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Role of Dopaminergic Mechanisms in the Regulation of Stress Responses in Experimental Animals

S. PURI, A. RAY,[1] A. K. CHAKRAVARTI* AND P. SEN

*Departments of Pharmacology and *Biochemistry, University College of Medical Sciences and GTB Hospital, Shahdara, Delhi-110095, India*

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PURI, S., A. RAY, A. K. CHAKRAVARTI AND P. SEN. *Role of dopaminergic mechanisms in the regulation of stress responses in experimental animals.* PHARMACOL BIOCHEM BEHAV 48(1) 53-56, 1994.—The effects of some dopaminergic agents were evaluated on stress responses in normal and immunized experimental animals. Restraint stress (RS) consistently induced gastric mucosal lesions and elevated plasma corticosterone in rats. Pretreatment with α-methyltyrosine (α-MT), haloperidol, or sulpiride aggravated both responses, whereas bromocryptine attenuated them. In rats immunized with sheep red blood cells (SRBCs), RS prevented the booster-induced rise in anti-SRBC antibody titre. This response was further suppressed by α-MT, haloperidol, or sulpiride pretreatment, whereas bromocryptine potentiated the humoral immune response. In mice immunized with SRBCs, antigen challenge-induced increase in footpad thickness was inhibited by RS. Similar inhibitions in this response were also seen after α-MT or haloperidol treatment. The results are discussed in light of complex dopaminergic mechanisms in the regulation of visceral, endocrinological, and immune responses during stress.

Restraint stress Gastric ulcers Corticosterone Immune response Dopamine

SEVERAL factors are known to participate in the maintenance of the physiological mileau of the organism, and "stress" is an aversive force which disrupts such homeostasis (24,28). Experimental and clinical data have shown that several biological parameters which act as useful indices of a stress response are modulated by a variety of stressors. For example, plasma corticosterone and gastric ulcer formotion are widely recognized as important markers of stress (8,14, 24,25). More recent data has shown that the neuroendocrine-immune axis is crucial during stress. The involvement of neural mechanism in the regulation of stress responsiveness has been suggested, and complex neurotransmitter interactions are proposed (12,20,25). Our studies have indicated that endogenous opioids and γ-aminobutyric acid (GABA) are important mediators during stress and that drugs modulating these transmitters influence stress markers in a complex manner (20,21).

The role of dopamine (DA) during stress is reported, and electrophysiological and neuropharmacological data show that brain DA neurons are actually activated during such aversive stimuli (4,23). Further, more recent studies have shown that DAergic transmission plays a crucial role in the gastric mucosal response to stress (5,7,16-19). However, the exact nature of DAergic involvement in the regulation of several other stress responses like the endocrinological and immunological is less extensively studied. The present study thus critically evaluated the involvement of DAergic mechanisms in the regulation of some stress responses. Accordingly, the effects of some modulators of DAergic transmissions are assessed on visceral (gastric mucosa), endocrinological (plasma corticosterone), and immune (humoral and cellular) responses in normal and immunized experimental animals.

METHODS

Subjects

Male Wistar rats (175-200 g) and Swiss albino mice (20-30 g) were used. They were housed in standard laboratory conditions of light (12-h light-dark schedule) and temperature (22 ± 2°C) and had free access to food and water.

[1] Requests for reprints should be addressed to Dr. A. Ray, Department of Pharmacology (Room # 527), University College of Medical Sciences and GTB Hospital, Shahdara, Delhi-110095, India.

Stress Procedure (19,20)

Restraint stress (RS) was applied in Plexiglas restrainers (INCO, Ambala) for 24 h at room temperature (RT). For the experiments with mice involving cell-mediated immune response, the mice were restrained in well-ventilated Plexiglas tubes for 1 h daily for five days.

Plasma Corticosterone

The animals were lightly anesthetised with ether, and blood was collected from the supraorbital plexus using the microcapillary technique. The plasma was assayed for corticosterone using the method of Glick et al. with some modifications (6,10). Three hundred microliters of isooctane was added to 100 μl of plasma. After mixing and centrifugation, the isooctane layer was discarded. Six hundred microliters of chloroform was now added to each tube, and after extraction 400 μl of chloroform was transferred to another stoppered tube. To this 800 μl of acid-alcohol (50%) solution (2 : 1) was added. After 1 h, acid layer fluorescence was measured at 462 nm (excitation) and 518 nm (emission) using a spectrofluorimeter (Shimadzu-RF 540, Japan).

Gastric Pathology

The rats were then sacrificed with an overdose of anesthetic ether. The stomachs were dissected out, cut open along the greater curvature, washed with cold water, and examined microscopically (×10) using a dissecting microscope with a micrometer. The number of mucosal erosions and their severity (cumulative length in mm, to the nearest 0.1 mm) were determined by an observer who was unaware of the treatment schedules.

Immunological Assay

Humoral immune response. Rats were immunized with sheep red blood cells (SRBCs) (0.5×10^9 cells/ml/100 g) on day 0. On day 7 they received a similar booster dose of the antigen and were food deprived for 18 h, and following this they were exposed to RS for 24 h. Subsequently, the rats were lightly anesthetised with ether and blood was collected from the supraorbital plexus using the microcapillary technique. A parallel "no RS" group was run and served as controls. The vehicle or drug treatments were made just prior to RS. The serum was assayed for hemagglutination titre as follows: Two-fold dilutions (0.025 ml) of sera were made in the microtitre plates with saline. To each well, 0.025 ml of 1% (v/v) SRBCs was added. The plates were incubated at 37°C for 1 h and then observed for hemagglutination. The highest dilution giving hemagglutination was taken as the antibody titre. The antibody titres were expressed in a graded manner, the minimum dilution (1/2) being ranked as 1, and the mean ranks of different groups were compared for statistical significance.

Cell-mediated immune response (CMI). Mice were immunized on day 0 with 0.1 ml SRBCs (5×10^8 cells/ml) on the back. They were then subjected to stress/vehicle/drug treatments from day 1 to day 5. On day 5 the mice were challenged by injecting 0.1 ml SRBCs into the left hind paw, whereas the right hind paw received 0.9% saline. Twenty-four hours after challenge the differences in paw volumes were measured by using the fluid displacement method (9). The changes in footpad thickness (as measured by differences in paw volumes) of different treatment groups were compared for statistical significance.

Drugs

The drugs used were α-methyltyrosine (α-MT), sulpiride, and bromocryptine mesylate (all from Sigma Chemical Co., St. Louis); haloperidol (Searle, India); and SCH 23390 (Schering Corporation, Kenilworth, NJ). They were dissolved/diluted in distilled water except for sulpiride, which was dissolved in 0.1 N HCl and pH neutralized to 5-5.5 with 0.1 N NaOH. They were injected twice daily (12 hourly) IP in a volume of 1 ml/kg, prior to RS.

Statistical Analysis

The data was analysed using the Mann-Whitney *U* test (two-tailed). A *p* value of at least 0.05 was used as the level of significance in all statistical tests.

RESULTS

Experiments in Normal Rats

RS (24 h at RT) consistently induced gastric nucosal erosions and elevated plasma corticosterone levels in 18-h food-deprived rats. As shown in Table 1, the ulcer severity (mm) and corticosterone levels in the RS group were markedly higher when compared to food-deprived, "nonstressed" rats (controls). Pretreatment of rats with the DA-synthesis inhibitor α-MT (300 mg/kg) clearly aggravated the ulcer severity (more than 100% increase) and also potentiated the corticosterone response (by 25%), when compared to the RS group. Similar effects were seen with the DA receptor (D_2) blockers haloperidol (0.5 mg/kg) and sulpiride (10 mg/kg), with the aggravations in the gastric mucosal response being more marked. The higher dose of sulpiride (50 mg/kg), however, attenuated RS-induced gastric ulcerogenesis. The D_1 receptor blocker SCH 23390 (0.05 mg/kg), however, did not appreciably influence any of the above stress responses. The DA (D_2) receptor agonist bromocryptine (2.5 mg/kg), on the other hand, attenuated both the ulcerogenic and corticosterone-elevating effects of RS. There was a near 80% reduction in

TABLE 1

EFFECTS OF SOME DOPAMINERIC DRUGS ON STRESS (RS)-INDUCED GASTRIC ULCEROGENESIS AND PLASMA CORTICOSTERONE IN RATS

Treatment (mg/kg, IP)	Stress Responses (mean ± SE)	
	Ulcer Severity (mm)	Plasma Corticosterone (μg/dl)
Controls*	0.4 ± 0.2	18.2 ± 0.9
RS	1.8 ± 0.6†	34.3 ± 1.4†
α-MT + RS‡	3.9 ± 0.9§	42.9 ± 2.4§
Haloperidol (0.5) + RS	3.2 ± 1.4¶	36.3 ± 1.6
SCH 23390 (0.05) + RS	1.6 ± 0.8	32.5 ± 1.0
Sulpiride (10) + RS	2.8 ± 1.0¶	36.1 ± 1.5§
Sulpiride (50) + RS	1.1 ± 0.2¶	28.5 ± 0.8¶
Bromocryptine (2.5) + RS	0.5 ± 0.1§	20.8 ± 1.6§

n = 6–8 per group. *No RS, only 18-h food deprived. †$p < 0.02$ (compared to controls). ‡α-MT (α-methyl-*p*-tyrosine)—300 mg/kg, then 30 min later 150 mg/kg; RS after 3½ h. §$p < 0.02$; ¶$p < 0.05$ (compared to the RS group).

ulcer severity and 60% reduction in the corticosterone response after bromocryptine pretreatment.

Experiments in Immunized Animals

In rats immunized with SRBCs a booster dose of the antigen clearly augmented the humoral immune response to the antigen (prebooster values were 5.8 ± 0.6). RS clearly attenuated the booster (SRBC)-induced rise in the secondary antibody titre. As shown in Table 2, the values in the RS group were significantly lower (by 20%) than those of the nonstress group ($p < 0.05$). Pretreatment with α-MT (300 mg/kg) further suppressed the anti-SRBC antibody titre when compared to the RS group ($p < 0.05$). Similar changes (i.e., suppression) were also seen when haloperidol (0.5 mg/kg) was given prior to RS. Both α-MT and haloperidol lowered the anti-SRBC antibody titres in nonstressed rats as well. However, these differences did not attain levels of statistical significance ($p > 0.05$). Further, the D_2 blocker sulpiride showed dose-related, bidirectional effects on the humoral immune response. Whereas the lower dose (10 mg/kg) of the drug further lowered the antibody titres (like α-MT and haloperidol), the higher dose (50 mg/kg) reversed the RS-induced suppression and even raised the antibody titre beyond control (nonstress vehicle) levels. Similar, facilitatory effects on the secondary immune response to SRBCs were also seen after bromocryptine (2.5 mg/kg) pretreatment.

In mice immunized with SRBCs and then challenged with SRBCs into the left paw (right paw received saline), there was a marked enhancement in the paw volume as measured by the fluid displacement method. The mean difference in paw volume in vehicle-treated (nonstressed) mice was 0.050 ± 0.006 ml. In the 1-h RS-treated mice there was significant reduction in paw volume difference when compared to the controls (by 40%). As shown in Table 3, the paw volume changes were much less marked in the RS group and significantly different from controls ($p < 0.05$). Similar effects on paw volume changes were also seen after α-MT pretreatment. Haloperidol (0.5 mg/kg) also attenuated the paw volume response to SRBCs, but the magnitude of the response was lesser in comparison to α-MT. Bromocryptine (2.5 mg/kg), on the other hand, did not influence appreciably the paw volume when compared to vehicle-treated mice. The changes in paw volume after bromocryptine treatment were, however, still significantly different from the same after RS treatment.

TABLE 2

EFFECTS OF RESTRAINT STRESS (RS) AND ITS MODULATION BY DOPAMINERGIC DRUGS ON HUMORAL IMMUNE RESPONSE IN RATS

Treatment (mg/kg, IP)	Mean Anti-SRBC Antibody Titre (±SE)	
	RS	No RS
Vehicle	5.3 ± 0.4*	6.7 ± 0.2
α-MT†	4.3 ± 0.3‡	5.8 ± 0.8‡
Haloperidol (0.5)	4.4 ± 0.3‡	6.1 ± 1.0
SCH 23390 (0.05)	6.0 ± 0.2	
Sulpiride (10)	4.6 ± 0.4	
Sulpiride (50)	7.7 ± 0.7‡	
Bromocryptine (2.5)	7.1 ± 0.3‡	7.0 ± 0.6

n = 6–8 per group.
*$p < 0.05$ (compared to vehicle-treated "no RS" group). †α-MT (α-methyl-*p*-tyrosine) 300 mg/kg + 150 mg/kg (after 30 min), then RS after 3½. ‡$p < 0.05$ (compared to respective vehicle-treated group).

TABLE 3

EFFECTS OF RESTRAINT STRESS (RS) AND DOPAMINERGIC AGENTS ON FOOTPAD THICKNESS IN MICE

Treatment (mg/kg, IP)	n	Mean Change in Paw Volume (±SE)
Vehicle*	15	0.050 ± 0.006
RS	18	0.040 ± 0.006†
α-MT‡	12	0.035 ± 0.008†
Haloperidol (0.5)	14	0.043 ± 0.010†
Bromocryptine (2.5)	12	0.054 ± 0.008

*No RS, only 18-h food deprived. †$p < 0.05$ (compared to vehicle group) ‡α-MT (α-methyl-*p*-tyrosine) 300 mg/kg + 150 mg/kg (after 30 min), then RS after 3½ h.

DISCUSSION

Complex neurochemical mechanisms are involved in the organism's biological response to noxious stimuli like stress, and several neurotransmitters/neuromodulators have been implicated (26). Our present data show that DA may be an important chemical transmitter regulating various stress responses. The CNS plays an important role in stress ulceration and regulation of plasma corticosterone (7). It has been shown that the CNS may also mediate immune responsiveness (25). The role of central DAergic pathways in the regulation of gastric mucosal integrity during stressful experiences is also suggested (16–18), and our present experiments suggest that DA may mediate endocrinological and immune responses as well. Neural control of endocrinological and immune function has been reported earlier (20,22). The DA-synthesis inhibitor α-MT aggravated both ulcer severity and plasma corticosterone levels when compared to stressed rats with normally/adequately functioning DA pathways. Similar findings were seen with haloperidol, the DA receptor blocker, and the lower dose of the specific D_2 receptor blocker, sulpiride (13). The above, and the observation that the specific D_2 agonist bromocryptine attenuated the observed stress responses, clearly indicate a possible protective role of DA during stress, and this may be via activation of the D_2 receptor. The D_1 receptor seems less involved in these stress responses (viz., gastric, endocrinological, and immunological). However, the possibility of an interaction between D_1 and D_2 receptors during elicitation of these stress responses cannot be totally discounted. In fact, an earlier study showed that D_2 receptor-mediated DAergic gastric cytoprotection during cold restraint stress was D_1 receptor-dependent (15).

Neural cortrol of immunity has been widely speculated in recent years, and several chemical mediators have been implicated (3). The biogenic amines (13), amino acids (20), and even neuropeptides (3,11) influence immune responses both in normal and in stressed animals. The role of DA in the regulation of immune responsiveness is less extensively studied, and our results show that DA depletion (by α-MT) or blockade (by haloperidol or sulpiride) suppresses both humoral and cell-mediated immune responses in both normal and stressed animals. On the other hand, the DA agonist (D_2 receptor-

directed) bromocryptine reversed the effects of stress on humoral immune response. Further, in nonstressed situations bromocryptine clearly facilitated humoral and, to some extent, cellular immune responsiveness. The relative lack of modulatory effects of the D_1 blocker SCH 23390 on immune response suggests the greater significance of D_2 receptors in this effect. The role of DA in the mediation of immune response has not been reported, though DA receptors have been identified on some immunocompetent cells (1). The dose-related effects of the specific D_2 blocker sulpiride are interesting. Whereas the lower dose aggravated RS effects, the higher dose reversed them. This differential nature of sulpiride effect has been reported on stress ulcerogenesis (19). An anxiolytic profile for this atypical neuroleptic has been suggested in behavioral models, and this probably could explain its stress-attenuating effects on visceral, endocrinological, and immune parameters. Antianxiety agents (e.g., benzodiazepines, buspirone, etc.) are known to attenuate stress effects (20,27). Further, behavioral factors are important determinants of immune responses (1,26). In view of the above, the effects with sulpiride are not surprising.

Taken together, it appears that DAergic mechanisms are crucial for the regulation of homeostatic mechanisms. This is evident from the modulatory effects of DAergic agents on stress-induced visceral, endocrinological, and immune responses. It is possible that both central and peripheral DAergic pathways/receptors could be involved. The role of the hypothalamo-pituitary-adrenal (HPA) axis in stress is amply demonstrated (24), and neural mechanisms like benzodiazepine-GABA, histamine, endogenous opioids, and so on have been proposed. It was suggested that corticosterone-induced suppression of immune function may covary with gastric ulcerogenesis during stress (20). The present findings reaffirm this contention and further implicate DA-neural systems in these HPA axis-mediated stress responses.

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