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Facilitation of Shock-Induced Fighting in the Rat After DSP-4, a Selective Noradrenergic Neurotoxin

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MOGILNICKA, E., D. J. DOOLEY, C. G. BOISSARD AND A. DELINI-STULA. *Facilitation of shock-induced fighting in the rat after DSP-4, a selective noradrenergic neurotoxin.* PHARMACOL BIOCHEM BEHAV 18(4) 625–628, 1983.— Rats treated with DSP-4 showed a marked enhancement of shock-induced fighting (SIF). Administration of l-propranolol attenuated or completely counteracted SIF in control animals, but only the highest dose (10 mg/kg) of this β-adrenergic antagonist was effective in reducing SIF in DSP-4 animals. Other behavioral experiments indicated that the responsiveness of DSP-4 rats to dopaminergic, serotoninergic, and cholinergic agonists was unchanged compared to that of control rats. The results confirm the participation of the noradrenergic system in SIF, and substantiate an involvement of β-adrenergic receptors in this kind of aggression.

DSP-4 Noradrenaline depletion l-Propranolol Shock-induced fighting

IRRITABLE aggression, as defined by the model of shock-induced fighting (SIF) in the rat or mouse, apparently involves the central noradrenergic system [4] though its exact role is still unknown. SIF is increased by treatments facilitating noradrenergic transmission; for example, rubidium ion [7], sleep deprivation [13], and immobilization stress [12]. Conversely, however, other data indicate that SIF is also enhanced by chemical or electrolytic destruction of noradrenergic neurons [6, 11, 18].

In the present study, we investigated if the lesion produced by DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine)], a selective noradrenergic neurotoxin [3,9], would have any effect on SIF. This new neurotoxin easily crosses the blood-brain barrier after peripheral administration [16], and produces a marked and long-lasting depletion of central noradrenaline (NA) in those brain regions innervated by the locus ceruleus [3, 9, 15]. One consequence of such NA depletion is supersensitivity of β-adrenergic receptors in neocortex [3,9] and hippocampal formation [3]. Because of this receptor supersensitivity and the selective action of DSP-4, we used rats treated with DSP-4 to further evaluate the role of the noradrenergic system, and particularly that of β-receptors, in SIF. In addition, we determined the responsiveness of DSP-4 animals to dopaminergic, serotoninergic, and cholinergic agonists in other behavioral experiments.

METHOD

Subjects

Male rats (Tif: RAI f (SPF), Tierfarm Sisseln, Switzerland, weighing 200–220 g) were housed in groups of 5 animals per cage under standard laboratory conditions (a continuous 12-hr light dark cycle, constant humidity and temperature) with free access to food and water.

Noradrenaline Depletion

The animals were prepared as previously described [3]. Briefly, they were pretreated with CGP 6085 A (2.7 mg/kg IP), a selective serotonin uptake inhibitor [19], 30 min before the injection of DSP-4 (63 mg/kg IP) or the control solution (0.9% saline). The control and DSP-4 rats were then used in experiments 10 days later. At 10 days after DSP-4 injection, results of previous neurochemical experiments [3] indicate substantial reductions of NE in neocortex, hippocampal formation, cerebellum and spinal cord (<25% of control ME remaining).

Behavioral Testing

Shock-induced fighting. Pairs of rats (DSP-4–DSP-4 and control-control) were formed at random and placed in a

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TABLE 1
SHOCK-INDUCED FIGHTING IN THE RAT AFTER DSP-4

Group	Dose (mg/kg)	Latency (sec)	Number of fights	Total time of fighting (sec)	n
Control		20	17	42	15
Control + l-Propranolol	5	70*	3.5*	5.5*	6
DSP-4		8**	38*	95*	15
DSP-4 + l-Propranolol	5	18.5	40*	100*	6
Control		78.5	13.5	33	10
Control + l-Propranolol	10	300	0	0	7
DSP-4		11*	29**	74.5**	10
DSP-4 + l-Propranolol	10	30.5*	5.5*	12.5	10

l-Propranolol was administered intraperitoneally 1 hr before the test.
All values are expressed as medians; n=number of pairs.
Asterisks indicate a significant difference (*$p<0.05$, **$p<0.02$) from the saline group as analyzed using the Mann-Whitney U-test.

shock box (24×24×42 cm). After a 1-min adaptation period, fighting was induced by electric footshock. A constant current, every 3 sec for 5 min at 1 mA intensity and 0.5 sec duration, was delivered by a shock scrambler (Ruedin LAS 8010) through an electrificable grid-floor. The latency to the onset of the first attack, the number of attacks, and the duration of fighting were assessed during the 5-min period. An attack was scored when an animal, in response to shock, assumed an upright posture and faced its partner with forepaws in touch. The duration of fighting was defined as the total time the rats spent in an upright position during the 5 min of stimulation.

Apomorphine stereotypy. Apomorphine-induced stereotypy (0.5 mg/kg SC) was rated according to Costal *et al.* [2] at 10 min intervals for 1 hr.

L-5 Hydroxytryptophan-syndrome. The L-5-HTP-syndrome (100 mg/kg IP) was scored as described by Ortmann *et al.* [14].

Oxotremorine-induced tremor. The intensity of tremor induced by oxotremorine (0.5 mg/kg SC) was evaluated according to Weinstock *et al.* [20] at 5-min intervals until 30 min had elapsed.

Cumulative scores for each rat were calculated for the 30-min (oxotremorine) or 1-hr (apomorphine, L-5 HTP) period. Results are expressed as means±S.E.M.

Drugs

DSP-4 hydrochloride and CGP 6085 A [4-(5,6)-dimethyl-2-benzofuranyl) piperidine hydrochloride] (CIBA-GEIGY) were injected in a volume of 5 ml/kg. Each solution of DSP-4, sufficient for 5 animals, was prepared immediately before use. Apomorphine hydrochloride (Sandoz), L-5-hydroxytryptophan (Sigma), oxotremorine (Fluka), and l-propranolol hydrochloride (CIBA-GEIGY) were administered in a volume of 2 ml/kg.

Data Analysis

The results were evaluated by the Mann-Whitney U-test. The minimal level of significance was $p<0.05$ (two-tailed criterion).

RESULTS

DSP-4 rats exhibited a marked enhancement of SIF compared to control rats. The latency to the first attack was significantly shortened, and the number of fights and total time of fighting were significantly increased (Table 1). DSP-4 rats fighted approximately twice as often as control animals; their attacks were more vigorous and accompanied by intense vocalization.

Depending on the dose, l-propranolol markedly reduced or prevented SIF in control rats. After 5 mg/kg of l-propranolol, the latency to the first attack was prolonged, and the number of fights and total time of fighting were reduced by ~80%. After 10 mg/kg, fighting was not observed and vocalization was not as frequent. In DSP-4 rats, however, l-propranolol at a dose of 5 mg/kg did not affect fighting frequency or duration; only the latency to the first attack was prolonged. At a dose of 10 mg/kg, though, this β-antagonist significantly reduced SIF (Table 1).

In comparison to control animals, the responsiveness of DSP-4 rats to apomorphine, L-5 HTP or oxotremorine was unchanged (mean±S.E.M., n=8): apomorphine-induced stereotypy—control=9.8±0.3, DSP-4=9.4±0.4; L-5 HTP syndrome—control=4.2±0.7) DSP-4=4.3±0.7; oxotremorine-induced tremor—control=6.2±1.0, DSP-4=8.2±1.0.

DISCUSSION

The results confirm previous findings regarding the facilitation of SIF after the destruction of noradrenergic

neurons [6, 10, 11, 17, 18]. Specifically, DSP-4 rats are more sensitive than control animals to footshock stimulation as indicated by the shortened latency to the first attack, the increases in the number of fights, and the total time of fighting.

The facilitation of SIF after DSP-4 is assumed to be entirely due to a deficit in central NA. This reasoning is based on the selective action of DSP-4 on noradrenergic neurons; dopaminergic, serotoninergic, and cholinergic neurons appear to be unaffected by DSP-4 as indicated by practically unchanged concentrations of the neurotransmitters associated with these neurons [3, 9, 15]. Moreover, DSP-4 rats have normal responsiveness to dopaminergic, serotoninergic and cholinergic agonists as demonstrated in the present study. The possibility that depletion of peripheral NA is responsible for the increase in SIF is also unlikely; peripheral NA depletion by DSP-4 is transitory with recovery occurring within 10–14 days [1, 3, 9].

An altered pain sensitivity may be involved in the increased SIF of DSP-4 animals. Evidence for or against this supposition is, at present, rather limited. What is known, however, is that the tail-flick and hot-plate responses of DSP-4 rats are not changed compared to those of control rats ([1]; D. Dooley, unpublished results). This fact indicates that the reflex response to painful stimuli is intact after DSP-4 treatment, yet does not exclude alterations in other types of pain sensitivity, especially those with a major supraspinal component.

The present results would suggest, in accordance with previous observations [6,8] that NA tonically inhibits SIF. However this assumption seems contradicted by other findings [18], which indicate that α-methyl-p-tyrosine (a tyrosine hydroxylase inhibitor) and disulfiram (a dopamine-β-hydroxylase inhibitor) do not change SIF. There is the distinct possibility, though, that neither of these substances have the selectivity of DSP-4.

As mentioned in the introduction, there are reports indicating a facilitatory role of NA in SIF. Eichelman *et al.* [7] found that enhanced NA metabolism by rubidium ion or antidepressants increased SIF. Enhanced noradrenergic transmission after sleep deprivation [13] or immobilization stress [12] was also assumed to be responsible for the increase of SIF. Again, however, none of these treatments selectively affects the noradrenergic system; consequently, an involvement of other neurotransmitter systems cannot be ruled out.

Some evidence indicates the involvement of β-receptors in SIF, Kantak *et al.* [10] suggested that these receptors were important for enhancing SIF in rats lesioned with 6-hydroxydopa. Since 6-hydroxydopamine produces β-receptor supersensitivity [17], the remaining endogenous NA may be sufficient to activate these receptors; thus, the increase of aggression produced by destruction of noradrenergic neurons may be consistent with the facilitatory role of NA in SIF. This mechanism—increase of SIF via supersensitive β-receptors—could also be relevant to lesions induced by DSP-4; Dooley *et al.* [3] found a substantial increase (~30%)of β-receptor density in rat neocortex and hippocampal formation 10 days after DSP-4 injection.

A participation of β-receptors in SIF is further supported by the results with the β-antagonist, l-propranolol. This drug, at a dose of 5 mg/kg, markedly attenuated SIF in control rats, but not in DSP-4 rats. Such a result could be interpreted to mean that the dose of l-propranolol was high enough to partially reduce the aggressive response of control animals, yet insufficient to alter the same response of DSP-4 animals having β-receptor supersensitivity. The SIF of DSP-4 rats was substantially reduced only by 10 mg/kg of l-propranolol, a dose which completely prevented irritable aggression in control animals. Of relevance is that chronic administration of propranolol increases SIF in rats, probably through β-receptor supersensitivity [5]. Considering that both chronic treatment with propranolol and depletion of central NA by DSP-4 facilitate SIF, it is entirely plausible that β-receptors mediate this phenomenon since supersensitive β-receptors are a common effect of the two treatments.

In conclusion, the results confirm a role of the noradrenergic system in SIF. These results, moreover, suggest the involvement of β-receptors in this kind of aggression.

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