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The Biphasic Effects of Centrally and Peripherally Administered Caffeine on Ethanol-Induced Motor Incoordination in Mice

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Abstract—The possible biphasic effect of caffeine on acute ethanol-induced motor incoordination by rotorod evaluation was investigated in mice. Caffeine in various doses was administered intracerebroventricularly (i.c.v.) to mice implanted with permanent indwelling stainless steel guide cannulae and intraperitoneally (i.p.) to non-cannulated animals. A motor incoordinating test dose of ethanol, 2 g kg^{-1}, was given i.p. in both cases. Caffeine < 25 μg administered i.c.v., dose-dependently attenuated while 75 μg i.c.v. potentiated ethanol (i.p.)-induced motor incoordination. Similarly, caffeine < 20 mg kg^{-1} given i.p., dose-dependently attenuated while 62·5 mg kg^{-1} potentiated ethanol (i.p.)-induced motor incoordination. The data obtained demonstrated that caffeine given either i.c.v. or i.p. exerted biphasic effects on ethanol-induced motor incoordination. The data also suggested that caffeine antagonized ethanol-induced motor incoordination when administered in appropriately low concentrations. At these low concentrations (< 25 μg i.c.v.; < 20 mg kg^{-1} i.p.) caffeine is well known to display high affinity for adenosine binding sites. Therefore, the present investigation lends further support to our earlier suggestion that adenosine may be involved in the motor impairing effect of ethanol.

In spite of the widespread use of the methylxanthines, caffeine and theophylline, as food constituents and as drugs, the molecular mechanisms that account for their pharmacological actions are not well understood. One of the first hypotheses, widely held until recently, that caffeine and theophylline act in-vivo mainly by inhibiting cyclic AMP (cAMP) phosphodiesterase is untenable given caffeine's relatively low potency (EC50 of 10^{-4} to 10^{-3} M) as an inhibitor of the enzyme compared with its potency in modulating physiological processes in-vivo. Even relatively high doses of methylxanthines fail to increase tissue cAMP levels in intact animals (Burg & Warner 1975). That methylxanthines might exert stimulant and perhaps anxiogenic effects by blocking the site at which benzodiazepines elicit anxiety reduction and sedation (Skolnick et al 1980) was also speculated but appeared not likely as considerably higher concentrations (in millimolar range) of caffeine are required to compete for the benzodiazepine receptors.

Recently, a variety of evidence has accumulated suggesting strongly that adenosine may mediate the behavioural influences of xanthines (Snyder et al 1981; Rall 1982). At present it is generally accepted that central nervous system (CNS) stimulation by methylxanthines involves blockade of adenosine receptors (Fredholm 1980; Snyder et al 1981). This hypothesis is further substantiated by evidence showing that endogenous adenosine levels may be high enough to exert a tonic modulatory effect in the brain (Dunwiddie et al 1981). We (Dar & Wooles 1986) as well as others (Fredholm 1982; Boulenger et al 1983) have reported increases of adenosine receptors in brain tissue following chronic methylxanthine treatment. This up-regulation of adenosine receptors in brain additionally suggests that methylxanthines also have other actions on the purinergic system and could also be the basis of tolerance to caffeine's CNS stimulant effects (Dar & Wooles 1986).

Caffeine is perhaps the most widely consumed behavioural stimulant drug in the world (Gilbert 1976) and therefore, the consequences of its ingestion could be potentially profound. Caffeine also has numerous pharmacological effects on a variety of organ systems (Ritchie 1985) and only the CNS stimulant effects of caffeine are accepted to result from antagonism of adenosine receptors. Caffeine is effective in antagonizing the two receptor subtypes, mediating A_1 and A_2 adenosine actions (Daly et al 1983; Phillis & Barraco 1985), although the behavioural effects of adenosine are generally believed to be mediated by high affinity A_1 binding sites that have nanomolar affinity and are associated with inhibition of adenylate cyclase activity (Phillis & Wu 1982; Coffin et al 1984).

Caffeine has been reported to exhibit biphasic effects on locomotor activity (Snyder et al 1981; Katims et al 1983) and sleep (Yanik et al 1987). It is the purpose of this study to investigate caffeine's possible biphasic effect on ethanol-induced motor incoordination. We have been interested in the study of possible adenosine involvement in the motor incoordinating effect of ethanol. The present study, therefore, will serve the dual purpose of testing the validity of the widely held belief that caffeine antagonizes the central depressant action of ethanol and subsequently the role of brain adenosine in this important CNS effect of ethanol.

Materials and Methods

Male Charles River mice (22–25 g) (Charles River, Research Triangle Park, NC) were used. After the permanent indwelling cannulae were implanted, the mice were housed individually in a controlled environment (ambient temperature 24 ± 1 °C; 12 h light/dark cycle) and were given tap water containing 0·3% tetracycline hydrochloride and free access to standard pellet food.

Chronic guide cannulae implantation

Stainless steel guide cannulae (23 gauge) aimed at the lateral ventricle of the brain, were stereotaxically (David Kopf Instruments, USA) implanted with the skull surface in the horizontal plane under chloral hydrate anaesthesia. Coordinates were according to Slotnik & Leonard (1975). Chronic guide cannulae were implanted monolaterally in the lateral ventricle (AP 0·2 mm (bregma); ML ± 1·4 mm; DV − 2·4 mm from skull surface). The guide cannulae tubes were lowered to the desired depth through appropriately located craniotomy holes. The cannulae were anchored with fast-drying carboxylate cement (Durelon, Premier Dental Products Company, Morristown, PA) to the cranial surface that had been scraped clean of periosteum. Aseptic conditions were maintained during the surgical implantation of the guide cannulae.

Microinjections

The injector cannula was connected to a 100 μL Hamilton microsyringe by PE-10 (Clay Adams) polyethylene tube. Five μL of caffeine solution of various concentrations were injected over 60 s using a multiple syringe automated micro syringe (Stoelting Co., Chicago, IL) and the animals were allowed to move freely within their individual cages. A minimum of five days were allowed for the mice to recover from the surgery and the effects of anaesthetic before they were used in the motor coordination experiments. Caffeine (Sigma Chemical Co., St. Louis, MO) solutions were freshly prepared just before intracerebroventricular (i.c.v.) injections in motor coordination experiments. Artificial cerebral spinal fluid (ACSF) containing (mM): NaCl 138·6, KCl 3·35, $CaCl_2$ 1·26, $MgCl_2$ 1·15, $NaHCO_3$ 20·94, NaH_2PO_4 0·58, urea 2·16, glucose 3·38, at pH 7·40 was used as the vehicle for i.c.v. administration of caffeine. Ethanol was injected i.p. in all motor coordination experiments in the present study. Caffeine was given i.p. only in those motor coordination experiments in which non-cannulated mice were used. 0·9% NaCl (saline) was the vehicle for making ethanol and caffeine solutions in these experiments.

Histology

Immediately following the completion of a motor coordination experiment, each mouse was injected with 5 μL of Evans Blue stain through the guide cannula, killed and its brain removed. The brains were then sectioned and the extent to which the stain spread within the ventricular system was assessed. Only those mice in which the histological confirmation was made based on even diffusion of the stain throughout the entire ventricular system (both lateral ventricles, third ventricle and aqueduct of Sylvius) were considered for the statistical evaluation of motor coordination data. Over 95% of cannulations were successful.

Motor coordination studies

Motor coordination in all experiments was evaluated by the use of a standard mouse rotorod treadmill (UGO Basile, Varese, Italy) which was calibrated for a fixed speed of 18 rev min^{-1}. Five mice could be placed on the rotorod and evaluated for motor coordination simultaneously. Mice were acclimatized to the treadmill 15–30 min before the actual experiments. Other experimental details were reported previously (Dar et al 1983; Dar & Wooles 1986). Based on a separate dose-response study between ethanol concentration and degree of motor incoordination (Fig. 1), a 2 g kg^{-1} test dose of ethanol was selected and routinely used in all motor coordination experiments. The test dose of ethanol was slightly sedative or subsedative yet produced significant motor incoordination. The mice were injected with the test dose of ethanol i.p. 5 min after caffeine 5, 10, 25, 50 and 75 μg injected i.c.v. and evaluated for motor coordination at 15, 30, 45 and 60 min post-ethanol, each mouse serving as its own control. Normal motor coordination was defined as the ability of each mouse to remain on the rotorod for an arbitrarily selected time of 180 s consecutively and any animal which failed to do so was excluded from this study. The degree of motor incoordination is expressed as a ratio called activity ratio which is defined as the ratio of time the mouse is able to stay on the rotorod after caffeine/ACSF/saline pretreatment followed by the administration of the test dose of ethanol compared with the time before the pretreatment (180 s). Thus the evaluation of the motor coordination started only after ethanol administration and lasted until 60 min post-ethanol. No evaluation of motor coordination was carried out between the time of caffeine/ACSF/saline administration and the ethanol injection. There was a fixed common denominator of 180 in all motor coordination experiments as the time animals stayed on the rotorod at each test period was divided by 180 to yield a ratio (activity). This permitted an intergroup statistical comparison of activity ratio and the activity ratio could not exceed 1. An activity ratio of 1 or near 1 would indicate no motor incoordination and a decreasing activity ratio would indicate increasing motor incoordination. At each caffeine dose at least two separate motor coordination experiments (total of 10 mice) were conducted. Motor coordination experiments in non-cannulated mice were similarly conducted and pretreatment with caffeine 2·5, 5, 10, 20 and 62·5 mg kg^{-1} i.p. or saline was followed 10 min later by the test dose, 2 g kg^{-1} i.p. of ethanol. The evaluation of motor coordination was conducted exactly in the same manner as explained above in the cannulated mice experiments. Statistical analysis of the data from motor coordination studies was done by one-way analysis of variance (ANOVA) for repeated measures followed by planned comparison of means. A $P \leqslant 0{\cdot}05$ was taken as a measure of significance.

Blood ethanol determination

Blood ethanol levels were determined by the method of Bonnichsen (1965) in separate groups of mice which received pretreatment with the lowest and the highest dose of caffeine used in the motor coordination studies with non-cannulated mice. Mixed venous-arterial blood samples from the sectioned tail of mice were collected at 0·5, 1, 1·5 and 2 h after 2 g kg^{-1} of ethanol. The control group received saline while the other groups received 2·5 and 62·5 mg kg^{-1} of caffeine followed 10 min later by the test dose of ethanol.

Results

Fig. 1 shows the dose response relationship between ethanol concentration and the degree of motor incoordination. The main objective of this dose response study was to select a

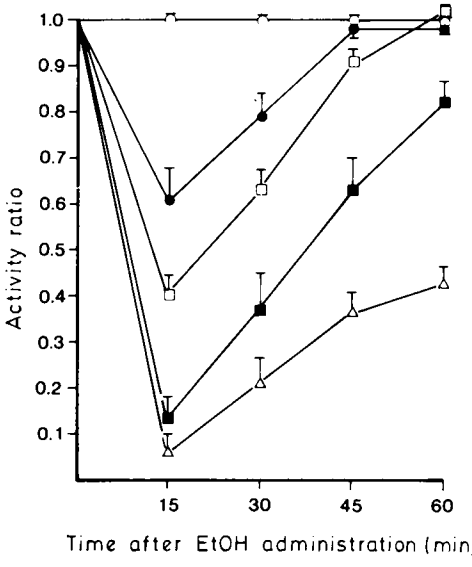

FIG. 1. Dose-response relationship between ethanol concentration and degree of motor incoordination. Data are expressed as the mean of the activity ratio ± s.e.m. of at least 10 animals. ○ saline + saline, ● saline + EtOH 1 g kg^{-1}, □ saline + EtOH 1·5 g kg^{-1}, ■ saline + EtOH 2 g kg^{-1}, △ saline + EtOH 2·5 g kg^{-1}.

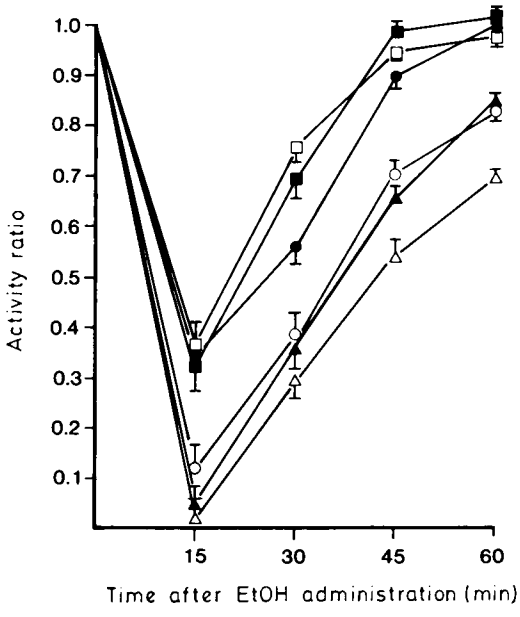

FIG. 2. The effect of various doses of caffeine administered i.c.v. on ethanol (i.p.)-induced motor incoordination in mice implanted with permanent indwelling stainless steel guide cannulae. Each point represents the mean ± s.e.m. of at least 10 mice. ○ ACSF 5 μL + EtOH 2 g kg^{-1}, ● caffeine 2·5 μg/5 μL + EtOH 2 g kg^{-1}, □ caffeine 5 μg/5 μL + EtOH 2 g kg^{-1}, ■ caffeine 25 μg/5 μL + EtOH 2 g kg^{-1}, △ caffeine 75 μg/5 μL + EtOH 2 g kg^{-1}, ▲ caffeine 150 μg/5 μL + EtOH 2 g kg^{-1}.

dose of ethanol for routine administration in all motor coordination experiments which would not be sedative but yet produced marked motor incoordination. The ethanol dose of 2 g kg^{-1} was selected as the test dose because it was subsedative or slightly sedative in some animals and the animals exhibited normal spontaneous motor activity. However, on rotorod evaluation the animals demonstrated significant motor incoordination which was maximum at 15 min post ethanol. Motor coordination was still 83% of normal at 60 min post ethanol and returned to normal level by 75 min post ethanol (data not shown in Fig. 1). The 2·5 g kg^{-1} dose of ethanol was sedative although the animals were still moving in their cages. The degrees of motor incoordination produced by this dose was marked, and greater compared with 2 g kg^{-1} ethanol dose at all time periods of evaluation and at 60 min post-ethanol animals regained only 43% of their normal motor coordination (Fig. 1).

The results of various doses of caffeine given i.c.v. on ethanol (i.p.)-induced motor incoordination is presented in Fig. 2. The lowest dose of caffeine used, 2·5 μg markedly antagonized ethanol (i.p.)-induced motor incoordination. The motor incoordination was 180% less at 15 min, 47% less at 30 min and 30% less at 45 min post-ethanol compared with animals that received ACSF pretreatment (control group) instead of caffeine followed by the same test dose of ethanol. The caffeine (2·5 μg)-pretreated mice regained 90 and 100% of their normal motor coordination by 45 and 60 min post-ethanol, respectively, while the control mice had regained 68 and 82% of their normal motor coordination at these times. The antagonism by 5 μg caffeine i.c.v. dose was also significant and produced 204 and 97% less motor incoordination at 15 and 30 min post-ethanol compared with control animals. The antagonism by this higher dose compared with 2·5 μg caffeine was significantly greater only at 30 min post-ethanol. The 25 μg dose of caffeine was as effective as 5 μg dose in antagonizing ethanol-induced motor incoordination.

A still higher dose of caffeine, 75 μg, produced qualitatively opposite effects and significantly potentiated ethanol-induced motor incoordination. There was 81, 24, 19 and 15% more motor incoordination than that produced by the test dose of ethanol when given alone. However, the highest dose of caffeine used, 150 μg, had potentiating effect only at 15 min post-ethanol (62% increased motor incoordination compared with the ACSF + ethanol group) and practically no significant effect at 30, 45 and 60 min post ethanol periods. The lack of an effect of relatively higher i.c.v. caffeine doses on CNS motor activity was similar to a previous observation by other investigators (Barraco et al 1983). The effect of each dose of caffeine on motor coordination used in these experiments was not different from ACSF treated mice when not followed by the test dose of ethanol (data not shown).

The effect of caffeine i.p. pretreatment on ethanol-induced motor incoordination is presented in Fig. 3. The most effective antagonism to ethanol-induced motor incoordination was observed with 5 mg kg^{-1} caffeine pretreatment. The motor incoordination was 184% less at 15 min post-ethanol compared with saline + ethanol group. The antagonism was significant at 30 and 45 min post-ethanol as the motor incoordination was 70 and 30% less, respectively, compared with the saline + ethanol group. Caffeine pretreated animals regained 91% of normal motor coordination at 45 min post ethanol and completely regained normal motor coordination at 60 min post ethanol whereas the saline + ethanol groups had 70 and 83%, respectively, at these time periods. The antagonism by 2·5 mg kg^{-1} caffeine was marked but quantitatively less compared with the 5 mg kg^{-1} dose. The decrease in ethanol-induced motor incoordination was 88, 34 and 12% at 15, 30, and 45 min, respectively, at post-ethanol

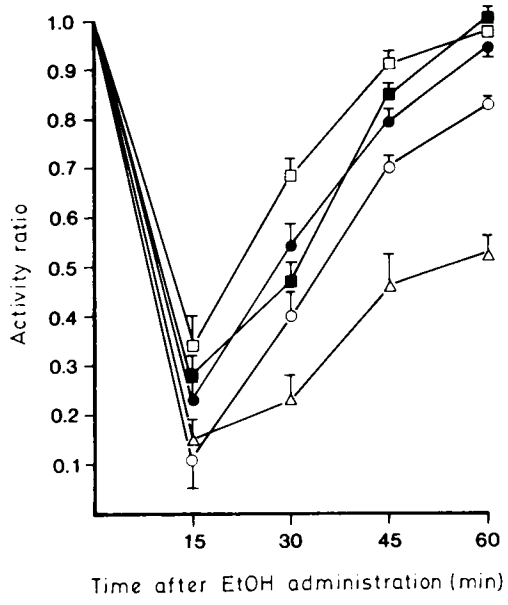

FIG. 3. The effect of various doses of caffeine administered i.p. on ethanol (i.p.)-induced motor incoordination in mice. Each point represents the mean ± s.e.m. of at least 10 mice. ○ saline + EtOH 2 g kg^{-1}, ● caffeine 2·5 mg kg^{-1} + EtOH 2 g kg^{-1}, □ caffeine 5 mg kg^{-1} + EtOH 2 g kg^{-1}, ■ caffeine 20 mg kg + EtOH 2 g kg^{-1}, △ caffeine 62·5 mg kg^{-1} + EtOH 2 g kg^{-1}.

time periods compared with the saline + ethanol group. At 60 min the animals practically regained their normal motor coordination. The 20 mg kg^{-1} dose of caffeine was less effective as an antagonist than both 2·5 and 5 mg kg^{-1} dose at 30 and less than 5 mg kg^{-1} at 45 min post-ethanol. The antagonistic effects were the same at 60 min post ethanol of all these caffeine pretreatment doses. The highest, 62·5 mg kg^{-1}, caffeine pretreatment dose exhibited qualitatively opposite effects on ethanol-induced motor incoordination and significantly potentiated the degree as well as the duration of ethanol-induced motor incoordination. There was marked increase, 43, 34 and 37% in ethanol-induced motor incoordination compared with saline + ethanol group. The animals in this group regained only 52% of their normal motor coordination compared with 83% of normal coordination regained by saline + ethanol control animals at 60 min post-ethanol. Blood ethanol determinations (Fig. 4) show no significant change in the ethanol clearance in the

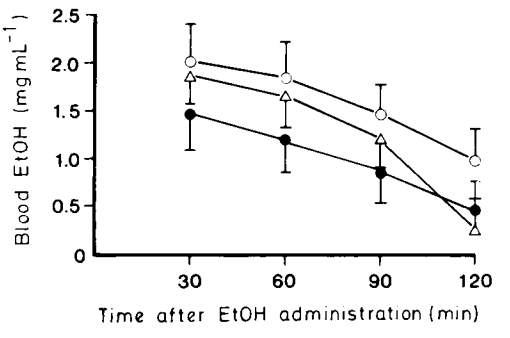

FIG. 4. Blood ethanol concentration after pretreatment with saline and a low and high dose of caffeine. Each point represents mean ± s.e.m. of 8 mice. ○ saline + EtOH 2 g kg^{-1}, ● caffeine 2·5 mg kg^{-1} + EtOH 2 g kg^{-1}, △ caffeine 62·5 mg kg^{-1} + EtOH 2 g kg^{-1}.

caffeine + ethanol and saline + ethanol groups at any time period of determination.

Discussion

The results of the present investigation indicated that caffeine exerts biphasic effects on ethanol-induced motor incoordination. Caffeine has also been reported to exert its biphasic effects on other behavioural responses (Snyder et al 1981; Katims et al 1983; Yanik et al 1987). The biphasic effects were observed (Figs 2, 3) when caffeine pretreatment was given i.c.v. or i.p. and ethanol injected i.p. only. Pretreatment with lower doses of caffeine decreased the degree and the duration of motor incoordination produced by a single test dose of ethanol but at higher doses it enhanced the ethanol-induced motor incoordination. The doses of caffeine when given alone did not alter the motor coordination. Doses of caffeine as low as 2·5 μg given i.c.v. and 2·5 mg kg^{-1} administered i.p. markedly attenuated the motor incoordinating effect of the test dose of ethanol. The antagonism by caffeine of ethanol-induced motor incoordination was dose related at the lower dose range but became unrelated to the dose as the caffeine dose (25 μg i.c.v.; 20 mg kg^{-1} i.p.) was increased (Figs 2, 3, respectively). The effect of further increases in the caffeine dose (75 μg i.c.v.; 62·5 mg kg^{-1} i.p.) on ethanol-induced motor incoordination was qualitatively opposite and resulted in a significant potentiation of it. A still higher caffeine dose (150 μg i.c.v.; 150 mg kg^{-1} i.p.) resulted in practically no effect on ethanol-induced motor incoordination (Figs 2, 3, respectively). The results of the present study also extend our earlier observations (Dar et al 1983; Dar & Wooles 1986) that brain adenosine mechanisms may be a participating factor in the ethanol-induced motor incoordination. However, the biphasic effects of caffeine observed in the present study differ from those observed by others (Snyder et al 1981; Yanik et al 1987) in that the lower doses of caffeine used in their studies did not significantly alter the ethanol-induced motor incoordination (data not shown). The higher doses of caffeine used by the same investigators were well within the range of lower doses of caffeine used in the present study and produced a similar motor stimulant-type effect, i.e. antagonized the motor incoordinating effect of ethanol. Still higher doses produced depressant-type effect, i.e. enhanced the ethanol-induced motor incoordination (Figs 2, 3) and this seems similar to an earlier observation (Waldeck 1974).

The specificity of caffeine as an antagonist of ethanol-induced motor incoordination could also be indirectly suggested by the biphasic nature of its effects on this CNS effect of ethanol. Non-methylxanthine CNS stimulants, such as bicuculline, have also been reported to antagonize the motor impairing effects of ethanol (Hakkinen & Kulonen 1976; Frye & Breese 1982) as well as by this laboratory (Dar & Wooles 1985). However, bicuculline's antagonism of ethanol-induced motor impairment was interpreted by all of those investigators to suggest a central GABA-mediation of this effect of ethanol. Additionally, there was no biphasicity observed in bicuculline's interaction with ethanol because a slightly higher than 3 mg kg^{-1} i.p. dose in CD-1 mice caused seizures, followed in some cases by death (unpublished observations).

The affinity of caffeine and methylxanthines is relatively much greater (20-fold) for the adenosine receptors than for other receptors such as the benzodiazepine binding sites (Marangos et al 1984). Therefore, it would be reasonable to assume that at the very low doses of caffeine, the observed dose-dependent antagonism of ethanol-induced motor incoordination very likely could be due to the blockade of adenosine receptors. As the dose of caffeine was increased (25 μg i.c.v.; 20 mg kg^{-1} i.p.) the non dose-dependent antagonism of ethanol-induced motor incoordination (Figs 2, 3) seem to suggest the additional involvement of yet unknown mechanism(s). At still higher doses (75 μg i.c.v.; 62·5 mg kg^{-1} i.p.) caffeine may be acting mainly via mechanism(s) not involving adenosine receptors. Obviously, the observed potentiation of ethanol-induced motor incoordination by caffeine which was qualitatively the opposite effect compared with that produced by lower caffeine doses (<20 mg kg^{-1} i.p.; <25 μg i.c.v.) cannot be explained based on blockade of presently known adenosine receptor subtypes (A_1 and A_2). The existence of biphasic effects of caffeine on ethanol-induced motor incoordination as observed in the present study as well as on other behavioural effects such as locomotor activity (Snyder et al 1981; Katims et al 1983) and sleep (Yanik et al 1987) may represent an example of an exception to the correlation between affinity for adenosine receptors and these behavioural effects. It is logical to assume that the biphasic effects of caffeine on ethanol-induced motor incoordination may be mediated via distinct mechanisms.

Caffeine and other methylxanthines are well known to inhibit phosphodiesterase, resulting in the accumulation of cAMP and the subsequent actions of cAMP on the neural membranes to produce an excitatory response which could antagonize the motor incoordinating effect of ethanol. However, as stated in the introduction section, this cannot explain the results of the present investigation because a higher concentration (usually in millimolar range) of caffeine than used in the present study is needed to inhibit phosphodiesterase enzyme. The lowest dose, 2·5 mg kg^{-1} (equivalent to 21 μM caffeine concentration in brain, if uniformly distributed) though below k_i(s) for caffeine at the A_1 and A_2 sites was still in the right range. The most effective dose in antagonizing ethanol-induced motor incoordination, 5 mg kg^{-1} (equivalent to 42 μM caffeine concentration in brain) was very much in range of K_i(s) for caffeine and so very likely involved the blockade of adenosine receptors.

The data from the present investigation also lend support to the popular belief that coffee or tea can help sober up a drunk person and that caffeine antagonizes the CNS depressant effects of ethanol. However, the biphasic nature of the effect of caffeine on ethanol-induced motor incoordination points to the importance of the dose of caffeine used or the amount of coffee or tea consumed to antagonize the CNS depressant effects of ethanol and perhaps this may also be the basis of confusion in the literature in this regard. The i.p. and i.c.v. dose of caffeine based on the present investigation should be below 20 mg kg^{-1} and 25 μg, respectively, to observe its optimal antagonistic effect on motor incoordination produced by ethanol.

The biphasic effects of caffeine on ethanol-induced motor incoordination, in the present investigation, as well as on locomotor behavior (Snyder et al 1981; Katims et al 1983), and sleep (Yanik et al 1987) could also be explained by differences in the sensitivity of subtypes of adenosine A_1 and A_2 receptors. It has been reported that the high and the low affinity subtypes of adenosine A_2 receptors are antagonized by caffeine with K_i values of 30 and 27 μM, respectively (Daly et al 1983). However, it antagonized the high affinity A_1 receptors with a higher K_i of 50 μM (Daly et al 1983). The biphasic effects of caffeine, thus could very well be due to differential sensitivity of a heterogeneous population of subtypes of adenosine receptors that mediate the various behavioural and receptor actions of adenosine (Coffin et al 1984). To be compatible with this explanation our data suggest that in the lower dose range (<20 mg kg^{-1} i.p.; <25 μg i.c.v.) caffeine may have greater affinity with those subtypes of A_1/A_2 adenosine receptors that mediate the depressant effects of adenosine such as motor incoordination (ataxia) produced by adenosine and its analogues. At higher caffeine doses (62·5 mg kg^{-1} i.p.; 75 μg i.c.v.) it may have greater affinity with the subtypes of A_1/A_2 adenosine receptors that mediate excitatory effects of adenosine. Still higher doses of caffeine may involve receptor mechanisms in addition to and/or other than adenosine. The convulsive doses of caffeine and theophylline have been suggested possibly to involve antagonism of the benzodiazepine receptor subtype which mediates the anticonvulsive effects of benzodiazepines (Marangos et al 1984). Even the highest caffeine dose (62·5 mg kg^{-1}) used in the present studies (equivalent to 525 μM caffeine concentration in brain, if uniformly distributed) appeared to be below the range (mM) which is known to inhibit benzodiazepine receptors as well as cause little or no inhibition of phosphodiesterase enzyme.

Finally, blood ethanol data suggest no significant effect of caffeine pretreatment on the clearance of ethanol (Fig. 4). There were no significant differences in blood ethanol levels in saline- and caffeine-pretreated animals. Thus the antagonism by low dose and potentiation by a high dose of caffeine, respectively, of ethanol-induced motor incoordination was due to mechanisms other than the alteration in the clearance of ethanol.

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