in the cortical region of the cerebellum, the brain area most prominent in motor coordination and motor functions. The cerebellum is a frequent site of neurological damage in human alcoholism [1, 29, 30] resulting in a variety of motor disturbances. In neonatal rats, brief exposure to ethanol vapors causes large losses of Purkinje cells [2,19]. In adult rats, exposure of five months to liquid ethanol diet followed by two months of withdrawal resulted in a loss of Purkinje cells [31]. In adult mice, the dendritic tree of Purkinje cells was reduced after four months of liquid ethanol diet followed by a two month recovery period [31].

The A_1 adenosine receptors in the cerebellum are apparently localized to granule cells, particularly their parallel fibers and terminals in the molecular layer [12]. Granule cells are the sole excitatory intrinsic neurons of the cerebellum. The localization of adenosine receptors to their processes suggests that adenosine might influence the release of the excitatory transmitter, presumably glutamic acid [32], from the granule cells. In most systems, adenosine inhibits the release of neurotransmitters [14]. Evidence has been presented that the inhibition of neuronal firing by adenosine is presynaptic, involving inhibition of the release of the excitatory transmitter [21]. This will go along very well with the localization of adenosine receptors to axons and terminals of the excitatory parallel fibers in the cerebellum. Thus, the increase in B_{max} of A_1 binding sites of adenosine in the molecular layer of cerebellum by a single acute test dose of ethanol (the dose that produced marked motor incoordination and inhibition of SMA in rats) may serve to elicit an increased presynaptic inhibition of release of the excitatory transmitter glutamic acid and therefore, may be a participating factor in the performance deficits on the rota-rod and in animal activity monitors. Thus it may also be inferred from this hypothesis as well as from results of the present study that adenosine may be a neurotransmitter and/or neuromodulator physiologically involved in the modulation of normal motor coordination and SMA controlled by the cerebellar cortex.

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this relationship is not reciprocal since D1 agonist-induced effects are not abolished, even by near total DA depletion. Taken together, the results of our behavioral and electrophysiological studies indicate that D1 receptor stimulation is necessary for the expression of postsynaptic DA receptor-mediated functional responses. Therefore, alterations of D1 receptor activity may play important roles in the pathophysiology of disorders of DA neurotransmission such as Parkinson's disease and schizophrenia as well as in their pharmacological treatment.

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