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Purine nucleoside – mediated immobility in mice: Reversal by antidepressants

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Abstract. In the forced swimming-induced immobility (despair) test model, adenosine, and 2-chloroadenosine treatment prolonged the immobilization period in mice. Dipyridamole, which is known to inhibit adenosine uptake, potentiated the adenosine effect. The purinoceptor antagonists caffeine and theophylline blocked purine nucleoside-induced enhancement of immobilization. Tricyclic antidepressants such as imipramine and desipramine, the MAO inhibitor tranylcypromine, and amphetamine, a psychostimulant, reversed purine nucleoside-induced immobility. On the other hand, quipazine, fluoxetine, and amitriptyline failed to reverse purine nucleosides-induced prolongation of immobility. None of the antidepressants in the doses investigated had any effect by themselves.

Reserpine also prolonged forced swimming-induced immobility in mice. The antidepressants fluoxetine and quipazine, but not methylxanthine pretreatment, reversed reserpine-induced immobility in this test model. These results indicate that adenosine and 2-chloroadenosine probably reduce norepinephrine outflow through their action on presynaptic purinoceptors on noradrenergic neurons and thereby cause prolongation of immobility in animals.

Key words: Forced swimming test – Adenosine – 2-Chloroadenosine – Purinoceptors – Antidepressants – Mice

Mice or rats, when forced to swim in a restricted space from which they cannot escape, after an initial period of vigorous activity become immobile, apart from small movements necessary to keep their heads above water. Porsolt et al. (1977a) suggested that the observed immobile behaviour (despair) resembled a state of depression, and can serve as a model for screening antidepressants (Porsolt et al. 1977a, b; 1978a, b). However, a variety of CNS active drugs and antidepressants have recently been shown to be effective in this model (Nagatani et al. 1984; Natan et al. 1984; Porsolt et al. 1979; Schechter and Chance 1979).

Adenosine and related nucleosides are reported to have a neuromodulator or neurotransmitter role (see Su 1983; Kulkarni and Mehta 1983, 1984b). These agents possessed sedative (Haulica et al. 1973; Dunwiddie and Worth 1982), anticonvulsant (Dunwiddie and Worth 1982), analgesic (Holmgren et al. 1983) and hypothermic activities (Mehta and Kulkarni 1983). However, the exact functional role of these nucleosides in the CNS is yet to be established. In the present study, the effect of purine nucleosides and its modification by antidepressants on forced swimming-induced immobility were investigated in mice. These results indicated that adenosine and 2-chloroadenosine, through their probable action on presynaptic purinoceptors on adrenergic nerve terminals, reduce norepinephrine outflow and thereby cause "beavioural despair" in mice.

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Materials and methods

Animals. Wistar mice (PGI, Chandigarh) of either sex (20–25 g) were used. They were acclimatized to the laboratory conditions preceding an experiment. They had free access to feed and water.

Induction and measurement of immobility. Mice were individually forced to swim inside a glass jar (25 × 12 × 25 cm) containing 15 cm water maintained at 23–25° C. The total duration of immobility was measured during a 6-min test. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water making only those movements necessary to keep its head above water.

Drugs. The following drugs were used: Adenosine (Loba-Chemie Wien-Fischamend), 2-chloroadenosine (Sigma), dipyridamole (C. H. Boehringer Sohn, Ingelheim am Rhein), caffeine (C. H. Boehringer Sohn, Ingelheim am Rhein), theophylline (Sigma), imipramine HCl (Ciba-Geigy), desipramine HCl (Ciba-Geigy), tranylcypromine sulphate (SK & F), amphetamine sulphate (SK & F), quipazine maleate (Miles Laboratories, Inc.), fluoxetine HCl (Eli Lilly & Co), amitriptyline (MSD), reserpine (Loba-Chemie Wien-Fischamend) and diazepam (Ranbaxy Laboratories).

Drugs administration. Drugs were dissolved in 0.9% w/v NaCl or, if insoluble, dispersed in a suspension of carboxymethyl cellulose (0.2% w/v in 0.9% w/v NaCl). All drugs were injected IP in a constant volume of 1 ml/100 g. Adenosine and 2-chloroadenosine were administered 15 min before the test, whereas reserpine was administered 5 h before the test. The other drugs were administered 30 min prior to purine nucleosides or after 4.5 h of reserpine treatment. Control animals received only vehicle. For the drug-purine nucleoside interaction study, adenosine and 2-chloroadenosine were employed in doses of 100 mg/kg and 2 mg/kg, respectively.

Statistics. The results, expressed as means ± SEM, were compared to the respective controls using the Mann-Whitney two-tailed test.

Results

Adenosine, as well as 2-chloroadenosine, prolonged the duration of immobility in mice in a dose-dependent manner (Table 1). Dipyridamole (5 mg/kg) did not have any effect by itself on immobility duration (Table 2) but potentiated the adenosine effect (Table 3). Caffeine (8 mg/kg) and theophylline (8 mg/kg) reversed the prolonged duration of immobility due to adenosine or 2-chloroadenosine treatment (Table 3) without having any effect alone (Table 2). However, these drugs failed to modify reserpine-induced enhancement of immobility (Table 5). Furthermore, pretreatment with imipramine, desipramine, tranylcypromine, and amphetamine reduced the enhancement of the immobilization period by purine nucleosides (Table 4) or reserpine (Table 5). On the other hand, quipazine (5 mg/kg), fluoxetine (5 mg/kg) and amitriptyline (5 mg/kg), although able to reverse the reserpine-induced increase in the immobility period (Table 5), failed to modify the effect of purine nucleosides on immobilization in mice (Table 4). None of the antidepressant drugs, at the doses employed, showed any effect by themselves (Table 2).

Table 1. Effect of adenosine and 2-chloroadenosine upon the total duration of immobility during the 6-min test

Treatment	Dose (mg/kg, IP)	*n*	Duration of immobility s ± SEM	*P**
Control	–	25	176.4 ± 5.96	
Adenosine	25	8	206.0 ± 7.92	< 0.01
	50	10	235.2 ± 8.74	< 0.01
	100	14	268.4 ± 7.72	< 0.01
2-Chloroadenosine	1	6	225.0 ± 9.15	< 0.01
	2	10	278.4 ± 3.68	< 0.01
	5	8	292.2 ± 4.87	< 0.01

* As compared to control group

Table 2. Effect of various drugs when administered alone upon the total duration of immobility during the 6-min test

Treatment	Dose (mg/kg, IP)	*n*	Duration of immobility s ± SEM	*P**
Control	–	8	166.5 ± 8.58	
Dipyridamole	5	6	178.0 ± 5.35	NS
Caffeine	8	5	169.2 ± 13.51	NS
Theophylline	8	5	165.0 ± 9.87	NS
Imipramine	5	6	180.5 ± 7.74	NS
Desipramine	5	6	171.8 ± 12.23	NS
Tranylcypromine	4	6	176.4 ± 5.68	NS
Amphetamine	1	6	163.0 ± 4.35	NS
Amitriptyline	5	6	168.5 ± 6.52	NS
Quipazine	5	6	172.0 ± 10.48	NS
Fluoxetine	5	6	164.8 ± 6.48	NS

* As compared to control group
NS: Not significant

Table 3. Modification by caffeine, theophylline, and dipyridamole of adenosine and 2-chloroadenosine-induced prolongation of immobility during the 6-min test

Treatment	Dose (mg/kg, IP)	*n*	Duration of immobility s ± SEM	*P**
Control	–	10	172.6 ± 8.14	
Adenosine	100	6	257.5 ± 6.38	< 0.01[a]
2-Chloroadenosine	2	6	282.5 ± 4.97	< 0.01[a]
Dipyridamole + adenosine	5 100	6	290.2 ± 4.43	< 0.02[b]
Caffeine + adenosine	8 100	6	180.2 ± 9.47	< 0.01[b]
Theophylline + adenosine	8 100	6	183.0 ± 11.89	< 0.01[b]
Caffeine + 2-chloroadenosine	8 2	6	178.1 ± 8.12	< 0.01[c]
Theophylline + 2-chloroadenosine	8 2	6	170.5 ± 6.87	< 0.01[c]

* As compared to (a) control, (b) adenosine, and (c) 2-chloroadenosine treatments, respectively

Table 4. Effect of various antidepressants upon adenosine- and 2-chloroadenosine-induced prolongation of immobility during the 6-min test

Treatment	Dose (mg/kg, IP)	*n*	Duration of immobility s ± SEM	*P**
Control	–	10	180.2 ± 5.47	
Adenosine	100	7	263.4 ± 8.96	< 0.01[a]
2-Chloroadenosine	2	8	285.0 ± 6.68	< 0.01[a]
Imipramine + adenosine	5 100	7	202.5 ± 6.09	< 0.01[b]
Desipramine + adenosine	5 100	8	193.2 ± 10.78	< 0.01[b]
Tranylcypromine + adenosine	4 100	8	198.6 ± 4.87	< 0.01[b]
Amphetamine + adenosine	1 100	6	205.5 ± 7.40	< 0.01[b]
Amitriptyline + adenosine	5 100	6	251.5 ± 11.48	NS[b]
Quipazine + adenosine	5 100	6	259.0 ± 4.50	NS[b]
Fluoxetine + adenosine	5 100	8	256.2 ± 6.40	NS[b]
Imipramine + 2-chloroadenosine	5 2	6	195.1 ± 5.82	< 0.01[c]
Desipramine + 2-chloroadenosine	5 2	6	188.5 ± 3.19	< 0.01[c]
Tranylcypromine + 2-chloroadenosine	4 2	6	199.5 ± 8.34	< 0.01[c]
Amphetamine + 2-chloroadenosine	1 2	6	208.0 ± 3.18	< 0.01[c]
Amitriptyline + 2-chloroadenosine	5 2	6	270.2 ± 12.69	NS[c]
Quipazine + 2-chloroadenosine	5 2	8	281.2 ± 7.17	NS[c]
Fluoxetine + 2-chloroadenosine	5 2	8	287.0 ± 5.25	NS[c]

* As compared to (a) control, (b) adenosine, and (c) 2-chloroadenosine treatments, respectively
NS: Not significant

Table 5. Effect of purinoceptor antagonists and antidepressants upon reserpine-induced prolongation of immobility during the 6-min test

Treatment	Dose (mg/kg, IP)	*n*	Duration of immobility s ± SEM	*P**
Control	–	8	169.5 ± 12.47	
Reserpine	2	7	317.4 ± 9.35	< 0.01[a]
Caffeine + reserpine	8 2	6	302.0 ± 8.79	NS[b]
Theophylline + reserpine	8 2	6	306.3 ± 11.05	NS[b]
Imipramine + reserpine	5 2	6	230.2 ± 4.04	< 0.01[b]
Desipramine + reserpine	5 2	8	213.4 ± 2.42	< 0.01[b]
Tranylcypromine + reserpine	4 2	6	239.5 ± 6.78	< 0.01[b]
Amphetamine + reserpine	1 2	6	269.0 ± 10.65	< 0.01[b]
Amitriptyline + reserpine	5 2	6	282.2 ± 6.79	< 0.05[b]
Quipazine + reserpine	5 2	6	265.8 ± 9.48	< 0.01[b]
Fluoxetine + reserpine	5 2	6	276.5 ± 10.86	< 0.05[b]

* As compared to (a) control, and (b) reserpine treatment, respectively
NS: Not significant

Discussion

The functional role of adenosine and related nucleosides and nucleotides in the CNS is being actively pursued. Attempts have been made to correlate the physiological and pharmacological actions observed as the peripheral (Mustafa 1980; Su 1977) and central (Daly et al. 1981; Stone 1981) specific receptor-mediated actions of purine nucleosides. Several drug actions (see Kulkarni and Mehta 1983, 1984a, b; Mehta and Kulkarni 1984; Mustafa 1979) have been explained in relation to purinergic substances.

Forced swimming-induced immobility is thought to be a depressed state, and various CNS active agents (Nagatani et al. 1984; Natan et al. 1984; Porsolt et al. 1979; Schechter and Chance 1979) and antidepressants (Porsolt et al. 1977a, b; 1978a, b) are reported to reverse this behaviour. We have also further demonstrated that diazepam (2 mg/kg) had no effect on swimming behaviour. However, a higher dose (5 mg/kg) prolonged immobility (243.0 ± 6.80 as compared to 182.2 ± 4.91 of control), probably through its muscle relaxant property, and was not reversed by antidepressants. Unlike the earlier reports in rats (Porsolt et al. 1979) and mice (Nagatani et al. 1984), reserpine significantly enhanced the period of immobility in our study. This discrepancy was probably due to species variation or the time elapsing after reserpine treatment before testing, as Nagatani et al. (1984) tested the animals just 1 h post reserpine treatment. Both the antidepressants, acting through noradrenergic or serotonergic mechanisms, and the serotonergic agents reversed reserpine-induced immobility, suggesting the involvement of both of these transmitters in reserpine-induced immobility in the behavioural despair model. This is in accordance with the fact that reserpine is known to deplete both noradrenaline and serotonin (Carlsson et al. 1957).

In contrast to reserpine-induced prolongation of immobility, adenosine- and 2-chloroadenosine-induced enhancement of immobility had a rapid onset, and was not reversed by quipazine, a 5-HT receptor agonist (Hong et al. 1969), fluoxetine, a 5-HT uptake inhibitor (Fuller and Wong 1977) or amitriptyline, which mainly inhibits the uptake of 5-HT. However, tricyclic antidepressants, tranylcypromine, and amphetamine pretreatment reversed the prolongation of immobility due to adenosine or 2-chloroadenosine. This indicates a noradrenergic, but not a serotonergic mediation of adenosine- and 2-chloroadenosine-induced enhancement of immobility in the behavioural despair test. Moreover, dipyridamole, an inhibitor of the uptake (Alfonso and O'Brien 1971; Hopkins 1973; Kalsner 1975) and metabolism (Klabunde 1983) of adenosine, potentiated the adenosine effect, whereas purinoceptor antagonists such as caffeine and theophylline, although failing to modify the reserpine effect, reversed the prolongation of immobility caused by adenosine or 2-chloroadenosine. These observations suggested that modulation of the noradrenergic system through a specific purinoceptor could be the mechanism involved in purine nucleoside-induced prolongation of immobility in mice. It is speculated that purine nucleosides presynaptically exert inhibition of transmitter release from noradrenergic nerve terminals (Clanachan et al. 1977; Paton 1981; Su 1981) thereby producing an enhancement of behavioural immobility in mice in the forced swimming test.

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