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Monoaminergic Changes in Locus Coeruleus and Dorsal Raphe Nucleus Following Noradrenaline Depletion

Tommaso Cassano,

Department of Biomedical Sciences, University of Foggia, Viale Luigi Pinto, 1, 71100 Foggia, Italy

Silvana Gaetani,

Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy

Maria Grazia Morgese,

Department of Biomedical Sciences, University of Foggia, Viale Luigi Pinto, 1, 71100 Foggia, Italy

Teresa Macheda,

Department of Biomedical Sciences, University of Foggia, Viale Luigi Pinto, 1, 71100 Foggia, Italy

Leonardo Laconca,

Department of Biomedical Sciences, University of Foggia, Viale Luigi Pinto, 1, 71100 Foggia, Italy

Pasqua Dipasquale,

Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy

Juan Taltavull,

Integrative Neuroscience Section, Behavioral Neuroscience Branch, National Institute on Drug Abuse, Baltimore, MD 21224, USA

Toni S. Shippenberg,

Integrative Neuroscience Section, Behavioral Neuroscience Branch, National Institute on Drug Abuse, Baltimore, MD 21224, USA

Vincenzo Cuomo, and

Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, Rome, Italy

Gabriella Gobbi

Department of Psychiatry, McGill University, Montréal, QC, Canada

Tommaso Cassano: tommaso.cassano@unifg.it

Abstract

The goal of our study was to assess the monoaminergic changes in locus coeruleus (LC) and dorsal raphe nucleus (DRN) following noradrenaline (NA) depletion. Seven days after a single *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) intraperitoneal administration in mice, we observed a decrease of NA in both the LC and DRN, as well as in prefrontal cortex (PFC) and hippocampus (HIPP). Moreover, an increase of serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) was detected at LC level, while no change was found in DRN. DSP-4 also caused a significant decrease of dopamine (DA) tissue content in HIPP and DRN, without affecting the LC and the PFC.

Correspondence to: Tommaso Cassano, tommaso.cassano@unifg.it.

T. Cassano and S. Gaetani have contributed equally to the present study.

A decrease of DA metabolite, homovanillic acid (HVA), was found in the DRN of NA-depleted mice. These results highlight that the neurotoxic action of DSP-4 is not restricted to LC terminal projections but also involves NA depletion at the cell body level, where it is paralleled by adaptive changes in both serotonergic and dopaminergic systems.

Keywords

Locus coeruleus; Dorsal raphe; Noradrenaline; Serotonin; Dopamine; DSP-4

Introduction

The brainstem monoaminergic nuclei, the noradrenergic locus coeruleus (LC) and serotonergic dorsal raphe nucleus (DRN), are characterized by their extensive efferent projections throughout the neuraxis [1]. Interactions between these two monoaminergic nuclei are of great functional importance. In fact, both systems are involved in the mediation of several important central nervous system functions including stress response, sympathetic control, regulation of attentional processes and memory consolidation [2–4]. Interactions between the LC and DRN impart a significant NA influence on the 5-HT system [5] and evidence has accumulated for a reciprocal relationship between these two nuclei as well [6,7].

In this regard, it is well established that adrenergic projections originating from the LC influence the activity of DRN 5-HT neurons [8]. In fact, pharmacological studies have suggested that the firing activity of 5-HT neurons in the DRN is dependent on a tonic activation by a noradrenergic input mediated via α_1 -adrenoceptors, and that α_2 -adrenoceptor [9,10] activation reduces 5-HT synthesis in the DRN [11]. Several studies have shown that the 5-HT system also influences brain NA neurons. In fact, electrophysiological and biochemical data have revealed an inhibitory role of 5-HT on the function of LC NA neurons. In particular, the activation of 5-HT₁ receptors reduces both glutamate-induced activation and glutamatergic synaptic potentials of LC NA neurons [12,13] and the 5-HT₂ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane induces an increased activation of the γ -aminobutyric acid (GABA) inhibitory input to the LC [14].

The selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) is widely used to lower brain NA to investigate the functions of the central noradrenergic system [15–17]. In fact, in vitro and in vivo studies showed that DSP-4, when administered systemically, can produce in the brain of rats and mice long-lasting reductions of endogenous NA, DA- β -hydroxylase activity and [³H]NA uptake [16,18,19]. The selectivity for noradrenergic neurons is mainly attributable to its high affinity for the NA re-uptake system. The observation that DSP-4 neurotoxicity is prevented by the NA transport (NAT) blocker, desipramine, strongly supports the hypothesis that DSP-4 enters neuron through the NA re-uptake mechanism, although it is not clear whether the primary deficits are mediated at the level of the axonal membrane or at intraneuronal sites [16,20].

Whilst the consequences of DSP-4 toxicity on the forebrain regions innervated by noradrenergic LC projections, particularly the hippocampus (HIPP) and cortex, have been quite well documented [21–23], no study has analysed, so far, the neurochemical alterations produced in the LC, where most of the noradrenergic cell bodies sending out these projections are localized. Based on the observation that NAT is highly expressed also in the LC complex [24], it is reasonable to hypothesize that DSP-4 can accumulate directly in the soma of noradrenergic neurons, thus causing a depletion of NA not only at distal axons and terminal level, but also at the cell body level.

Moreover, as 5-HT neurons of the DRN receive dense noradrenergic innervation originating from the LC and are under tonic activation by noradrenergic input [25–28], the question arises of whether DSP-4 lesion might affect also 5-HT transmission at the DRN level. This hypothesis is supported by previous research demonstrating that the expression of NAT within the DRN is very high [24,29].

The role of noradrenergic tone in the DRN is of considerable clinical importance as it is thought to affect the efficacy of a range of antidepressant treatments. In particular, afferent noradrenergic input to the DRN may modify the pharmacological effects of selective serotonin reuptake inhibitors (SSRIs) [30]. On the other hand, studies in mice unable to synthesize NA, due to targeted disruption of the DA- β -hydroxylase gene (*Dbh* $-/-$), demonstrated that some SSRIs may also require NA for their acute behavioural and neurochemical effects [31]. These studies further confirm the tight functional interactions between NA and 5-HT systems.

Moreover, there is growing interest for DA in the field of mood disorders, since drugs that enhance its transmission are clinically effective on their own. For example, the selective D₂/D₃ agonist pramipexole, used in the treatment of Parkinson's disease (PD), was shown to be effective in depression as a monotherapy [32], as well as an augmentation strategy for SSRI-resistant patients [33]. Conversely, the prevalence of depression can reach 50% in PD patients [34]. Taken together, these observations suggest that an attenuation of DA transmission could participate in the pathogenesis of mood disorders, possibly in part through interactions with the 5-HT and/or the NA system(s).

NA has a prominent role in the rewarding and addictive properties of DA-releasing drugs [35] and DSP-4-induced damage to noradrenergic neurons enhanced the neurotoxic effects of methamphetamine to the DA system [35–37]. Therefore, it appears crucial to examine the reciprocal interactions of these three types of neurons to understand the effects of medications and drugs of abuse acting on monoaminergic systems. In this regard, it is well documented that DA neurons of the ventral tegmental area (VTA) send projections to the DRN [38] and the LC [39], while in turn, receiving important inputs from the latter nuclei [40].

This study attempted to investigate the effects of DSP-4 on the noradrenergic system. In particular we attempted to address the following questions: (1) does DSP-4 deplete NA in the LC-noradrenergic and DRN-serotonergic cell bodies (or nuclei) as well as their distal axons/terminal and (2) does DSP-4 induce compensatory changes in both the 5-HT and DA systems, by affecting the reciprocal interactions between the VTA, DRN and the LC.

To this aim, all our experiments were performed in adult mice, 7 days after the intraperitoneal (i.p.) administration of DSP-4 (25 or 50 mg/kg), and procedures adhered to a well characterised protocol described by Hallman and Jonsson [15].

Experimental Procedure

Drugs

DSP-4 (25 mg/kg or 50 mg/kg) was purchased from Tocris (Cookson Ltd, Bristol, UK) and diluted in saline to the appropriate concentration. All reagents were analytical grade and were obtained from Sigma Chemical Co (St. Louis, MO, USA) and Fluka BioChemica (Ronkonkoma, NY, USA).

Animals and Drug Treatment

Male Swiss mice (Harlan, S. Pietro al Natisone, Udine, Italy) weighting 20–30 g were housed 4–5 per cage in a temperature-controlled room (22 $\pm$ 1°C) and maintained on a 12:12 h cycle (lights on 06:00–18:00). Food in pellet form and water were available ad libitum. The study

was performed in accordance with guidelines released by the Italian Ministry of Health (D.L. 116/92), the declaration of Helsinki, and the guide for the care and use of laboratory animals, published by the National Institutes of Health. All efforts were made to minimize the number of animals used in the study and their suffering.

Mice were injected i.p. with DSP-4 (25 or 50 mg/kg) or vehicle (saline), and returned to their cages until being tested. 7 days later, mice were killed by cervical dislocation.

Monoamine and Monoamine Metabolites Assay in Brain Tissues

After the animals were killed, their brains were rapidly excised and either immediately frozen on dry ice for LC and DRN collection, or freshly dissected to isolate HIPP and prefrontal cortex (PFC). LC and DRN were collected following a procedure obtained by modifying a previous published protocol [41]. Each frozen brain was placed on its dorsal surface in the trough of an ice-cold brain cutting block. Single razor blades (kept on ice) were carefully inserted through the cutting channels slicing the brain at right angles to its sagittal axis. The brain was positioned so that a razor blade was initially inserted tangentially to the most posterior aspects of the olfactory tubercles. This initial razor blade sliced through the sagittal plane of the brain at the level of the main body of the anterior commissure. The position of the initial razor blade served as a reference point from which further brain sections were obtained. Three razor blades were inserted posterior to this first blade, along the caudal extent of the brain, as shown in Fig. 1. The third and fourth razor blades were removed from the block with the coronal brain slices adhering to their surfaces and were placed on the surface of a petri dish containing crushed ice. The brain regions were then dissected from these slices, under a stereomicroscope using a needle of 0.3 mm internal diameter. Such a small needle was chosen to minimize the contamination of dissected structures by surrounding tissue, as the tissue punches obtained were considerably smaller than the full size of the region of interest [28].

Soon after collection, all tissue samples were sonicated in ice-cold perchloric acid 0.1 M and then centrifuged at $10,000\times g$ for 8 min at 4°C. Aliquots of both HIPP and PFC homogenates were saved for protein analysis, which was not performed for LC and DRN due to the small tissue size. Supernatants were collected and used for monoamine and monoamine metabolites assay.

The endogenous levels of monoamines (NA; 5-HT; DA) and both 5-HT and DA metabolites (5-HIAA, HVA and 3,4-dihydroxyphenylacetic acid, DOPAC) were assayed by HPLC coupled to an electrochemical amperometric detector, with the working electrode set at +650 mV versus an Ag/AgCl reference electrode. Analytes extracted from LC and DRN were separated using a SphereClone 150-mm $\times$ 2-mm column (3- μ m packing) and a mobile phase composed of 85 mM of sodium acetate, 0.34 mM EDTA, 15 mM sodium chloride, 0.81 mM of octanesulphonic acid sodium salt, 5% methanol (v/v) (pH = 4.85) delivered at a flow rate of 220 μ l/min. Chromatographic separation of analytes extracted from HIPP and PFC was conducted under the following conditions: (1) a BAS C-18 100 $\times$ 1.0 mm column (5- μ m packing); (2) a mobile phase consisting of 10 mM citric acid, 21 mM Na₂HPO₄, 0.2 mM EDTA, 0.75 mM sodium octanesulfate, 5 mM TEA and 13% methanol (v/v) adjusted to pH 5.3; (3) a flow rate of 60 μ l/min.

For each analysis, a set of standards containing various concentrations of each compound (monoamines and their metabolites) was prepared in the acid solution to obtain appropriate calibration curves. The concentrations of compounds in the supernatant were determined by linear interpolation from standard curves and were normalized either to the weight of the wet tissue sample, (for analytes extracted from LC and DRN) or to the protein content (for analytes extracted from HIPP and PFC). Results were expressed as mean $\pm$ SEM.

Statistical Analysis

Statistical comparisons of the average monoamine levels were performed initially by one-way ANOVA, after verifying the normality of distribution by a Kolmogorof-Smirnov test. If any statistical change was observed, data were further analysed using post hoc comparison, with a Dunnett's multiple comparison test to detect eventual differences between control and DSP-4 treated groups.

Results

Effects of DSP-4 Administration on Endogenous Monoaminergic Levels in the LC and DRN

In the LC, NA levels were significantly reduced following DSP-4 administration ($F_{(2,26)} = 5.5$, $P < 0.01$, $n = 8-10$) in the following dose dependent manner (Fig. 2a): vehicle = 15.9 ± 3.2 pmol/mg of tissue; DSP-4 25 mg/kg = 8.3 ± 1.2 pmol/mg of tissue (a reduction of 48% vs. vehicle); DSP-4 50 mg/kg = 6.0 ± 1.2 pmol/mg of tissue (a reduction of 62% vs. vehicle). Post hoc Dunnett's test indicated that both doses were significantly different from control group ($P < 0.05$ and $P < 0.01$, respectively).

In the DRN, NA levels were also significantly reduced following DSP-4 administration ($F_{(2,28)} = 19.21$, $P < 0.0001$, $n = 8-12$) in the following dose dependent manner (Fig. 2b): vehicle = 10.2 ± 0.9 pmol/mg of tissue; DSP-4 25 mg/kg = 6.6 ± 1.1 pmol/mg of tissue (a reduction of 36% vs. vehicle); DSP-4 50 mg/kg = 2.7 ± 0.5 pmol/mg of tissue (a reduction of 73% vs. vehicle). Post hoc Dunnett's test indicated that both doses were significantly different from control group ($P < 0.05$ and $P < 0.01$, respectively).

Following DSP-4 administration 5-HT levels were increased in the LC but were unaffected in the DRN. In particular, in the LC 5-HT levels were significantly increased following the DSP-4 administration at the highest dose ($F_{(2,27)} = 6.3$, $P < 0.01$, $n = 7-12$) (Fig. 2c): vehicle = 2.5 ± 0.3 pmol/mg of tissue; DSP-4 25 mg/kg = 2.3 ± 0.4 pmol/mg of tissue; DSP-4 50 mg/kg = 4.7 ± 0.8 pmol/mg of tissue (an increase of 84% vs. vehicle; $P < 0.01$, Dunnett's test).

In the DRN 5-HT levels were unchanged by DSP-4 treatment ($F_{(2,29)} = 0.05$, n.s., $n = 9-12$). In particular, the following concentrations were found (Fig. 2d): vehicle = 6.8 ± 1.6 pmol/mg of tissue; DSP-4 25 mg/kg = 6.1 ± 0.9 pmol/mg of tissue; DSP-4 50 mg/kg = 6.6 ± 1.6 pmol/mg of tissue.

The level of the 5-HT metabolite, 5-HIAA, also mimicked this pattern, remaining unchanged in DRN ($F_{(2,28)} = 0.06$, n.s., $n = 8-12$) but significantly increasing (+82%) in LC ($F_{(2,27)} = 3.7$, $P < 0.05$, $n = 7-12$) (Table 1).

In DRN, DA levels were also significantly decreased following DSP-4 administration ($F_{(2,28)} = 5.9$, $P < 0.01$, $n = 9-13$) at the highest dose (Fig. 2f): vehicle = 1.4 ± 0.2 pmol/mg of tissue; DSP-4 25 mg/kg = 1.5 ± 0.4 pmol/mg of tissue; DSP-4 50 mg/kg = 0.5 ± 0.1 pmol/mg of tissue (a decrease of 62% vs. vehicle; $P < 0.05$, Dunnett's test).

At the 50 mg/kg dose of DSP-4 a significant reduction (-42%, $P < 0.01$, Dunnett's test) of HVA, the main metabolite of DA, was also observed ($F_{(2,27)} = 6.8$, $P < 0.01$, $n = 7-12$) (Table 1).

In the LC, DA level was unchanged at both 25 and 50 mg/kg concentrations of DSP-4 ($F_{(2,28)} = 3.3$, n.s., $n = 9-13$). In particular, the following concentrations were found (Fig. 2e): vehicle = 1.0 ± 0.1 pmol/mg of tissue; DSP-4 25 mg/kg = 1.4 ± 0.3 pmol/mg of tissue; DSP-4 50 mg/kg = 0.8 ± 0.1 pmol/mg of tissue. The content of DA metabolite, HVA, was also unaffected at both doses of DSP-4 ($F_{(2,29)} = 3.1$, n.s., $n = 8-13$) (Table 1).

The concentration of the other DA metabolite, DOPAC, remained unchanged in both DRN ($F_{(2,29)} = 2.5$, n.s., $n = 8-13$) and LC ($F_{(2,27)} = 1.6$, n.s., $n = 7-13$) of DSP-4 treated mice (Table 1).

Effects of DSP-4 Administration on Endogenous Monoaminergic Levels in PFC and HIPP

As expected, DSP-4 at both doses (25 and 50 mg/kg) caused, 7 days after its administration, a marked reduction of endogenous NA levels in a number of LC-innervated regions.

One-way ANOVA confirmed that DSP-4 significantly decreased tissue content of NA in the HIPP ($F_{(2,13)} = 31.5$; $P < 0.0001$, $n = 4-5$) and PFC ($F_{(2,13)} = 26.4$; $P < 0.0001$, $n = 4-5$) (Fig. 3a, b). Hippocampal NA levels were 29.6 ± 0.85 pmol/ μ g protein in samples from vehicle-treated mice and resulted decreased by 58% (12.5 ± 3.1 pmol/ μ g protein) and by 77% (6.7 ± 2.4 pmol/ μ g protein) following DSP-4 25 and 50 mg/kg, respectively. Post hoc Dunnett's test indicated that both doses were significantly different ($P < 0.01$) from control group.

NA levels in PFC (vehicle = 22.4 ± 1.4 pmol/ μ g protein) were reduced by 33% (DSP-4 25 mg/kg = 15 ± 2.0 pmol/ μ g protein) and 65% (DSP-4 50 mg/kg = 7.8 ± 1.1 pmol/ μ g protein). The effects of the 25 and 50 mg/kg doses were significantly different ($P < 0.01$, Dunnett's test) from vehicle-treated group.

On the other hand, DSP-4 did not alter 5-HT levels in either HIPP ($F_{(2,12)} = 3.6$; n.s., $n = 4-5$) (vehicle = 41.6 ± 1.6 pmol/ μ g protein; DSP-4 25 mg/kg = 34.1 ± 2.9 pmol/ μ g protein; DSP-4 50 mg/kg = 34.0 ± 2.0 pmol/ μ g protein) or PFC ($F_{(2,15)} = 0.08$; n.s., $n = 4-6$) (vehicle = 56.9 ± 3.3 pmol/ μ g protein; DSP-4 25 mg/kg = 57.6 ± 4.8 pmol/ μ g protein; DSP-4 50 mg/kg = 58.8 ± 3.4 pmol/ μ g protein) (Fig. 3c, d). As well as 5-HT, DA in PFC was not significantly different from control at both doses of DSP-4 ($F_{(2,13)} = 0.8$; n.s., $n = 4-5$) (vehicle = 73.7 ± 42.2 pmol/ μ g protein; DSP-4 25 mg/kg = 92.0 ± 37.2 pmol/ μ g protein; DSP-4 50 mg/kg = 32.9 ± 12.5 pmol/ μ g protein) (Fig. 3f). On the contrary, one-way ANOVA revealed a significant decrease in hippocampal DA levels, 7 days after DSP-4 treatment ($F_{(2,13)} = 4.7$, $P < 0.05$, $n = 4-5$) (vehicle = 3.0 ± 0.2 pmol/ μ g protein; DSP-4 25 mg/kg = 2.2 ± 0.4 pmol/ μ g protein; DSP-4 50 mg/kg = 1.8 ± 0.3 pmol/ μ g protein) (Fig. 3e). The highest dose administered (50 mg/kg) produced a 41% reduction on DA endogenous level that resulted statistically significant ($P < 0.05$, Dunnett's test).

Discussion

To our knowledge, no study has fully investigated the neurochemical alterations and adaptive changes occurring in the LC and DRN of mice systemically administered with the selective noradrenergic toxin DSP-4. Thus, the most novel finding of our study is that DSP-4 causes a significant dose-dependent depletion of endogenous NA concentrations at both noradrenergic and serotonergic cell body levels and that profound adaptive changes in the monoaminergic system accompany NA depletion. The same treatment causes a destruction of noradrenergic projections (distal axons and terminals) ascending from the LC, such as PFC and HIPP, thus confirming previous results observed in a range of species and experimental approaches [26, 42].

Fritschy and Grzanna [43] hypothesised that LC neurons are susceptible to retrograde degeneration, following the induction of axonal lesions in adult rats within 2 weeks from DSP-4 administration. Here we show that LC alterations can be observed as early as 1 week following DSP-4 treatment, which suggests that measuring NA tissue levels might be a more sensitive approach to detect neurotoxic effects of the drug in LC perikarya. In this regard, some evidence suggests that the neurotoxic effects of this drug at noradrenergic cell body levels

might be not only due to retrograde degeneration but also to direct action at the LC itself, where NAT is highly expressed [24].

The neurotoxicity of DSP-4, although most thoroughly documented in rodents, has been demonstrated also in other species, including Zebra finches and in the songbirds. In the latter study, authors found that DSP-4 treatment profoundly decreased the number of dopamine- β -hydroxylase immunoreactivity cell bodies by 60% in the LC and 80% in the ventral subcoeruleus [44].

The highly significant dose-dependent decrease of NA levels found in the DRN may be due to the direct effects of DSP-4 on the dense plexus of noradrenergic fibres that arise in the pontine noradrenergic cell group and innervate the DRN [25,45]. In this regard, NAT has been identified also in the DRN [24] where it contributes to DSP-4-induced NA-depletion.

In addition, our study demonstrates that profound adaptive changes in other monoaminergic systems occur following NA depletion. Previous electrophysiological studies, focusing on the interactions between NA and 5-HT systems, found that LC exerts an excitatory control on 5-HT neurons of the DRN, mediated via α_1 -adrenoceptors [10,46]. Conversely, in our study the selective lesion of NA neurons elicited by DSP-4 (50 mg/kg) induced a significant increase in endogenous 5-HT levels (+84%) and in its metabolite, 5-HIAA (+82%) in the LC, thus suggesting that NA input might exert a tonic inhibitory effect on 5-HT neurons in the intact brain. Previous reports demonstrated that descending projections from the DRN to the LC account for at least 50% of the 5-HT innervation of this nucleus [47] and that LC receives afferents also from the median raphe nucleus [1]. We hypothesize that the increased 5-HT content in the LC of DSP-4 treated mice might be the consequence of enhanced 5-HT release not from DRN projections, but rather from median raphe-5-HT axons. This hypothesis is based on the observation that both 5-HT and 5-HIAA remained unchanged in the PFC and HIPP, which receive 5-HT innervations mostly from DRN. In support of this hypothesis, Saavedra et al. have demonstrated that 5-HT synthesis is enhanced in the median raphe nucleus, but not in the DRN, when NA transmission is attenuated in the LC [28]. In this context, a LC vs median-raphe-5-HT antagonism has been exhaustively demonstrated [42]. Likewise, Svensson and colleagues demonstrated that the spontaneous firing rate of DRN 5-HT neurons after 6-OHDA lesion remains unaltered, thus suggesting that a putative loss of NA would not have a sustained impact on 5-HT neuronal activity [10]. In conclusion, our results indicate that the adaptive 5-HT changes observed in the NA-depleted LC might be due to a disinhibition of the median raphe nucleus rather than the DRN.

Moreover, both doses of DSP-4 did not significantly alter 5-HT levels in the DRN. It is well known that the LC sends excitatory axons to the DRN; the excitatory effect is mediated directly through α_1 -postsynaptic receptors located at 5-HT neurons and indirectly inhibitory (GABA mediated) through α_2 -postsynaptic receptors [46,48]. In our work, the levels of 5-HT and 5-HIAA remain unchanged in the DRN, thus suggesting that a balanced disruption of both adrenoceptors might occur at the DRN somatodendritic level.

Furthermore, our study demonstrates that profound adaptive changes occur also at DA level following NA depletion. In particular, mice systemically treated with the highest dose of DSP-4 (50 mg/kg) showed a significant decrease of DA tissue levels in the HIPP (-41%) and DRN (-62%). A reduction in DA levels in the PFC (-55%) and LC (-22%) were observed as well, although they did not reach statistical significance. Devoto et al. [49] have suggested the possibility that DA might be released from sites other than classical dopaminergic terminals including noradrenergic synapses, where DA may act as both a NA precursor and a co-transmitter. This hypothesis is supported by a number of observations: (1) DA concentrations in areas scarcely innervated by the meso-cortical dopaminergic pathway were found to be only

slightly lower than in areas with massive dopaminergic afferents [49]; (2) DA in different cortical areas can be modified by drugs acting on noradrenergic transmission but not, or only modestly, by drugs that directly modify dopaminergic activity [50,51]; (3) local infusion into the LC of drugs activating noradrenergic neurons, as well as electrical stimulation of the LC, increased the extracellular levels of both NA and DA in the projecting areas [52,53]. Taken together, these findings suggest an involvement of dopaminergic transmission in the neurotoxicity of DSP-4, particularly evident at higher doses. However, divergent results have also been reported. For example, the lesion of LC caused by local injection of 6-OHDA, in addition to the NA depletion, induced an increase of DA neuronal activity rather than a reduction [54]. It is likely that such discrepancy with our results might be due to the different experimental protocol (e.g. routes of administration, animals and neurotoxin used). Nevertheless, our results are in line with a recent study showing that NA exerts a neuroprotective effect on DA neurons. In particular, it has been demonstrated that pharmacological stimulation of DA release is typically attenuated when NA signalling is blocked [35] and the baseline DA release is reduced following NA depletion [55].

Conclusions

The present study led to two major findings: (1) DSP-4 induces a significant dose-dependent depletion of endogenous NA in the LC-cell body area, that is comparable with those observed in other projection areas, including the PFC, HIPPO and DRN; (2) the neurochemical alterations induced by high doses of the selective noradrenergic toxin cause adaptive changes in both DA and 5-HT systems.

Although our study does not provide specific information about the mechanism by which the lesioning of noradrenergic neurons modulates other monoamine systems, it highlights the intimate interactions among these systems. Dissecting such complex interactions may be particularly important to better understand the pathophysiology of psychiatric disturbances and multiple degenerative disorders where a noradrenergic depletion is involved. In fact, the major neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases, are characterized by the loss of NA neurons in the LC [56,57].

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Fig. 1. Diagrammatic representation of coronal brain sections from which brain regions were dissected. *Circle lines* indicate borders of brain regions and *vertical lines* in the sagittal brain section indicate the four razor blades inserted through the cutting block

Fig. 2. Effect of DSP-4 (25 and 50 mg/kg i.p.) 7 days after treatment on NA, DA and 5-HT levels in LC and DRN of mice. Each bar represents the mean $\pm$ SE. Differences between groups were analysed via a one-way ANOVA followed by Dunnett's multiple comparison test where ANOVA was significant: * $P < 0.05$, ** $P < 0.01$ in comparison with vehicle group

Fig. 3. Effect of DSP-4 (25 and 50 mg/kg i.p.) 7 days after treatment on NA, DA and 5-HT levels in *HIPP* and *PFC* of mice. Each bar represents the mean $\pm$ SE. Differences between groups were analysed via a one-way ANOVA followed by Dunnett's multiple comparison test where ANOVA was significant: ** $P < 0.01$ in comparison with vehicle group

Table 1

Effect of DSP-4 (25 and 50 mg/kg i.p.) 7 days after treatment on endogenous 5-HIAA, HVA and DOPAC levels in DRN and LC of mice

	5-HIAA			HVA			DOPAC		
	Vehicle	DSP 25	DSP 50	Vehicle	DSP 25	DSP 50	Vehicle	DSP 25	DSP 50
DRN	2.13 ± 0.29	2.22 ± 0.59	2.00 ± 0.49	0.97 ± 0.07	0.97 ± 0.14	0.56 ± 0.07**	0.38 ± 0.06	0.51 ± 0.05	0.29 ± 0.06
LC	1.47 ± 0.15	1.41 ± 0.45	2.68 ± 0.53*	0.62 ± 0.05	0.82 ± 0.12	0.51 ± 0.09	0.14 ± 0.02	0.19 ± 0.04	0.12 ± 0.3

Each value (mean ± SE) was expressed as pmol/mg weight of the DRN and LC tissues. Differences between groups were analysed via a one-way ANOVA followed by Dunnett's multiple comparison test where ANOVA was significant:

* $P < 0.05$,
** $P < 0.01$ in comparison with vehicle group