Pergamon

Pharmacology Biochemistry and Behavior, Vol. 50, No. 3, pp. 481–484, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00300-9

BRIEF COMMUNICATION

Noradrenergic Denervation Attenuates Desipramine Enhancement of Aggressive Behavior in Isolated Mice

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Received 12 May 1994

MATSUMOTO, K., K. OJIMA AND H. WATANABE. *Noradrenergic denervation attenuates desipramine enhancement of aggressive behavior in isolated mice.* PHARMACOL BIOCHEM BEHAV **50**(3) 481–484, 1995.—The effects of denervation of central noradrenergic system on the desipramine-induced enhancement of aggressive behavior were examined in long-term isolated mice. Consistent with previous reports, desipramine (10 mg/kg, IP) significantly increased the duration of aggressive behavior in isolated mice. Pretreatment of isolated mice with DSP-4, a selective noradrenaline neurotoxin, significantly attenuated the enhancing effect of desipramine on aggressive behavior without affecting the basal aggressive responses. This effect appeared concomitant with decrease of noradrenaline levels in the cortex, hippocampus, cerebellum and hypothalamus (78%, 75%, 57%, and 17%, respectively). DSP-4 did not significantly affect dopamine or 5-HT levels in these regions. These results suggest that noradrenergic terminals originating mainly from the locus coeruleus play an important role in the desipramine enhancement of aggressive behavior, but not in the basal aggressive behavior of isolated mice.

Aggressive behavior Desipramine DSP-4 Isolation Noradrenaline Locus coeruleus Mice

LONG-TERM social isolation induces aggressive behavior in nonaggressive laboratory mice. The functional changes, in catecholaminergic or serotonergic systems or both, in the brain appear to occur following this manipulation and participate in the appearance of aggressive behavior, although the roles of these neuronal systems in the long-term social isolation-induced aggressive behavior are still controversial [for a review, see (3)]. We have previously reported that desipramine, a selective noradrenaline uptake blocker, increases the duration of aggressive behavior in socially isolated mice, and that this effect is mediated by α_2- and β_2-adrenoceptors but not by α_1- or β_1-adrenoceptors (9,11). Other antidepressant drugs with ability to block noradrenaline uptake have been shown to have biphasic effects on the isolation-induced aggressive behavior; i.e., increase in aggressive behavior at lower doses and decrease at higher doses (4). These findings raise the possibility that functional changes in the central noradrenergic system may be induced by long-term social isolation, and that such changes may be closely related to the antidepressant-induced enhancement of aggressive behavior (4,9).

In the present study, we examined effects of DSP-4, a selective noradrenergic neurotoxin, on the desipramine-induced enhancement of aggressive behavior to further clarify the role of noradrenergic system in the social isolation-induced behavioral changes. Part of the present study has appeared in a preliminary form elsewhere (10).

MATERIALS AND METHODS

Isolated Housing

Animals were housed in isolation as described in our previous report (4,9). Briefly, male ddY strain inbred mice, weighing approximately 18–20 g (SLC Co., Shizuoka, Japan) were obtained at the age of 28 days. Mice were isolated in 24 × 17

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TABLE 1

EFFECT OF DSP-4 ADMINISTRATION ON NORADRENALINE, DOPAMINE, AND SEROTONIN CONTENTS IN THE BRAIN

Regions		Housing Condition: Group-Housed	Isolated: Without DSP-4	Isolated: With DSP-4
Experiment I, 3 days after DSP-4 injection				
Cortex	NA	0.382 ± 0.012	0.379 ± 0.023	0.086 ± 0.004†
	DA	0.393 ± 0.038	0.360 ± 0.024	0.276 ± 0.042
	5-HT	0.613 ± 0.057	0.712 ± 0.040	0.771 ± 0.050
Hippocampus	NA	0.529 ± 0.023	0.521 ± 0.026	0.137 ± 0.017†
	DA	nd	nd	nd
	5-HT	0.886 ± 0.041	0.874 ± 0.035	0.821 ± 0.045
Hypothalamus	NA	2.137 ± 0.065	1.983 ± 0.068	1.774 ± 0.070*
	DA	0.614 ± 0.047	0.677 ± 0.031	0.794 ± 0.048
	5-HT	1.519 ± 0.090	1.767 ± 0.084	1.779 ± 0.110
Cerebellum	NA	0.328 ± 0.020	0.329 ± 0.015	0.142 ± 0.015†
	DA	nd	nd	nd
	5-HT	0.157 ± 0.022	0.180 ± 0.012	0.163 ± 0.013
Experiment II, 14 days after DSP-4 injection				
Cortex	NA	0.388 ± 0.016	0.425 ± 0.012	0.179 ± 0.023†
	DA	0.192 ± 0.034	0.246 ± 0.042	0.115 ± 0.014
	5-HT	0.798 ± 0.039	0.901 ± 0.017	0.890 ± 0.026
Hippocampus	NA	0.537 ± 0.027	0.576 ± 0.020	0.231 ± 0.037†
	DA	nd	nd	nd
	5-HT	0.943 ± 0.042	1.029 ± 0.037	1.101 ± 0.037
Hypothalamus	NA	2.146 ± 0.061	2.354 ± 0.090	1.800 ± 0.072†
	DA	0.926 ± 0.066	0.775 ± 0.080	0.805 ± 0.044
	5-HT	1.993 ± 0.095	2.094 ± 0.082	2.156 ± 0.068
Cerebellum	NA	0.336 ± 0.015	0.378 ± 0.016	0.176 ± 0.027†
	DA	nd	nd	nd
	5-HT	0.194 ± 0.015	0.233 ± 0.030	0.249 ± 0.058

Each mouse was isolated or group-housed (five mice per cage) for 7 weeks before the experiments. Mice were treated with saline or DSP-4 (50 mg/kg, IP). Three and 14 days after DSP-4 administration, the contents of monoamines in the brain were measured. Each value (μg/g tissue) represents mean ± SEM from 7–8 mice. *$p < 0.05$, †$p < 0.01$ compared with DSP-4 untreated group; nd = not detected.

× 12 cm cages for 6–7 weeks before the experiments. Housing conditions were thermostatically maintained at 24 ± 1°C, with a 12 h light/dark cycle. Food and water were given ad lib.

Measurement of Aggressive Responses

Measurement of aggressive behavior has been carried out as previously reported. Briefly, when testing aggressive behavior between isolated mice, two isolated mice were placed in the neutral cage (24 × 17 × 12 cm), which was the same size as their home cage. The duration of biting attacks, wrestling, or both, observed during a 20-min period was measured. The latency to the first attack was also recorded using a keyboard interfaced with a computer (PC 9801NS, NEC, Tokyo, Japan). Effects of drugs on aggressive behavior were evaluated using 7–8 pairs of mice per group.

Treatment with DSP-4

DSP-4 (50 mg/kg) was intraperitoneally (IP) injected to isolated animals to denervate noradrenergic system. Three and 14 days after DSP-4 treatment, the animals were used to test the aggressive behavior, or decapitated to measure noradrenaline levels in the brain.

Measurement of Noradrenaline Levels in the Brain

Three days after the DSP-4 (50 mg/kg, IP) treatment, animals were decapitated, and the brains rapidly removed and washed in ice-cold saline. The frontal cortex, hypothalamus, hippocampus, and cerebellum were dissected on an ice-cold glass plate, and frozen in liquid nitrogen. Noradrenaline contents in these tissues were measured as described in detail in our previous article (2). Briefly, each tissue was homogenized in 1-ml ice-cold 0.25M $HClO_4$ solution containing 0.3-mg cysteine and an internal standard 3,4-dihydroxybenzylamine. After centrifugation (10,000 g at 4°C for 10 min), the supernatant was mixed with 1 ml of 1.5M Tris-HCl buffer (pH 8.6) solution containing 30-mg acid-washed alumina. After shaking at 4°C for 10 min, the alumina was washed three times with distilled water. Catecholamines adsorbed to the alumina were eluted with 0.3 ml of 0.25M $HClO_4$ solution containing

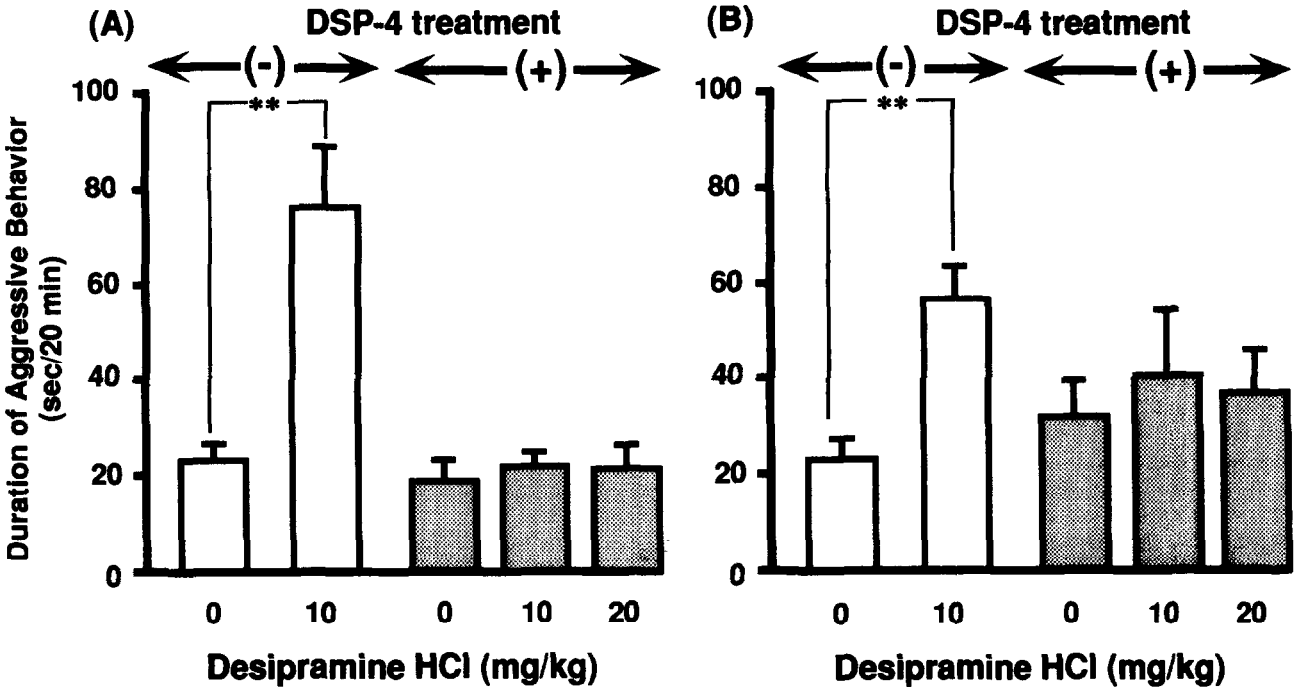

FIG. 1. Attenuation of desipramine-induced enhancement of aggressive behavior by DSP-4 treatment. DSP-4 (50 mg/kg; dottted columns) or saline (open columns) was intraperitoneally (IP) injected to isolated mice 3 (A) or 14 days (B) before the experiments. When testing aggressive behavior, either saline or desipramine was IP injected 60 min before the experiments. Each datum represents the mean ± SEM of eight pairs of animals. $^{**}p < 0.01$ compared with DSP-4 untreated control.

0.1 mg/ml cysteine, and determined using a high performance liquid chromatography with electrochemical detector.

Drugs

Desipramine HCl and DSP-4 HCl [N-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine hydrochloride; Sigma, St. Louis, MO] were dissolved in saline and injected IP in a constant volume of 0.01 ml/g body weight. Other chemicals were obtained from commercial sources. Desipramine was administered 60 min before the experiments.

Statistics

The data were analyzed with the nonparametric Kruskal-Wallis analysis of variance followed by the Mann-Whitney *U*-test for multiple comparisons between groups. Neurochemical data were analyzed with two-tailed Student's *t*-test. Differences with $p < 0.05$ were considered statistically significant.

RESULTS

Effect of DSP-4 Treatment on the Noradrenaline Levels in the Brain

As summarized in Table 1, the noradrenaline levels in the cortex, hippocampus, hypothalamus, and cerebellum significantly decreased by 78%, 75%, 17%, and 57%, respectively, at 3 days after administration of DSP-4 (50 mg/kg, IP). The noradrenaline levels in these regions of the DSP-4-treated mice slightly recovered at 14 days after administration, but they were still significantly lower than those of the DSP-4-untreated isolated mice (cortex: 57.9%; hippocampus: 60%; hypothalamus: 23.5%; cerebellum: 53.4%, percent decrease).

Effects of DSP-4 Treatment on the Desipramine-Induced Enhancement of Aggressive Behavior in Isolated Mice

We previously reported that desipramine (5–20 mg/kg) dose dependently increased the duration of aggressive behavior in isolated mice. Consistent with these data, administration of desipramine (10 mg/kg, IP) significantly increased the duration of aggressive behavior in DSP-4-untreated isolated mice (Fig. 1). When tested the aggressive behavior 3 days after administration of either saline or DSP-4 (50 mg/kg, IP) (Fig. 1A), no significant difference in the basal duration of aggressive behavior between DSP-4-treated and vehicle-treated mice was observed. However, desipramine (10 and 20 mg/kg, IP) did not increase the duration of aggressive behavior in the DSP-4 treated isolated mice ($H = 0.275$; $p > 0.05$). Moreover, when examined 14 days after DSP-4 treatment, the basal aggressive responses of the DSP-4-treated mice were slightly but not significantly higher than those of vehicle-untreated mice. Desipramine administration did not significantly affect aggressive behavior in the DSP-4-treated animals ($H = 0.166$; $p > 0.05$)(Fig. 1B).

DISCUSSION

The neurotoxic effect of DSP-4 has been found to be rather selective for noradrenaline-containing nerve terminals, since systemic administration of DSP-4 does not severely affect serotonergic or dopaminergic systems (7,8,12). Consistent with these findings, the present data demonstrated that DSP-4 treatment selectively decreased noradrenaline levels in various regions of brain of isolated mice without affecting dopamine and serotonin contents. Moreover, the effects of the treatment in these regions appeared in the following rank of order: cor-

tex ≥ hippocampus > cerebellum > > hypothalamus. These regional differences in noradrenaline depletion are consistent with previous reports by other groups (8), and they are probably due to the fact that DSP-4 treatment preferentially degenerates noradrenaline nerve terminals projecting from the locus coeruleus (1,5–8).

As previously reported (9–11), desipramine administration significantly increased the duration of aggressive behavior in long-term isolated mice. This finding disagrees with the data reported by other groups. For example, Valzelli (15) reported that the same dose of desipramine, as well as imipramine and nortriptyline, did not affect the aggressive behavior induced by isolation in mice. On the other hand, Sofia (14) demonstrated that both desipramine and imipramine attenuated isolation-induced aggressive behavior in mice. The discrepancy between our and their findings still remains unclear, but it may be due to the difference in the analyzing methods of aggressive behavior, or animals strains used, or both.

In the present study, the lesion of noradrenaline nerve terminals produced by DSP-4 markedly attenuated the desipramine-induced enhancement of aggressive behavior. These findings support the hypothesis that noradrenaline accumulated in the synaptic cleft by desipramine plays important roles in the enhancement of aggressive behavior in isolated mice (4), but also suggest the possibility that serotonergic system in the brain does not participate in the antidepressant-induced increase in aggressive behavior in isolated mice.

The functional changes in catecholaminergic system in the brain have been proposed to participate in the appearance of aggressive behavior following long-term social isolation in mice. However, in contrast to the effect on desipramine-induced enhancement of aggressive behavior, the DSP-4 treatment failed to change the basal aggressive responses in isolated mice. These findings disagree with the data reported by Ross and Ogren (13). These authors demonstrated that dopamine-β-hydroxylase inhibitors decreased the aggressive behavior in isolated mice, and that the isolation-induced aggressive behavior required an intact synthesis of noradrenaline. The reason for this discrepancy between their and our findings remains unclear, but it may be due to difference in the selectivity of action of drugs used. Nevertheless, taken into account the fact that the locus coeruleus innervates the regions most severely degenerated by DSP-4 treatment, the noradrenergic nerve terminals, especially those projecting from the locus coeruleus, do not seem to be involved in the pathophysiological mechanisms underlying the appearance of basal aggressive behavior in isolated mice.

ACKNOWLEDGMENTS

The present work was supported in part by a Grant-in-Aid (#04671346) for Science Research from the Ministry of Education, Science and Culture, Japan, and by Foundation for the Promotion of Higher Education in Toyama Prefecture.

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