Pharmacology Biochemistry and Behavior, Vol. 42, pp. 431–435, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Effects of Lithium and Purinergic Compounds on the Behavioral and Physiological Aspects of Restraint Stress in Rats

ITSUKO USHIJIMA,[1] YASUSHI MIZUKI, TAKAHIDE HARA, NAMI OBARA, NORIO MINEMATSU AND MICHIO YAMADA

Department of Neuropsychiatry, Yamaguchi University School of Medicine, 1144 Kogushi, Ube 755, Japan

Received 9 December 1991

USHIJIMA, I., Y. MIZUKI, T. HARA, N. OBARA, N. MINEMATSU AND M. YAMADA. *Effects of lithium and purinergic compounds on the behavioral and physiological aspects of restraint stress in rats.* PHARMACOL BIOCHEM BEHAV **42**(3) 431–435, 1992.—This study investigates the effects of lithium and caffeine on psychomotor activities, defecation, and gastric lesions induced by restraint stress. Rats exposed to restraint stress typically exhibited a biphasic response consisting of an initial hypermotility (such as tail-flipping, body-rolling, jaw movement, and vocalization) accompanied by defecation, and followed by hypomotility (decrease in motility) accompanied by gastric ulceration. Lithium chloride (150 μg, ICV; 50 and 100 mg/kg, IP) significantly attenuated these responses while N^6-cyclohexyl adenosine (CHA; 1.5 μg, ICV; 0.3 mg/kg, IP), a potent adenosine A_1 receptor agonist, attenuated the behavioral effects but potentiated the gastric ulceration. Caffeine (3 μg, ICV; 1.0 mg/kg, IP), an adenosine receptor antagonist, inhibited the effects of CHA in animals exposed to 3 h of stress, but aggravated the effects in animals exposed to 6–12 h of stress. These results suggest that caffeine consumption may produce supersensitivity of adenosine receptors, which potentiate the actions of adenosine or CHA. Lithium may modulate the effects of stress by indirectly inhibiting central adenosine receptor activity.

Stress Lithium Caffeine Ulceration N^6-cyclohexyladenosine Psychomotility

SEVERAL theories have been proposed to account for the therapeutic efficacy of lithium in manic-depressive disorders (22), which are generally characterized by hyperaggressiveness (24). In humans and animals, lithium salts have been reported to exert an inhibitory effect on aggressive behavior induced by stress (7,23). Various stressors have been shown to be associated with stress-related diseases such as gastric/duodenal ulcers, hypertension, cardiovascular/cerebrovascular disease (15), and affective disorders such as depression (2).

Adenosine potentiates stress-induced gastric lesions. When administered either systemically or centrally, adenosine A_1 receptor agonists such as N^6-cyclohexyl adenosine (CHA) and N^6-(*L*-phenylisopropyl) adenosine (L-PIA) produce gastric lesions even in nonstressed animals (28,29). Furthermore, Anderson et al. (1) reported that [^{3}H]CHA binding to A_1 receptors increases in hypothalamic membrane preparations from stressed rats. From these data, one may conclude that he stimulation of central adenosine A_1 receptors may be a causative factor of stress-induced gastric lesions. On the other hand, hypermotility and defecation observed during stress were attenuated by morphine (11,27), adenosine, adenosine analogs, benzodiazepines, and GABA agonist (28). The present studies were carried out to examine how lithium influenced stress-evoked hypermotility and gastric ulceration, and whether it acted via central purinergic systems.

METHOD

Animals

Healthy, male, adult Wistar rats, 90–100 days of age (weighing 300–350 g) were obtained from Kyudo Animal Laboratory (Saga, Japan) and maintained in an animal room with a 12 L:12 D cycle (lights on 7:00 a.m.–7:00 p.m.). Commercial food (Oriental Japan Ltd., Fukuoka, Japan) and tap water were available ad lib except during the time of the experiments. All experiments were carried out at an environmental temperature of 23 ± 1°C.

Procedure for Restraint Stress and Measurement of Its Physiological Effects

Restraint stress was produced by enclosing rats in a flexible wire mesh (3 × 3 mm) cone that had been bent to conform to the size of the individual animals. Rats restrained in this man-

[1] To whom requests for reprints should be addressed.

ner were placed on a plastic platform and their behaviors were observed. They were assessed for the presence of hypermotility and defecation. Hypermotility was rated according to the following scale: 1, jaw movement, body-rolling, or tail-flipping; 2, jaw movement and body-rolling, jaw movement and tail-flipping, or body-rolling and tail-flipping; 3, simultaneous jaw movement, body-rolling, and tail-flipping; 4, simultaneous jaw movement, body-rolling, tail-flipping, and vocalization. Stress-induced responses were measured during each 0-1, 1-2, 3-4, 5-6, or 11-12 h after ICV injection of drugs (Fig. 1). However, in the IP drug injection studies the cumulative scores attained during the first 3 h of stress exposure after drug administration were used (Table 1). Scores of these responses were counted manually by a different observer for each rat. The observers were blind with respect to treatment. The total number of fecal pellets deposited during the 3-h stress period was also used as the index of defecation (Table 1).

Measurement of Gastric Lesions

The stomach was opened along the greater curvature and pinned on a cork board and fixed in 10% formalin solution. The sum of the area (mm^2) of hemorrhagic ulcerative lesions in the gastric mucosa was measured by a particle analyzer (Luzex 450, Toyo Ink MFG, Co., Ltd., Tokyo, Japan), an image analyzing system to autoanalyze characteristic lesion values.

Administration of Drugs

To observe drug effects on stress-evoked hypermotility and gastric lesions, we exposed rats to immobilization stress immediately after IP injection of CHA (0.3 mg/kg), lithium chloride (50 and 100 mg/kg), and caffeine (1.0 mg/kg). To examine the effects of combined treatment with CHA and lithium chloride, caffeine, or vehicle on these responses, we administered CHA (0.3 mg/kg, IP) immediately after lithium chloride (100 mg/kg, IP), caffeine (1.0 mg/kg, IP), or vehicle. Rats were then exposed to restraint stress immediately after CHA (0.3 mg/kg, IP) treatment. To examine the effects of multiple caffeine doses on CHA-aggravated gastric lesions, we administered caffeine (1.0 mg/kg, IP) twice immediately before and 6 h after stress. In other experiment, rats were exposed to restraint stress immediately after ICV injection of CHA (1.5μg), lithium chloride (150 μg), caffeine (3 μg), and vehicle through cannulae previously implanted according to the method of de Wied (6).

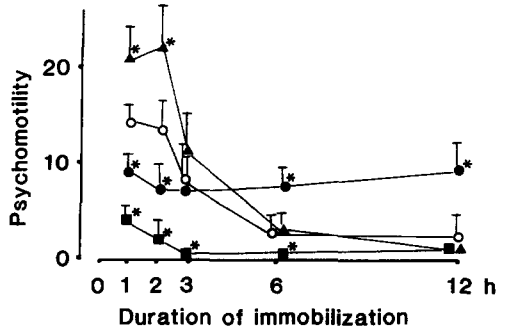

FIG. 1. Time responses of stress-induced psychomotility to ICV injection of CHA, lithium, and caffeine in rats. Each point represents the number of responses upon exposure of vehicle- and drug-treated animals to restraint stress for the designated times. Each value shows the mean ± SE from six rats. (○), vehicle 1.0 μl ICV; (■), CHA 3 μg ICV; (●), lithium 150 μg ICV; (▲), caffeine 3 μg ICV. *$p < 0.02$, significant difference from vehicle group, determined by Mann-Whitney *U*-test.

Drugs

Drugs used were CHA (Boehringer-Mannheim, Mannheim), caffeine (Sigma Chemical Co., St. Louis, MO), and lithium chloride (Katayama, Osaka). CHA was dissolved in ethanol (0.05 ml) and subsequently diluted with saline (ad. 10 ml), and lithium chloride was dissolved in distilled water; an equal volume of vehicle (1.0 ml/kg, IP; 1.0 μl, ICV) was injected in control animals. Doses are expressed in terms of the salt. Injection of vehicle did not produce any abnormal symptoms.

Statistical Analysis

Data were analyzed by means of analysis of variance (ANOVA) and subsequent Tukey tests for the sum of the area of gastric lesions. The Mann-Whitney *U*-test was used for analysis of struggling and defecation scores. The level of significance chosen was $p < 0.05$.

RESULTS

Effects of CHA, Lithium, and Caffeine on Stress-Evoked Hypermotility and Defecation

When rats were restrained with wire-mesh immediately after saline (1.0 ml/kg, IP; 1.0 μl, ICV) injection, they exhibited a biphasic response, that is, an initial hypermotility accompanied by defecation and a subsequent hypomotility. Initially, they struggled with tail-flipping and occasionally vocalized, rolled, or tried to chew the restrainer (hypermotility) and deposited feces (defecation). Subsequently, these responses progressively decreased over a period of 2 h and completely disappeared within 6 h of continuous restraint.

CHA (1.5 μg, ICV) inhibited completely stress-induced hypermotility and defecation but did not affect the subsequent hypomotility. Lithium chloride (150 μg, ICV) significantly inhibited hypermotility and defecation but did not induce the subsequent hypomotility that was observed after exposure to stress for 6-12 h. Caffeine (3 μg, ICV) significantly potentiated hypermotility during 1-3 h of restraint, but this response returned to the level of the vehicle-injected group after 6-12 h of restraint. These results are summarized in Fig. 1.

CHA (0.3 mg/kg, IP) and lithium (50 and 100 mg/kg, IP) completely inhibited hypermotility and defecation during 3 h stress, but caffeine (1.0 mg/kg, IP) potentiated them. Caffeine (1.0 mg/kg, IP) and lithium (100 mg/kg, IP) antagonized the inhibitory effect of CHA on stress-induced hypermotility and increased defecation (Table 1).

Effects of Lithium and Caffeine on CHA-aggravated Stress Lesions

Lithium chloride (50 and 100 mg/kg, IP) completely inhibited CHA-aggravated gastric lesions, even after 12 h stress. Caffeine (1 mg/kg, IP; 3 μg, ICV) antagonized CHA-aggravated gastric lesions after 3 h stress but potentiated the lesions in 6- and 12-h stress-exposed animals in a time-dependent manner (Table 2). However, when caffeine (1.0

TABLE 1

EFFECTS OF CHA, LITHIUM, AND CAFFEINE ON PSYCHOMOTILITY AND DEFECATION IN RATS EXPOSED TO 3-h STRESS

Drugs	Dose	Stress (h)	Psychomotility (total scores/3 h)	Defecation (no. of feces/3 h)	(n)
IP injection	(mg/kg)				
Vehicle		0	–	0.8 ± 0.0	(6)
Vehicle		3	35.4 ± 3.5	5.4 ± 0.9[a]	(8)
CHA	(0.3)	3	5.4 ± 1.2[b]	0.0 ± 0.0[b]	(12)
Lithium	(50)	3	25.0 ± 2.8[b]	0.0 ± 0.0[b]	(6)
Lithium	(100)	3	22.3 ± 2.9[b]	0.0 ± 0.0[b]	(6)
CHA + lithium	(100)	3	17.2 ± 1.2[bc]	0.0 ± 0.0[b]	(6)
Caffeine	(1.0)	3	58.3 ± 5.2[b]	7.8 ± 1.5[b]	(6)
CHA (0.3) + caffeine	(1.0)	3	30.7 ± 5.4[c]	4.3 ± 1.2[c]	(6)
ICV injection	(μg)				
Vehicle		0	–	0.3 ± 0.0	(5)
Vehicle		3	32.1 ± 3.4	4.8 ± 0.5[a]	(8)
CHA	(1.5)	3	4.3 ± 1.1[b]	0.0 ± 0.0[b]	(6)
Lithium	(150)	3	22.3 ± 2.8[b]	0.0 ± 0.0[b]	(6)
Caffeine	(3.0)	3	54.4 ± 5.9[b]	7.0 ± 1.2[b]	(6)

Statistical significance: [a]vs. vehicle in nonstressed rats; [b]vs. vehicle 3-h stressed rats; [c]vs. CHA. Statistical significance level is $p < 0.02$.

mg/kg) was readministered after 6 h of immobilization stress, gastric lesions did not occur. The effects of lithium chloride (150 μg, ICV) and caffeine (3 μg, ICV) on stress lesions aggravated by CHA (0.3 mg/kg, IP) were similar to those of IP injection (Table 2).

DISCUSSION

In this study, when rats were exposed to restraint stress, hypermotility and defecation appeared initially, followed by a decrease in these responses with concomitant ulcer formation. Defecation rate is widely used to measure some aspects of emotionality (11,12,27). The increase of defecation observed during restraint stress is completely inhibited by opioids (27), anxiolytic drugs (benzodiazepine), and GABA (28), suggesting that increases of defecation may be an indicator of fear and anxiety. The effects of CHA on stress-induced hypermotility, defecation, and ulceration were compatible with previous data, that is, an inhibition of hypermotility and defecation, and a stimulation of ulcer formation (28,29). Adenosine and its analogs—benzodiazepine, GABA, and morphine—inhibit

TABLE 2

EFFECTS OF LITHIUM AND CAFFEINE ON CHA-AGGRAVATED GASTRIC LESIONS IN RATS EXPOSED TO 0-, 3-, 6- OR 12-h STRESS

		Gastric Lesions (mm^2)				
Drugs	Dose	Stress (h)	Saline	(n)	CHA (0.3 mg/kg)	(n)
IP injection	(mg/kg)					
Saline		0	0.0 ± 0.0	(4)	10.3 ± 4.5	(6)
Saline		3	0.0 ± 0.0	(6)	20.8 ± 7.2[a]	(12)
Saline		6	0.0 ± 0.0	(6)	28.3 ± 8,5[a]	(6)
Saline		12	2.3 ± 0.4[a]	(6)	32.4 ± 9.1[a]	(6)
Lithium	(50)	12	0.0 ± 0.0[b]	(6)	0.0 ± 0.0[ab]	(6)
Lithium	(100)	12	0.0 ± 0.0[b]	(6)	0.3 ± 0.0[ab]	(6)
Caffeine	(1.0)	3	0.0 ± 0.0	(6)	0.5 ± 0.0[ab]	(6)
Caffeine	(1.0)	6	0.0 ± 0.0	(6)	24.5 ± 8.3[a]	(6)
Caffeine	(1.0)	12	8.4 ± 0.8[ab]	(6)	43.3 ± 9.8[ab]	(6)
Caffeine	(1.0) × 2	12	0.0 ± 0.0[bc]	(5)	0.6 ± 0.0[abc]	(6)
ICV injection	(μg)					
Saline		3	0.0 ± 0.0	(5)	22.5 ± 5.3	(5)
Lithium	(150)	3	0.0 ± 0.0	(5)	0.0 ± 0.0[a]	(8)
Caffeine	(3)	3	0.0 ± 0.0	(5)	0.0 ± 0.0[a]	(8)

Statistical significance level ($p < 0.05$): [a]vs. saline-injected group in nonstressed rats; [b]vs. respective correspondent saline group in stressed rats; [c]vs. caffeine (1.0) group in 12-h stressed rats.

release of transmitters (noradrenaline, acetylcholine, and serotonin) in various central and peripheral neuronal preparations (3,8,9,13,14,20,21). Stress-induced hyperemotional responses accompanied with noradrenaline release are inhibited by morphine (27). CHA, benzodiazepines, and GABA also inhibit stress-induced hypermotility and defecation. Since adenosine and benzodiazepine, but not morphine and GABA, increase stress-induced gastric lesions, it seems unlikely that a relationship exists between the inhibition of neurotransmitter release and exacerbation of stress lesions by adenosine and its analogs. These results suggest that the neuronal mechanism involved in ulcer formation induced by restraint stress may be different from that in hyperemotional responses (28).

Treatment with caffeine in the stressed state potentiated the initial hypermotility and defecation, and antagonized the subsequent ulcerative effect, but did not the subsequent hypomotility. Lithium also antagonized both the initial hypermotility and defecation, and the subsequent hypomotility with concomitant ulceration. It appears that lithium may normalize hyper- and hypoemotional responses, as well as gastric ulceration, in stressed states.

Adenosine receptors have been classified into two different types—A_1 and A_2 receptors—on the basis of inhibitory and stimulatory effects, respectively, of adenosine on rat brain adenylate cyclase activity (30). CHA is a potent agonist of the A_1 receptor, which is involved in presynaptic autoinhibition mechanisms (18). The receptors are blocked by methylxanthines such as theophylline and caffeine in a nonselective manner (5). Recent experiments have implied that a variety of the central stimulant effects of acute caffeine administration were related to a blockade of adenosine receptors (25) and that methylxanthine was more potent at competing for adenosine receptors than in mediating any other biochemical effect (e.g., phosphodiesterase inhibition) (5).

Caffeine inhibited CHA-aggravated gastric lesions after 3 h of stress, as previously reported (29), but potentiated the ulcerations after 6–12 h of stress. When caffeine was administered again after 6 h of restraint stress, gastric lesions did not occur. These results suggest that caffeine consumption following a time lapse potentiates stress-induced ulcer formation. The long-lasting CHA may be substituted for caffeine at the adenosine receptors activated following caffeine consumption.

Chronic exposure to the adenosine receptor antagonists, caffeine and theophylline, has previously been shown to produce an increase in the density of adenosine A_1 receptors in rat cerebral cortex (4,10,26). Thus, a prolonged blockade of these receptors is likely to induce a long-lasting decrease in adenosine interaction with its receptors and a compensatory increase in receptor number or sensitivity (17). Most recently, there is evidence that chronic theophylline exposure increases agonist and antagonist binding to adenosine A_1 receptors in rat brain (19). However, in this study it was suggested that the acute treatment with caffeine may induce early development of supersensitivity to adenosine receptors, presumably via caffeine consumption, under the stressed states.

On the other hand, since lithium has no effect on [^{3}H]CHA binding in brain tissue (16) it may not function as a direct purinoceptor antagonist but rather act indirectly at adenosine A_1 receptors. Thus, lithium seems to normalize abnormal symptoms and ulceration induced by stress.

REFERENCES

1. Anderson, S. M.; Lew, J. R.; Kant, G. J. Effects of stress on [^{3}H]cyclo-hexyladenosine binding to rat brain membranes. Pharmacol. Biochem. Behav. 26:829–833; 1987.
2. Anisman, H.; Zacharko, R. Depression: The predisposing influence of stress. Behav. Brain Sci. 5:89–137; 1982.
3. Bhargava, K. P.; Daas, M.; Gupta, G. P.; Guputa, M. B. Study of central neurotransmitters in stress-induced gastric ulceration in albino rats. Br. J. Pharmacol. 68:765–772; 1980.
4. Chou, D. T.; Khan, S.; Forde, J.; Hirsh, K. R. Caffeine tolerance: Behavioral, electrophysiological and neurochemical evidence. Life Sci. 36:2347–2358; 1985.
5. Daly, J. W.; Bruns, R. F.; Snyder, S. H. Adenosine receptors in the central nervous system: Relationship to the central actions of methylxanthines. Life Sci. 28:2083–2097; 1981.
6. De Wied, D. Behavioral effects of intraventricularly administered vasopressin and vasopressin fragments. Life Sci. 19:685–690; 1976.
7. Eichelman, B.; Seagraves, E.; Barchas, J. Alkari metal cations: Effects on isolation-induced aggression in the mouse. Pharmacol. Biochem. Behav. 7:407–409; 1977.
8. Enna, S. J. The role of neurotransmitters in the pharmacologic actions of benzodiazepines. In: Mathew, R. J., ed. The biology of anxiety. New York: Brunner/Mazel; 1982:107–122.
9. Feuerstein, T. J.; Hertting, G.; Jackisch, T. Modulation of hippocampal serotonin (5-HT) release by endogenous adenosine. Eur. J. Pharmacol. 107:233–242; 1985.
10. Fredholm, B. B. Adenosine actions and adenosine receptor after 1 week treatment with caffeine. Acta Physiol. Scand. 115:283–287; 1982.
11. Glavin, G. Subject emotionality and coping responses as predisposing and precipitating factors in restraint ulcer in rats. In: Umehara, S.; Ito, I., eds. Advances in experimental ulcer. Tokyo: Tokyo Medical College Press; 1982:76–91.
12. Hall, C. S. Emotional behavior in the rat: 1. Defecation and urination as measures of individual differences in emotionality. J. Comp. Physiol. Psychol. 81:385–403; 1934.
13. Hedqvist, P.; Fredholm, B. B. Effects of adenosine on adrenergic neurotransmission. Prejunctional inhibition and post-junctional enhancement. Naunyn Schmiedebergs Arch. Pharmacol. 293: 217–223; 1976.
14. Jackisch, R.; Stritmatter, H.; Fehr, R.; Hertting, G. Modulation of hippocampal noradrenaline and acetylcholine release by endogenous adenosine. Naunyn Schmiedebergs Arch. Pharmacol. 324:R20; 1983.
15. Natelson, B. Stress, predisposition and the onset of serious disease: Implications about psychosomatic etiology. Neurosci. Biobehav. Rev. 7:511–527; 1983.
16. Newman, M.; Zohar, J.; Kalian, M.; Belmaker, R. H. The effects of chronic lithium and ECT on A_1 and A_2 adenosine receptor systems in rat brain. Brain Res. 291:188–192; 1984.
17. Overstreet, D. H.; Yamamura, H. I. Receptor alteration and drug tolerance. Life Sci. 25:1865–1877; 1979.
18. Paton, D. M. Structure-activity relations for presynaptic inhibition of noradrenergic and cholinergic transmission by adenosine. J. Auton. Pharmacol. 1:287–290; 1981.
19. Sanders, R. C.; Murray, T. F. Chronic theophylline exposure increases agonist and antagonist binding to A_1 adenosine receptors in rat brain. Neuropharmacology 27:757–760; 1988.
20. Scatton, B.; Bartholini. G. γ-Aminobutyric acid (GABA) receptor stimulation IV. Effect of progabide (SL-76002) and other GABA-ergic agents on acetylcholine turnover in rat brain areas. J. Pharmacol. Exp. Ther. 220:689–695; 1982.
21. Scatton, B.; Zivkovic, B.; Dekek, J.; Lloyd, K. G.; Constantinidis, J.; Tissot, R.; Bartholini, G. γ-Aminobutyric acid (GABA) receptor stimulation III. Effect of progabide (SL-76002) on norepinephrine, dopamine and 5-hydroxytryptamine turnover in rat brain areas. J. Pharmacol. Exp. Ther. 220:678–688; 1982.

22. Schou, M.; Thomsen, K. Lithium prophylaxis of recurrent endogenous affective disorders. In: Johnson, F. N., ed. Lithium research and therapy. London: Academic Press; 1975:63–84.
23. Sheard, M. H. Effect of lithium on foot shock aggression in rats. Nature 228:284–285; 1970.
24. Sheard, M. H. The effect of lithium on human aggression. Nature 230:113–114; 1971.
25. Snyder, S. H.; Katims, J. J.; Annau, Z.; Bruns, R. F.; Daly, J. W. Adenosine receptors and behavioral actions of methylxanthines. Proc. Natl. Acad. Sci. USA 78:3260–3264; 1981.
26. Szot, P.; Sanders, R. C.; Murray, T. F. Theophylline-induced upregulation of A_1 adenosine receptors associated with reduced sensitivity to convulsants. Neuropharmacology 26:1173–1180; 1987.
27. Tanaka, M.; Kohno, Y.; Tsuda, A.; Nakagawa, R.; Ida, Y.; Iimori, K.; Hokai, Y.; Nagasaki, Y. Differential effects of morphine on noradrenaline release in brain regions of stressed and non-stressed rats. Brain Res. 275:105–115; 1983.
28. Ushijima, I.; Mizuki, Y.; Hara, T.; Kudo, R.; Watanabe, K.; Yamada, M. The role of adenosinergic, GABAergic and benzodiazepine systems in hyperemotionality and ulcer formation in stressed rats. Psychopharmacology (Berl.) 89:472–476; 1986.
29. Ushijima, I.; Mizuki, Y.; Yamada, M. Development of stress-induced gastric lesions involves central adenosine A_1 receptor stimulation. Brain Res. 339:351–355; 1985.
30. Van Calker, D.; Muller, M.; Hamprecht, B. Adenosine inhibits the accumulation of cyclic AMP in cultured brain cells. Nature (Lond.) 276:839–841; 1987.