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Functional Correlation Between Subclasses of Brain Adenosine Receptor Affinities and Ethanol-Induced Motor Incoordination in Mice[1]

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DAR, M. SAEED. *Functional correlation between subclasses of brain adenosine receptor affinities and ethanol-induced motor incoordination in mice.* PHARMACOL BIOCHEM BEHAV **37**(4) 747–753, 1990.—To further investigate if the modulation of ethanol-induced motor incoordination is by brain adenosine A_1 and/or A_2 receptor, adenosine analogs with wide variability in their affinity for A_1 and A_2 subtypes were administered ICV and their effect on ethanol-induced (IP) motor incoordination was evaluated by rotorod. A dose-dependent marked accentuation of ethanol-induced motor incoordination by adenosine agonists (CHA, NECA, CPA, DCCA) tested, with nearly no effect on normal motor coordination in the absence of ethanol, was observed. There was a positive correlation between A_2 affinity, A_2/A_1 affinity ratio but a negative correlation between A_1 affinity and the potency (ED_{50}) of adenosine agonists to accentuate ethanol-induced motor incoordination. However, with the high potency of CHA and NECA, both having significant affinity for A_1 and A_2 receptors, together with the well known membrane perturbation by ethanol, it seems difficult to rule out until more information becomes available the contribution of A_1 receptor activation to adenosine modulation of ethanol-induced motor incoordination. The high density of high affinity A_2 (A_{2a}) in the striatum and of A_1 in the cerebellum and several brain areas and the known importance of these two brain areas in the motor control, indirectly supports or at least provides a circumstantial evidence for a functional correlation between ethanol-induced motor incoordination and brain adenosine receptors.

Adenosine analogs Ethanol Motor incoordination Adenosine A_1 receptor Adenosine A_{2a} receptor Intracerebroventricular

SOME of the possible physiological functions of adenosine within CNS include modulation of neuronal activity, inhibition of release of neurotransmitters and interaction with the function and metabolic effects of various neurotransmitters (25). Many effects of adenosine have been attributed to its action at receptors located on the external neuronal surface. Although some adenosine responses may be indirect, it is generally accepted that adenosine exerts direct inhibitory effects upon cellular activity because adenosine maintains its effects even in the absence of synaptic transmission (20). Adenosine receptors have been classified into two main subtypes based on interaction of adenosine and its analogs with adenylate cyclase as well as the differential affinities of a variety of synthetic analogs of adenosine (15,17). The A_1 (Ri) receptor subtype mediates inhibition while A_2 (Ra) subtype mediates stimulation of adenylate cyclase activity, respectively. It is generally accepted that at A_1 receptors adenosine analogs mediate inhibition of adenylate cyclase with the potency order of N^6-R-1-phenyl-2-propyladenosine (R-PIA), N^6-cyclohexyladenosine (CHA) >>> S-PIA. On the other hand, at A_2 adenosine receptors, adenosine analogs mediate stimulation of adenylate cyclase with a typical potency order of 5′-N-ethylcarboxamidoadenosine (NECA) > 2-chloroadenosine > R-PIA = CHA > S-PIA. It has also been suggested that adenosine receptors not linked to adenylate cyclase but which exhibit potency orders for adenosine analogs characteristic of A_1 or A_2 receptors also exist (26). In biochemical studies, the structure-activity relationships for adenosine analogs point to obvious differences between the subtypes of adenosine receptors. However, such differences have not been easily observed in physiological responses (5). Nevertheless, the interaction of adenosine with the receptors is very specific and requires both the adenine and ribose moieties. The affinity for A_1 subtype is very high (in nM range) while for A_2 receptors, the affinity lies in μM range.

Much of the evidence would suggest that the behavioral and the CNS depressive actions of adenosine are mediated via A_1 receptors (12,20), since these are observed when very low concentrations of adenosine agonists are used both peripherally and centrally, consistent with actions at high-affinity sites. Similarly,

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we have demonstrated that very low doses of adenosine agonists/antagonists, peripherally and centrally produce alteration of ethanol-induced motor incoordination suggesting again the probable involvement of A_1 adenosine receptors in this behavioral interaction between ethanol and adenosine (4, 7–9). The question of involvement of A_1 or A_2 receptor subtype in the behavioral effects of adenosine is controversial and A_2 receptor activation has also been linked to the sedative and locomotor effects of adenosine (1, 11, 24). During the course of our studies related to adenosine modulation of ethanol-induced motor disturbances (4, 7–9), we developed considerable interest in investigating whether or not the modulation of ethanol-induced motor disturbances by adenosine involves A_1 and/or A_2 receptor subtypes or if a yet unknown adenosine receptor is involved. The receptor subtypes A_1 and A_2 pharmacologically, physiologically as well as anatomically are distinct from one another (10,26), especially in terms of the potencies of agonists. Thus, one way to differentiate the relative involvement of A_1 and A_2 binding sites in modulation of ethanol-induced motor disturbances would be to compare the relative potencies of adenosine receptor agonists in potentiating ethanol-induced motor disturbances with their relative affinities for A_1 and A_2 receptor subtypes. The purpose of this study, therefore, was to observe a correlation between A_1, A_2 and A_2/A_1 ratio of adenosine receptor equilibrium dissociation rate constants (K_is), obtained from (3,6), of adenosine agonists tested and the ability of these drugs to accentuate the ethanol-induced motor incoordination. These correlation data may indicate the extent of involvement of A_1 and/or A_2 adenosine receptor subtype(s) in the accentuation of ethanol-induced motor incoordination. The adenosine agonists tested differ widely in their affinities for A_1 and A_2 receptor subtypes and were the highly A_1-selective, (N^6-cyclopentyl-2-chloro-1-deazaadenosine [DCCA]), N^6-cyclopentyladenosine [CPA] (6), the potent A_2 analog NECA, that displays almost equal affinity for the A_1 subtype and CHA which even though an A_1-selective also binds to A_2 receptors (3). The accentuation of ethanol-induced motor incoordination by these adenosine agonists was investigated by conducting a dose-response study. In view of known peripheral hemodynamic effects of the adenosine agonists, administration of these drugs was made directly into lateral cerebral ventricles by intracerebroventricular (ICV) route.

METHOD

Subjects

Male CD-1 mice (Charles River Labs, Raleigh, NC) weighed 24–28 g at the time of experimentation and were maintained on commercial pellet food and tap water ad lib. After the surgical implantation of permanent indwelling cannulas, mice were individually housed in plastic cages in a controlled environment (ambient temperature 24 ± 1°C) and exposed to a 12-h light/12-h dark cycle (lights on at 0800 h). All motor coordination experiments were scheduled and performed between 0800 and 1230 h.

Surgery

Stainless steel guide cannulas (23 gauge) were stereotaxically (David Kopf Instruments, USA) implanted into lateral cerebral ventricle with the skull surface in the horizontal plane (flat skull) under chloral hydrate (450 mg/kg IP) anesthesia. Coordinates were according to Slotnik and Leonard (23) and consisted of: AP, 0.2 (bregma); ML, ±1.4 mm; and DV, −2.4 mm from the surface of the skull. The guide cannulas were lowered to the desired depth through the appropriately located craniotomy holes. A fast drying carboxylate cement, Durelon® (Premier Dental Products, Co., Norristown, PA) was used to anchor these cannulas to the cranial surface that has been scraped clean of periosteum. At least five days were allowed for recovery of animals from anesthesia and surgery prior to their use in rotorod experiment. Aseptic conditions were maintained during the surgical implantation of the guide cannulas. The cranial surface was routinely cleaned by swab sticks of providone-iodine solution, Operand® (Redi Products, Prichard, WV). The burrs of drill, surgical tools as well as guide cannulas were sterilized by autoclaving prior to their use.

Drugs

All drug solutions were prepared either on the day of experiment or a day earlier and kept frozen. The vehicle for drug solution was artificial cerebrospinal fluid (ACSF) containing: NaCl, 138.6 mM; KCl, 3.35 mM; $CaCl_2$, 1.26 mM; $MgCl_2$, 1.15 mM; $NaHCO_3$, 20.94 mM; NaH_2PO_4, 0.58 mM; urea, 2.16 mM; and glucose, 3.38 mM at pH 7.4 (18). The drugs DCCA, CPA, CHA and NECA used in the present study were dissolved in ACSF with the aid of dimethylsulfoxide (DMSO). The volume of ICV injection of each drug was kept constant at 5 μl administered over 30 s followed by another 30-s period during which the animals were free to move within their individual cages. In order to make an ICV injection, the cannula insert (injector cannula) was connected to 25 μl Hamilton glass microsyringe by PE-10 polyethylene tubing (Clay Adams). The syringe and part of PE-10 tubing were separately filled with water and the latter was separated in the PE-10 tubing by a small air bubble from the drug solution. The movement of the air bubble during ICV drug injection was monitored as an indication of proper drug administration into cerebral ventricles.

Histology

Following the completion of each motor coordination experiment, 5 μl of Fast Green stain were injected into each mouse via the guide cannula. The mouse was sacrificed by cervical dislocation and the brain removed. The brains were sectioned and the extent of dye spread within the ventricular system was assessed. Only those animals in which a histological confirmation, made on an even diffusion of the stain throughout the entire ventricular system (both lateral ventricles, third ventricle and aqueduct of Sylvius), were included in the calculation of data. Less than 5% of guide cannulas failed to deliver the stain.

Motor Coordination

Standard mouse rotorod treadmill (UGO Basile, Verese, Italy) calibrated for a fixed speed of 20 rpm was used for measurement of degree of motor incoordination as reported previously (7–9). Mice were acclimated to the treadmill by placing them on it two to three times a few min prior to the actual experiment. It is important to test each mouse for its motor coordination before its use in a rotorod experiment in case of an inborn defect (e.g., cerebellar). The screening test used requires each mouse to remain on the rotorod for 180 s. Less than 3% of all mice tested fail to meet this criterion. The successfully screened animals received the pretreatment with vehicle or drug (DCCA, CPA, CHA or NECA) by ICV injection, followed within two to three min by the IP injection of a test dose of ethanol. The index of motor coordination was always evaluated every 15 min for 60 min starting from the moment of ethanol injection.

The preset criterion of 180-s stay on the rotorod was the basis to evaluate the effect of a drug pretreatment on ethanol-induced motor incoordination. The effect of a drug pretreatment on etha-

nol-induced motor incoordination was determined by whether the animals stayed on the rotorod for a period of (a) less than, (b) greater than, or (c) nearly the same as the period they stayed on the rotorod when saline + ethanol (control) treatment was given. If the drug pretreatment causes an enhanced ethanol-induced motor incoordination, this will be indicated, according to our criterion, by a stay on the rotorod for a period less than when they received control treatment. On the other hand, if the drug pretreatment induces a reversal of ethanol-induced motor incoordination, the animals would remain on the rotorod for a period greater than when control treatment was given to them, or up to full time of 180 s, which according to our criterion reflects normal motor coordination. The animals in each rotorod experiment acted as their own control and were not used again after the conclusion of each experiment.

The rotorod data are expressed as activity ratio, which is defined as the ratio of the time the animal is able to remain on the rotorod after one of the drugs and/or ethanol administration, compared to predrug and/or ethanol values. Since 180 s was the selected cut off time, we always had 180 as the common denominator in every motor coordination experiment. Although the ratio scores were used to present the rotorod data, actual time periods rather than activity ratios were used for statistical analyses according to the procedure which we have always followed previously (4, 7–9). The ratio scores cannot exceed one (normal coordination) and a ratio of less than one or decreasing will indicate increasing motor incoordination. At each dose of a drug, at least two separate motor coordination experiments (total of 10 mice) were conducted. Statistical analysis of the data from motor coordination studies was performed by analysis of variance (ANOVA) with repeated measures to test for the significance of interaction between treatment and evaluation times. This was followed by one-way ANOVA and Newman-Keuls post hoc analysis at each rotorod evaluation time to determine significant differences between treatment groups. These statistics were performed using the Crunch Statistical Package version 3 (Crunch Software Corporation, Oakland, CA). A $p<0.05$ was taken as a measure of significance. In the correlation analysis of the motor coordination data and the K_i values of the adenosine agonists, the determination of the correlation coefficients as well as the levels of significance from the t-values was carried out as described by Gad and Weil (13).

RESULTS

A dose-response study between various concentrations of ethanol and motor incoordination was carried out (data not shown) in order to select a dose of ethanol that produced a significant motor incoordination with little or no sedation and change in overt behavior. Based on such a study, a dose of ethanol, 2 g/kg IP, was selected as the test dose and used in the entire study. The onset of motor incoordination after the test dose of ethanol in normal mice was quick, reaching to peak level within 15 min and the animals generally regained their normal motor coordination by 45 or 60 min postethanol injection.

The protocol for motor coordination experiments was similar to the one previously reported (8). Each animal served as its own control. Figure 1 shows the accentuation by various (9, 18, 36 and 72 pmoles) ICV doses of CHA of ethanol-induced (IP) motor incoordination in a nearly dose-dependent fashion with a significant evaluation time and drug treatment interaction, $F(15,198) = 10.57$, $p<0.0001$. Doses of CHA, as low as 9 pmoles, significantly (ANOVA, followed by planned comparisons of the means gave $p<0.01$) enhanced the ethanol-induced motor incoordination at 15, 30, 45 and 60 min postethanol time periods (71, 58, 56 and 21%, respectively). The accentuation of ethanol-induced motor

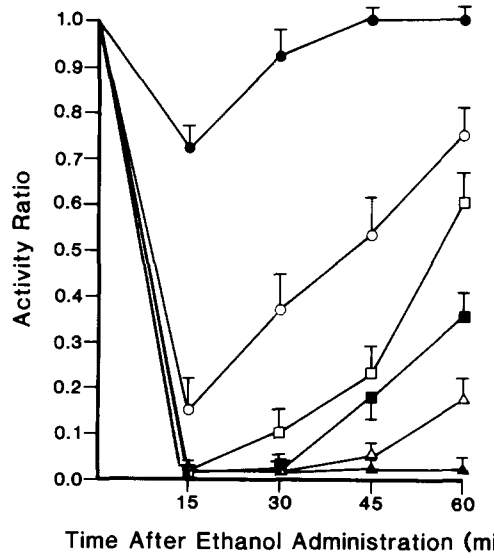

FIG. 1. Dose-response relationship between various concentrations of CHA administered ICV and the degree of ethanol-induced (IP) motor incoordination. Each point represents mean ± S.E.M. of at least 10 mice (○) ACSF 5 μl + EtOH 2 g/kg; (□) CHA 9 pmoles/5 μl + EtOH 2 g/kg; (■) CHA 18 pmoles/5 μl + EtOH 2 g/kg; (△) CHA 36 pmoles/5 μl + EtOH 2 g/kg; (▲) CHA 72 pmoles/5 μl + EtOH 2 g/kg; (●) CHA 36 pmoles/5 μl + saline.

incoordination further increased with escalating (18 to 72 pmoles) doses of CHA. The highest dose (72 pmoles) of CHA markedly accentuated motor incoordinating effect of ethanol resulting in the complete failure of animals to stay on the rotorod during the 60-min postethanol (Fig. 1). There was a 28% decrease in the motor coordination at 15 min when CHA, 36 pmoles, was injected alone followed by saline instead of test dose of ethanol (Fig. 1). The accentuation of ethanol-induced motor incoordination by CPA (300, 600, 900 pmoles) was similar to CHA and dose-dependent. There was a significant interaction between evaluation time and drug treatment, $F(15,354) = 4.96$, $p<0.001$. Nevertheless, a much lower potency of CPA, compared to CHA (ED_{50} 800 pmoles vs. 34 pmoles respectively), was exhibited in accentuation of ethanol-induced motor incoordination (Figs. 1 and 2). Even at the highest dose (900 pmoles) of CPA, the animals regained 30% of their normal motor coordination at 60-min time period compared to 0% in case of CHA (72 pmoles) under similar experimental conditions. When CPA, 900 pmoles, was given alone followed by saline instead of ethanol, a relatively small (30%) decrease in motor coordination at 15 min postinjection was observed (Fig. 2). The accentuating effect of DCCA (542, 813, 1356 pmoles) on ethanol-induced motor incoordination was also observed in a dose-related fashion (Fig. 3), associated with a significant evaluation time-drug treatment interaction, $F(15,243) = 3.45$, $p<0.001$. The lowest dose (542 pmoles) of DCCA exhibited little ($p<0.2$) effect on ethanol-induced motor incoordination. Significant (ANOVA, followed by planned comparison of the means yielded $p<0.01$) accentuation of ethanol-induced motor incoordination was, however, observed after 813 pmoles and 1356 pmoles pretreatment with DCCA; the animals regained nearly 40% and 30% respectively of normal motor coordination at 60-min postethanol. There was no decrease in motor coordination when 1356 pmoles DCCA was injected alone followed instead by saline (Fig. 3). The accentuating effect of NECA (32, 81, 130

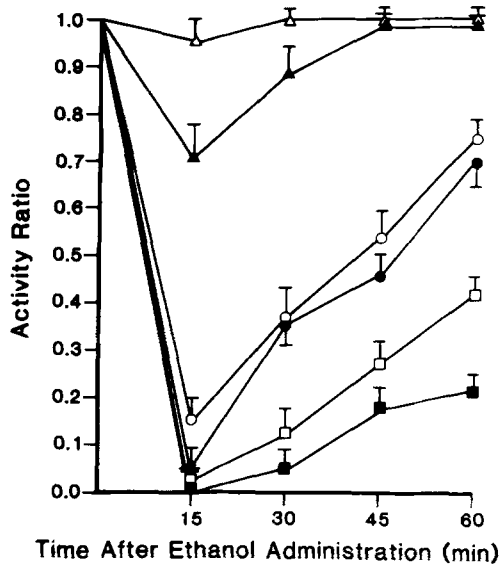

FIG. 2. Dose-response relationship between various concentrations of CPA injected ICV and the degree of ethanol-induced (IP) motor incoordination. Each point represents mean ± S.E.M. of at least 10 mice. (○) ACSF 5 μl + EtOH 2 g/kg; (●) CPA 298 pmoles/5 μl + EtOH 2 g/kg; (□) CPA 596 pmoles/5 μl + EtOH 2 g/kg; (■) CPA 894 pmoles/5 μl + EtOH 2 g/kg; (△) CPA 596 pmoles/5 μl + saline; (▲) CPA 894 pmoles/5 μl + saline.

pmoles) on ethanol-induced motor incoordination was observed in a dose-related fashion (Fig. 4) with a significant interaction between evaluation time and drug treatment, $F(9,210) = 12.25$, $p<0.0001$. Even the lowest (32 pmoles) dose significantly (ANOVA, followed by planned comparison of the means gave $p<0.001$) enhanced the motor incoordinating effect of ethanol. The two higher (81 and 130 pmoles) doses of NECA markedly (ANOVA, followed by planned comparison of the means gave $p<0.001$) accentuated the ethanol-induced motor incoordination at all evaluation time periods compared to ethanol controls (Fig. 4). After pretreatment with 81 and 130 pmoles of NECA, the ethanol-induced motor incoordination at 15-, 30- and 45-min evaluation periods was nearly total and the animals were unable to stay on the rotorod (Fig. 4). Even at 60 min postethanol, the animals in 32, 81 and 130 pmoles pretreated groups regained 60, 18 and 6% respectively of their motor coordination. NECA, 81 and 130 pmoles administered alone, followed by saline instead of the test dose of ethanol, produced 30 and 40%, respectively, decrease in normal motor coordination at 15 min regaining their normal motor coordination within 45 min postinjection (Fig. 4).

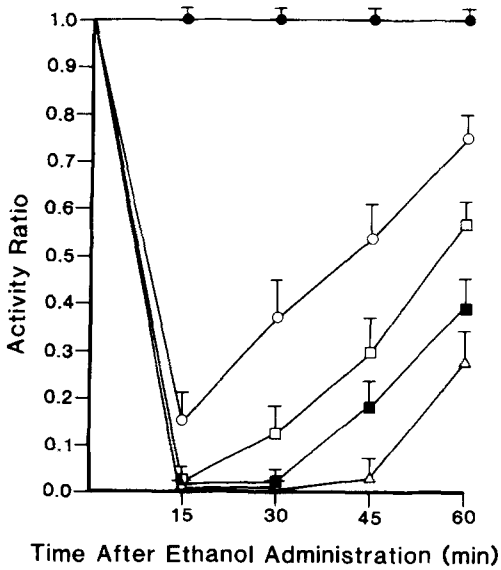

FIG. 3. Dose-response relationship between various concentrations of DCCA injected ICV and the degree of ethanol-induced (IP) motor incoordination. Each point represents mean ± S.E.M. of at least 10 mice. (○) ACSF 5 μl + EtOH 2 g/kg; (□) DCCA 542 pmoles/5 μl + EtOH 2 g/kg; (■) DCCA 813 pmoles/5 μl + EtOH 2 g/kg; (△) DCCA 1356 pmoles/5 μl + EtOH 2 g/kg; (●) DCCA 1356 pmoles/5 μl + saline.

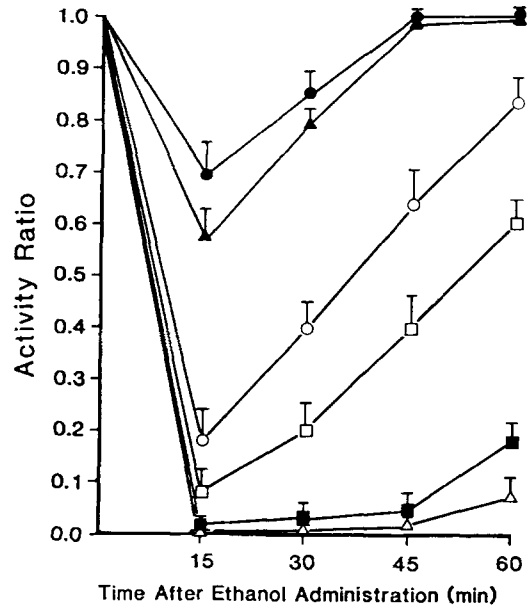

FIG. 4. Dose-response relationship between various concentrations of NECA administered ICV and the degree of ethanol-induced (IP) motor incoordination. Each point represents mean ± S.E.M. of at least 10 mice. (○) ACSF 5 μl + EtOH 2 g/kg; (□) NECA 32 pmoles/5 μl + EtOH 2 g/kg; (■) NECA 81 pmoles/5 μl + EtOH 2 g/kg; (△) NECA 130 pmoles/5 μl + EtOH 2 g/kg; (●) NECA 81 pmoles/5 μl + saline; (▲) NECA 130 pmoles/5 μl + saline.

The ED_{50} values (in pmole), which were determined at 60 min postethanol period of the dose-response curves, of drugs used in the present study are as follows: CHA = 34.2; NECA = 76; CPA = 800; DCCA = 980. The motor coordination data from the dose-response rotorod studies and the K_i values for $A_1$and A_2 as well as A_2/A_1 ratios for each adenosine agonist were compared in order to observe any quantitative relationship between the affinities of these receptor subtypes and the accentuation by these drugs of ethanol-induced motor incoordination. ED_{50}s for accentuating effect on ethanol-induced motor incoordination for each drug were calculated using the probit analysis. The log ED_{50} values for the accentuation by NECA, CHA, CPA and DCCA of ethanol-induced motor incoordination were plotted against log A_1, A_2 and A_2/A_1 ratio of adenosine receptor equilibrium dissociation rate constant (K_is) obtained from Bruns et al. (3) and Cristalli et al. (6) (Figs. 5, 6, 7, respectively). A significant positive relation-

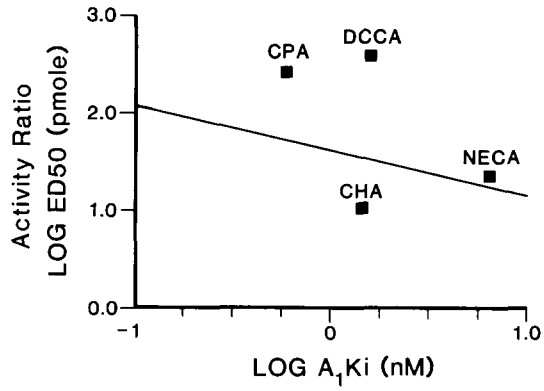

FIG. 5. Correlation of K_i values for A_1 receptor of the adenosine analogs tested, with the ED_{50} for the accentuation of ethanol-induced motor incoordination in mice. Binding affinities are obtained from (3,6). Correlation coefficient (r) is for log activity ratio ED_{50} versus log binding K_i and was obtained after data analysis by linear regression on 9–10 animals per each drug treatment group ($df = 37$).

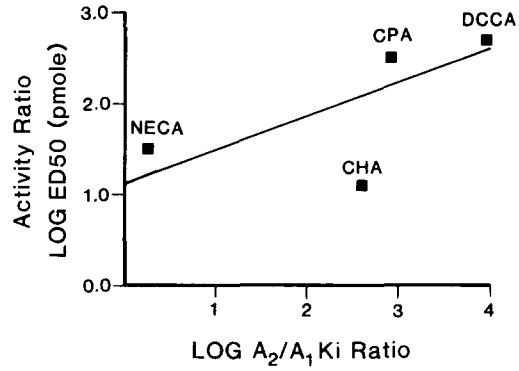

FIG. 7. Correlation of K_i values for A_2/A_1 receptor ratio of the adenosine analogs tested, with the ED_{50} for the accentuation of ethanol-induced motor incoordination in mice. Binding affinities are obtained from (3,6). Correlation coefficient (r) is for log activity ratio ED_{50} versus log binding K_i and was obtained after data analysis by linear regression on 9–10 animals per each drug treatment group ($df = 37$).

ship was observed between the log A_2 and A_2/A_1 ratio of K_i values and the log ED_{50}s for the accentuation of ethanol-induced motor incoordination ($r = .62$, $p<0.003$; $r = .64$, $p<0.002$, respectively). There was, however, a significant negative correlation between the A_1 receptor affinity and the potency (ED_{50}) of the adenosine analogs to accentuate ethanol-induced motor incoordination ($r = .51$, $p<0.005$). The data points shown in each of the correlation figure (Figs. 5–7) represented the mean of 9–10 animals per experimental group. Therefore, for each figure (5, 6, and 7), the correlation coefficients and values for the level of significance were determined based on 39 pairs of the data ($df = 37$).

DISCUSSION

The involvement of brain adenosine in the modulation of CNS effects of ethanol has been demonstrated by several reports from our laboratory (4, 7–9) as well as others (14,21). The biochemical characteristics and autoradiographic distributions of A_1 binding sites in the brain were aided by the availability of high specific activity, ^{3}H-labeled CHA and R-PIA which exhibit selective and specific binding with A_1 subtypes at low concentrations. Ligands selective for A_2 receptor have been less abundant. No previous study has been performed in order to evaluate the quantitative relationship between the binding affinities of adenosine A_1 and/or A_2 receptors or their subtypes and the motor depressive effects of adenosine agonists, except for a recent report (11) that appeared during the preparation of this manuscript. A significant correlation has been demonstrated between the drugs that inhibit high affinity, saturable binding of adenosine agonist and antagonist radioligands to A_1 and A_2 receptor subtypes and their corresponding in vitro and in vivo pharmacological potency thereby strongly suggesting that the radioligand binding is indeed to specific adenosine receptors (2, 19, 22).

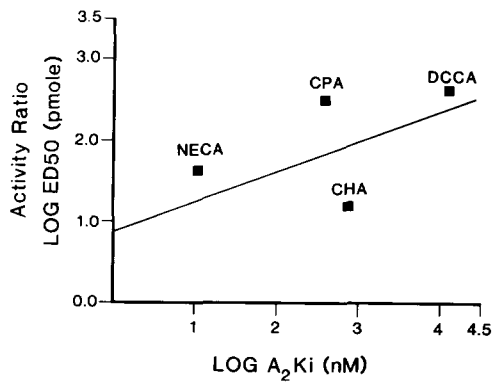

FIG. 6. Correlation of K_i values for A_2 receptor of the adenosine analogs tested, with the ED_{50} for the accentuation of ethanol-induced motor incoordination in mice. Binding affinities are obtained from (3,6). Correlation coefficient (r) is for log activity ratio ED_{50} versus log binding K_i and was obtained after data analysis by linear regression on 9–10 animals per each drug treatment group ($df = 37$).

The adenosine agonists used in the present investigation differ markedly in their selectivity for the adenosine receptor subtype and accentuated ethanol-induced motor incoordination dose-dependently. The rank order of their potency is CHA > NECA > CPA > DCCA. From their rank order it appears that the accentuation of ethanol-induced motor incoordination by adenosine agonists primarily will be determined by their affinities for the specific adenosine receptor subtype. Relatively high potency of NECA in the accentuation of ethanol-induced motor incoordination suggested the involvement of a high affinity form of A_2 [most likely the proposed (3) A_{2a}] receptor. NECA is a potent but non-selective ligand and can bind approximately as much to adenosine A_1 as to A_2 receptors. Therefore, a simultaneous activation of adenosine A_1 receptor by NECA should be expected. In a different study (J. Pharmacol. Exp. Ther.; in press), 5′-cyclopropylcarboxamidoadenosine (CPCA), a potent A_2 agonist which also binds to A_1 receptor subtype, accentuated ethanol-induced motor incoordination with its potency falling between those of CHA and NECA, further supporting the significance of the adenosine A_2 receptors. Again, an appreciable activation of A_1 receptor should also occur with CPCA. The potency (ED_{50}'s) of CHA, an A_1 agonist that also binds to A_2 receptor and NECA, exhibited significant positive correlation with the affinity of A_2 receptor (Fig. 6). The accentuation of ethanol-induced motor incoordination was produced by relatively much smaller ($\times 76$ pmoles NECA and $\times 34$ pmoles CHA for ED_{50}) dose of these agonists. Similar significant positive correlation was observed between A_2/A_1 recep-

tor affinity ratio and ED_{50}'s for the accentuation of ethanol-induced motor incoordination (Fig. 7). The importance of adenosine A_2 receptor subtype in the accentuation of ethanol-induced motor incoordination was thus demonstrated by the significance of these positive correlations.

The highly selective A_1 adenosine agonists, CPA and DCCA, exhibited least potency ($\times 800$ pmoles and $\times 980$ pmoles respectively for ED_{50}) in accentuating ethanol-induced motor incoordination, the ED_{50}'s displayed a significant negative correlation with A_1 receptor affinity suggesting a lack of A_1 involvement in the accentuation of ethanol-induced motor incoordination. The relationship between A_1 receptor affinity and relatively weaker accentuation of ethanol-induced motor incoordination can be exemplified by comparing CPA and DCCA, which have similar A_1 receptor affinities (K_is 0.59 nM and 1.6 nM respectively) but differ markedly in their A_2 receptor affinities of 462 nM and 13,200 nM respectively. On the other hand, A_1-selective adenosine agonist CHA was found to be the most potent of the four agonists tested in this study. However CHA, in addition to A_1 activation, also significantly binds to A_2 receptor subtype which suggests that CHA-produced activation of high affinity A_2 (A_{2a}) subtype in the striatum may be adequate to explain the adenosine modulation of ethanol-induced motor incoordination. Although the drugs were administered by ICV route, still the differences in the ED_{50} values or in their behavioral potencies, especially the highest potency of CHA, may likely be the result of differences in their lipophilic to hydrophobic ratio characteristics which may very well affect the rate of their diffusion in the brain tissue.

The above interpretation and conclusion that the high affinity A_2 (A_{2a}), but not A_1, receptor subtype is involved in the accentuation of ethanol-induced motor incoordination may appear simplistic for several reasons. First, the data with CHA and NECA suggested otherwise. CHA is A_1 selective and NECA displays almost same selectivity for both A_1 and A_2 receptors. Therefore, a significant activation of A_1 receptor by both NECA and CHA must have occurred when high affinity A_2 (A_{2a}) receptors were being activated by these drugs. Since drugs were administered ICV, overall activation of A_1 receptors, distributed widely in brain, will be quantitatively much greater compared to the activation of high affinity A_2 (A_{2a}) receptors located mainly in the striatum (3). Second, the doses of CHA and NECA used in the study, when uniformly distributed in the brain, will yield nM concentration. At this concentration, only the high affinity adenosine receptor subtypes, A_1 and A_2 (A_{2a}) would be activated. Third, the adenosine agonists tested produced little or no decrease in normal motor coordination and the marked motor incoordination was seen only in presence of ethanol. Ethanol is well known to cause perturbation of neuronal membrane and has been shown to increase the maximum number of adenosine A_1 receptors in the cerebellar cortex without altering the receptor affinity (K_d) (4). It is, therefore, conceivable that in spite of a negative correlation between affinity of A_1 subtype and the ED_{50} of drugs for the accentuation of the ethanol-induced motor incoordination, membrane perturbation by ethanol could result in profound conformational and/or functional changes in A_1 receptor other than a change in its affinity resulting in their significant participation in the modulation of ethanol-induced motor incoordination. Cerebellum, a key brain area controlling the normal motor coordination, has a high density of adenosine A_1 (but low of A_2) receptors primarily located in the granular layers of the cerebellar cortex. Striatum also controls normal motor functions (16) and has a high concentration of both high-affinity A_2 (A_{2a}) and A_1 adenosine receptors. In view of these facts and in light of observed positive correlation between ED_{50} values of each drug for accentuation of ethanol-induced motor incoordination and K_is of A_2 and A_2/A_1 ratio, it may be tempting to suggest that the predominant involvement of A_2 with some possible participation of A_1 subtype in the adenosine modulation of motor-incoordinating effect of ethanol could be due to the physiological and anatomical characteristics of the brain areas, cerebellum and striatum.

The results of this investigation, therefore, could be useful in predicting the CNS, and perhaps other biological, effects of adenosine agonists based on their affinity for A_1 and A_2 subtypes as well as in the subclassification of adenosine receptors. The data suggest that adenosine A_1 and A_2 receptor binding affinities, even though determined in brain tissue in vitro (3), are related to accentuation of ethanol-induced motor incoordination in mice. Modulation by adenosine agonists of ethanol's motor incoordination seems to depend predominantly on the activation of high affinity A_2 (A_{2a}) subtype in agreement with the observed significant positive relationship between log ED_{50} values of adenosine agonists and receptor affinities of A_2 and A_2/A_1 ratio.

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