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The Role of the Purinergic System in the Control of Stereotypy: Relationship to D-1/D-2 Dopamine Receptor Activity

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POPOLI, P., M. G. CAPORALI AND A. SCOTTI DE CAROLIS. *The role of the purinergic system in the control of stereotypy: Relationship to D-1/D-2 dopamine receptor activity.* PHARMACOL BIOCHEM BEHAV 32(1) 203–206, 1989.—A behavioral study on the stereotypy induced by caffeine and carbamazepine or caffeine and haloperidol was assessed in adult male rabbits. The stereotypy induced by caffeine + carbamazepine was not reduced by pretreatment with haloperidol (0.1 mg/kg) or SCH 23390 (0.01 mg/kg). N-ethylcarboxamidoadenosine (NECA, 0.01 mg/kg), an A_2 adenosine receptor agonist, completely prevented the appearance of caffeine + carbamazepine-, but not of caffeine + haloperidol-induced stereotypy. An EEG investigation was also performed in order to evaluate the influence of the blockade of D-1 and D-2 dopamine receptors on the desynchronized tracing induced by caffeine (50 mg/kg). Neither haloperidol (0.1 mg/kg) nor SCH 23390 (0.01 mg/kg) were able to influence this EEG effect of caffeine. Present data support the hypothesis that A_2 adenosine receptors may be involved in the control of pathological movements. The relationship between the purinergic system and D-1/D-2 dopamine receptors is also discussed.

A_2 Receptors D-1/D-2 Receptors Stereotyped behavior

CAFFEINE has many stimulant effects on the CNS (12). In rabbits, a dose of 50 mg/kg IV causes cortical desynchronization associated with synchronous theta waves in the hippocampus (5–6 Hz; 200–500 μV) (18). This picture lasts 45–60 min; behaviorally, the animals show mydriasis and excitation (6). The excitatory effects of caffeine have been attributed to an antagonism of the adenosine receptors (3, 9, 10).

Carbamazepine is a tricyclic anticonvulsant currently used to treat clinical seizures. The mechanism of the anticonvulsant action of carbamazepine is still unknown. It has been suggested that this drug may act *via* an influence on the purinergic receptors. The nature of carbamazepine interactions at the A_1 receptors, occurring at therapeutic doses, is not clearly assessed. At high doses, carbamazepine seems to act as an A_2 adenosine receptor antagonist (23).

It has been reported that both caffeine and carbamazepine have dopaminergic actions (4,22). However, in a previous paper (6), we demonstrated that the combined administration of caffeine (50 mg/kg IV) and carbamazepine (20 mg/kg IV) produces a stereotyped behavior in rabbits. This effect cannot be attributed to D-2 dopaminergic stimulation because pretreatment with haloperidol (0.1 mg/kg IV) does not prevent stereotyped behavior.

As both caffeine and carbamazepine have an antagonistic activity on the A_2 adenosine receptors, we have hypothesized that A_2 receptors may play a role in the control of stereotyped behavior. Our hypothesis is in agreement with the data of Green *et al.* (14), suggesting that endogenous adenosine may exert a tonic inhibition on the dopaminergic system, and with those of Spealmann and Coffin (21), demonstrating the importance of A_2 adenosine receptors in the control of motility.

We reported that the combined administration of haloperidol (0.1 mg/kg IV) and caffeine (50 mg/kg IV) is also able to induce stereotyped behavior (6). Recently, the importance of D-1 dopamine receptors in dopamine-dependent behaviors has been emphasized. D-1 receptors may play a role in "abnormal perioral movements" (1,20). Several authors suggested a close connection between D-1 and D-2 receptors involved in motor functions (5,19).

In the present work, we have further investigated the role of A_2 adenosine receptors on the stereotyped behavior induced by the combination of caffeine and carbamazepine in rabbits. An EEG investigation was also carried out in order to evaluate whether a blockade of dopamine receptors could modify the desynchronized tracing induced by caffeine (6).

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The influence of the blockade of dopamine receptors on caffeine + carbamazepine-induced stereotypy has been further studied in order to ascertain the role of D-1 and D-2 dopamine receptors. The relationship between dopaminergic and purinergic systems in the genesis of abnormal movements has also been investigated.

METHOD

Experimental Procedure

Experiments were carried out on male adult rabbits weighing 2.2–2.5 kg. Electrodes were implanted, under local anaesthesia (2% xylocaine), on the skull of the frontal, parietal, and occipital cortices of both hemispheres. A lateral ear vein was cannulated for drug administration. The animals were then partially restrained and accommodated in the recording room, which was electrically-shielded and sound-attenuated. Experiments were started one hour later. The EEG was recorded for 60 min from the last drug injection. Evaluation of the EEG recordings was made using standard criteria for recognition of periods of desynchronization as evidenced by low-amplitude fast-frequency waves in the cortical leads (16). The time spent in the desynchronized activity was calculated for periods of 15 min over the 60-min test.

Stereotyped Behavior

Behavior was systematically observed during the experiments and the intensity of stereotypy was scored at 15-min intervals over the 60-min test. The intensity was scored according to the Costall and Naylor scale (7): 0) no stereotypy; 1) periodic sniffing; 2) continuous sniffing; 3) periodic licking or biting; 4) continuous licking or biting. Individual data were the mean score from the four periods of observation of each animal.

Drugs

Caffeine sodium benzoate (Sigma Chemical Company, USA) and N^6-L-phenylisopropyladenosine (L-PIA, Sigma) were dissolved in distilled water. SCH 23390 hemimaleate (Schering-Plough Corp., USA) was dissolved in 0.1% tartaric acid. N-ethyl-carboxamidoadenosine (NECA, Sigma) was dissolved in saline. Carbamazepine (Ciba-Geigy) was dissolved in a mixture of 40 parts of polyethyleneglycol and 60 parts of water. The commercial injectable preparation of haloperidol (Serenase, Lusofarmaco, Italy) was used. Drug doses refer to the weight of the base. All drugs were administered intravenously.

Data Analysis

Student's *t*-test and Mann-Whitney U-test were used to assess the statistical significance of the results.

RESULTS

EEG

In a previous paper (6) we demonstrated that caffeine (50 mg/kg IV) induces a significant EEG desynchronization (45.6–60 min in mean) which is not reduced by pretreatment (10 minutes before) with haloperidol (0.1 mg/kg IV) or CBZ (20 mg/kg IV). As shown in Fig. 1, pretreatment (10 minutes before) with SCH 23390 (0.01 mg/kg), A D-1 dopamine receptor antagonist, is also unable to influence the duration of the caffeine-induced EEG desynchronization. The doses of haloperidol and SCH 23390 used have been shown to have a clear antagonistic activity at the level of the dopamine receptors (D-2 and D-1 respectively), but no significant effect on the EEG patterns as compared to controls (17). On the contrary, N-ethylcarboxyamido-adenosine (NECA, 0.25 mg/kg), an adenosine receptor agonist, is able to significantly reduce the duration of the caffeine-induced EEG effect (Fig. 1). The dose of NECA used does not significantly influence the mean EEG desynchronization with respect to the control (saline).

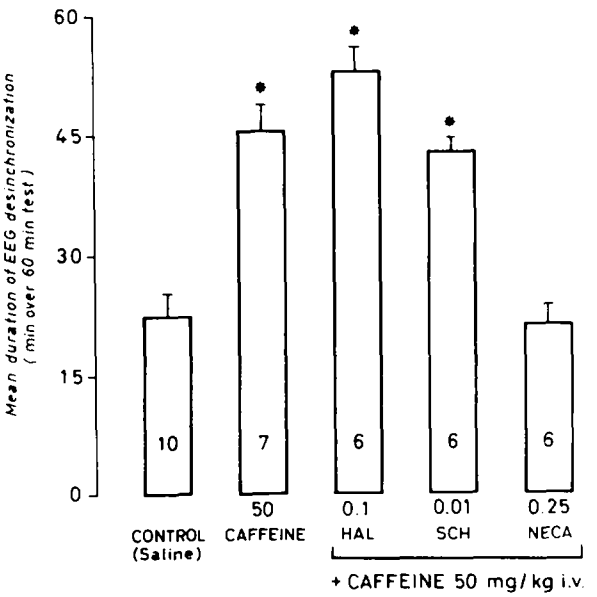

FIG. 1. Mean duration of EEG desynchronization induced by caffeine alone or in combination with haloperidol, SCH 23390, and NECA in rabbits. The mean of the time spent in desynchronized activity was calculated for periods of 15 minutes over the 60 minute test period. The numbers of the bars indicate the number of animals in each experiment. Doses are expressed in mg/kg IV. *Differs from saline at $p<0.01$ according to Student's *t*-test.

Behavior

Caffeine + carbamazepine-induced stereotypy. The stereotyped behavior (sniffing, licking, chewing) induced by caffeine + carbamazepine (50 and 20 mg/kg respectively) is not reduced by pretreatment (10 minutes before) with haloperidol (0.1 mg/kg) (6). As shown in Table 1, SCH 23390 (0.01 mg/kg, 10 minutes before) is also unable to affect the caffeine + carbamazepine-induced stereotypy. Conversely, pretreatment with NECA (0.01 mg/kg) completely prevents the appearance of this behavior.

Caffeine + haloperidol-induced stereotypy. The combined administration of caffeine and haloperidol (50 and 0.1 mg/kg respectively) induces a stereotyped behavior closely similar to the kind of stereotypy described above [see Caporali *et al.* (6)]. This effect is completely reverted by pretreatment (10 minutes before) with SCH 23390 (0.01 mg/kg). On the contrary, NECA (0.01 mg/kg), administrered 10 minutes before the injection of caffeine and haloperidol, does not significantly affect the appearance of stereotyped behavior (see Table 2). In a separate series of experiments, the influence of the combined treatment with caffeine and SCH 23390 (50 and 0.01 mg/kg respectively) on behavior has been studied. Rabbits treated with these two drugs do not show a significant stereotyped behavior (see Table 2).

TABLE 1

EFFECTS OF HALOPERIDOL, SCH 23390, AND NECA ON THE STEREOTYPED BEHAVIOR INDUCED BY CARBAMAZEPINE + CAFFEINE IN RABBITS

Treatment (IV)	Doses (mg/kg)	Number of Animals	Stereotypy Mean Intensity (±SEM)
Saline		10	0
Caffeine	50	7	0.5 ± 0.1
Carbamazepine	20	7	0
Haloperidol	0.1	7	0
NECA	0.01	6	0
Carbamazepine + Caffeine	20 + 50	6	5.3 ± 0.8*
Haloperidol + Carbamazepine + Caffeine	0.1 + 20 + 50	6	14.3 ± 3.2†
SCH 23390 + Carbamazepine + Caffeine	0.01 + 20 + 50	6	5.9 ± 0.9*
NECA + Carbamazepine + Caffeine	0.01 + 20 + 50	6	0.4 ± 0.2

Intensity is represented as mean of scores.
*Differs from caffeine at $p<0.01$; †at $p<0.005$ according to Mann-Whitney U-test.

TABLE 2

INFLUENCE OF CAFFEINE + HALOPERIDOL AND/OR SCH 23390 ON THE BEHAVIOR OF RABBITS

Treatment (IV)	Doses (mg/kg)	Number of Animals	Stereotypy Mean Intensity (±SEM)
Caffeine	50	7	0.5 ± 0.1
Haloperidol + Caffeine	0.1 + 50	6	9.8 ± 1.1*
NECA + Haloperidol + Caffeine	0.01 + 0.1 + 50	6	9.6 ± 1.8*
SCH 23390 + Caffeine	0.01 + 50	6	2.6 ± 2.0
SCH + Haloperidol + Caffeine	0.01 + 0.1 + 50	6	0.4 ± 0.2

Intensity is represented as mean of scores.
*Differs from caffeine at $p<0.005$ according to Mann-Whitney U-test.

DISCUSSION

Present experiments confirm and extend our preliminary data on the importance of the purinergic modulation of motor behavior (6). Our EEG study shows that the EEG desynchronization induced by caffeine cannot be attributed to a direct action of this drug on D-1 or D-2 dopamine receptors. In fact, the pretreatment with D-2 (haloperidol) or with D-1 (SCH 23390) dopamine receptor antagonists does not reduce the duration of the caffeine-induced EEG desynchronization. Obviously, this EEG effect of caffeine is prevented by the administration of an adenosine receptor agonist (NECA).

In agreement with these data, our behavioral investigation demonstrates that caffeine + carbamazepine-induced stereotypy cannot be explained by an interaction of these two drugs on dopamine receptors. Both haloperidol and SCH 23390, in fact, do not prevent the appearance of sniffing, licking or chewing. These results agree with those of Watanabe and Uramoto (22), who found that caffeine mimics dopamine receptor agonists without stimulation of dopamine receptors, and with those of Barros and Leite (4), suggesting that CBZ potentiates the apomorphine-induced stereotypy without directly acting on the dopaminergic system. The fact that haloperidol enhances the caffeine + carbamazepine-induced stereotypy is not surprising. In fact, the administra-

tion of haloperidol and caffeine in itself evokes a clear stereotyped behavior.

The finding that the stereotyped behavior induced by caffeine + carbamazepine is completely prevented by pretreatment with NECA confirms our hypothesis, suggesting that caffeine + carbamazepine may induce pathological movements *via* a blockade of A_2 adenosine receptors. This is also in agreement with the data of Spealmann and Coffin (21) suggesting that stimulation of A_2 adenosine receptors may exert an inhibition on motility.

Previously, Green *et al.* (14) demonstrated that local injection of NECA in the nucleus caudatus of rats inhibits apomorphine-induced effects, suggesting that adenosine might be involved in the modulation of dopaminergic function in the striatum. Thus, our data, as well as much of the data in literature, indicate that the purinergic system may play an important role in the control of movements.

The finding that the combined administration of haloperidol and caffeine induces stereotypy cannot be explained by a blocking action of haloperidol on A_2 adenosine receptors, since pretreatment with NECA does not prevent the appearance of stereotypy. On the other hand, the combined administration of caffeine and SCH 23390 does not induce stereotyped behavior. Thus, stereotypy should appear after a strong blockade of A_2 adenosine receptors or after a combined blockade of both A_2 and D-2 receptors. On the contrary, stereotyped behavior does not appear when A_2 and D-1 receptors are simultaneously blocked. Interestingly enough, the stereotypy induced by caffeine + haloperidol is prevented by SCH 23390. Probably, D-1 receptors may be under tonic D-2 and A_2 inhibition, and the combined blockade of the A_2 (caffeine) and D-2 (haloperidol) receptors may enable the D-1-mediated responses. This hypothesis may appear surprising in light of the fact that DA-dependent behaviors have been traditionally attributed to a stimulation of D-2 dopamine receptors (8, 13, 15). Nevertheless, several investigations have demonstrated a close connection between D-1 and D-2 receptors in the behavioral effects of dopamine. D-1 and D-2 receptors appear, in some cases, to exert synergistic behavioral activation (2, 11, 24), and it has been suggested that D-1 receptors may play an "obligatory" role in mediating all dopamine-dependent behaviors (2).

In conclusion, our results seem to indicate that A_2 adenosine receptors control the expression of the dopaminergic behavior *via* an inhibition of dopamine receptors.

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