Selective Lifelong Destruction of Brain Monoaminergic Nerves Through Perinatal DSP-4 Treatment

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Abstract *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) is a highly selective neurotoxin for noradrenergic projections originating from the locus coeruleus (LC). The outcome of the systemic DSP-4 treatment of newborn rats is an alteration in postnatal development of the noradrenergic system, involving the permanent denervation of distal noradrenergic projection areas (neocortex, hippocampus, spinal cord), accompanied by noradrenergic hyperinnervation in regions proximal to the LC cell bodies (cerebellum, pons–medulla). DSP-4 is well tolerated by developing rats and does not increase the mortality rate. Permanent noradrenergic denervation in the cerebral cortex and spinal cord is present at all developmental stages, although this effect is more pronounced in rats treated with DSP-4 at an early age, i.e., up to postnatal day 5 (PND 5). Notably, regional hyperinnervation is a hallmark of neonatal DSP-4 treatment, which is not observed after either prenatal or adult DSP-4 application. In contrast to robust biochemical changes in the brain, DSP-4 treatment of newborn rats has a marginal effect on arousal and cognition functions assessed in adulthood, and these processes are critically influenced by the action of the noradrenergic neurotransmitter, norepinephrine (NE). Conversely, neonatal DSP-4 does not significantly affect 5-hydroxytryptamine (serotonin; 5-HT), dopamine (DA), gamma-aminobutyric acid (GABA), and histamine levels in brain. However, as a consequence of altering the functional efficacy of 5-HT_{1A}, 5-HT_{1B}, DA, and GABA receptors, these neurotransmitter systems are profoundly affected in adulthood. Thus, the noradrenergic lesion obtained with neonatal DSP-4 treatment represents a unique neurobiological technique for exploring the interplay between various neuronal phenotypes and examining the pathomechanism of neurodevelopmental disorders.

Keywords DSP-4 · Neurotoxin · Degeneration · Depletion · Locus coeruleus · Noradrenergic neurons · Perinatal period

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1 Introduction

During the search of tertiary haloalkylamines related to bretylium and capable, once in the brain, of cyclizing by an intramolecular reaction to quaternary ammonium derivative with distinctive adrenergic neuron blocking activity, a compound was identified with a long-lasting inhibitory effect on [^{3}H]-labeled noradrenaline (NE) uptake by brain slices (Ross et al. 1973; Ross and Renyl 1976). This compound, *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), interacts with the NE transporter (NET), whereby DSP-4 is then accumulated intraneuronally to trigger the degeneration of noradrenergic terminals (Lyons et al. 1989). On the molecular level, a cellular energy collapse, bringing about lack of ATP, may serve as an explanation of DSP-4 neurotoxicity (Wenge and Bönisch 2009). Another hypothesis assumes that DSP-4 down-regulates the noradrenergic phenotypes by its actions on DNA replication, leading to replication stress and cell cycle arrest (Wang et al. 2014). However, the precise mechanism of DSP-4s' action still remains unclear. Markers of terminal loss are observed as a reduction in NE brain tissue content (Jonsson et al. 1982; Dabrowska et al. 2007; Nowak et al. 2008), decrease in NET density (Jonsson et al. 1981; Sanders et al. 2011), and temporary increase in α_2-adrenergic autoreceptor (Wolfman et al. 1994; Sanders et al. 2011). The changes

in these noradrenergic markers in specific brain regions suggest a selective loss of afferents from locus coeruleus (LC) noradrenergic neurons (Jonsson et al. 1981). The animal age at the time of DSP-4 administration is crucial for its biological response and long-lasting changes in noradrenergic system and other neuronal phenotypes (e.g., serotoninergic and dopaminergic) (Jonsson et al. 1981, 1982; Nowak et al. 2006; Dabrowska et al. 2008).

2 DSP-4 Treatment of Newborn Rats

DSP-4 is a highly selective neurotoxin for noradrenergic projections originating from the LC. DSP-4 is typically injected intraperitoneally (i.p.), occasionally subcutaneously (s.c.), or intravenously (i.v.) in adult rats or mice. Considering technical and methodological limitations, newborn rats, for systemic administration, are routinely administered s.c. injections (Donnerer et al. 1990; Jonsson et al. 1981). The systemic administration of this compound has a rapid dose-related effect on brain NE levels (Jaim-Etcheverry and Zieher 1980), sustained throughout the remaining lifetime (Jonsson et al. 1981). In contrast to the central nervous system (CNS), the absence of alterations in sympathetic neurons in the periphery (superior cervical ganglia) has been reported (Jaim-Etcheverry and Zieher 1980). The lowest tested DSP-4 dose (10 mg/kg s.c.) produces a NE reduction of approximately 50 % in the cerebral cortex and spinal cord, with negligible changes in the cerebellum and pons–medulla. Conversely, a large DSP-4 dose (50 mg/kg s.c.) incurs near complete NE depletion in the cerebral cortex and spinal cord, with concomitant increases in NE levels, both in the pons–medulla and cerebellum, although the effects are more pronounced in the latter region (Jonsson et al. 1982). A dose of 50 mg/kg of DSP-4 is well tolerated by developing rats, does not increase the mortality rate, and is the most efficient in producing a long-lasting reduction of NE reuptake in the brain, both in male and female rats (Jonsson et al. 1981). Similarly, DSP-4 administration schedules also differ between studies. A single injection scheme at postnatal days (PND) 1–3 is the most effective and frequently employed technique for the selective generation of NE phenotypes. However, DSP-4 can be administered to newborn rats once on postnatal day 1 (PND 1), twice on PND 1 and 3, and three times on PND 1, 3, and 5, but this compound loses selectivity with increased frequency dosing (Brus et al. 2004).

3 Noradrenergic System

3.1 Biochemical Alterations

The outcome of systemic DSP-4 treatment of newborn rats is an alteration in the postnatal development of the noradrenergic system involving the permanent denervation of distal NE projection areas, accompanied by NE hyperinnervation in

regions proximal to the LC cell bodies, likely 1980reflecting the collateral sprouting of noradrenergic neurons. Notably, the total number of NE nerve terminals remains unaltered (Jaim-Etcheverry and Zieher ; Jonsson and Sachs 1982).

Neonatal DSP-4 treatment leads to persistent changes of endogenous NE levels in several tested brain regions. In adulthood, NE is depleted approximately 90–98 % in the cerebral cortex (frontal, cingulate, parietal, occipital), hippocampus, spinal cord (lumbar) (Jonsson et al. 1982; Nowak et al. 2008, 2009), and olfactory bulb (Cornwell et al. 1996), while only 35 % depletion is observed in the striatum, following neonatal treatment (Dabrowska et al. 2007). Negligible effects are observed in the hypothalamus (Jonsson et al. 1982; Dabrowska et al. 2007). The hypothalamus receives the main noradrenergic input from the lateral tegmentum (LT) (Cryan et al. 2002), supporting previous findings that systemic pretreatment with DSP-4 selectively destroys noradrenergic terminals emanating from the LC. No significant changes in NE concentration are noted in the thalamus and medulla oblongata (Jonsson et al. 1982; Korossy-Mruk et al. 2013), while a pronounced increase in NE levels is observed in the cerebellum, brainstem, mesencephalon, and pons–medulla after neonatal DSP-4 treatment (Jonsson et al. 1982; Bortel et al. 2008c). Basically, following neonatal DSP-4 treatment, the level of the major NE metabolite, 3-methoxyl-4-hydroxyphenylethylene glycol (MHPG), is not affected in frontal cortex, hippocampus, hypothalamus, and striatum; consequently, MOPEG/NE metabolic ratios are significantly increased in frontal cortex, hippocampus, and striatum (Dabrowska et al. 2007) or unchanged in the brain stem and cerebellum (Bortel et al. 2008c).

Treating neonatal animals (on PND 3) with DSP-4 and harvesting brains on PND 32 produce a near complete elimination of NE in the cerebral cortex and hippocampus with a parallel reduction in the noradrenergic transporter (NET), as determined through autoradiographic analysis with [^{3}H]nisoxetine, a highly specific ligand for the NET (Sanders et al. 2011). When administered at birth, DSP-4 also reduces [^{3}H]NE reuptake in the spinal cord (93 %) but increases [^{3}H]NE reuptake in the cerebellum (94 %), with no significant alterations in the striatum. However, when measuring NE uptake in the entire brain, no significant changes are observed, suggesting that similar to neonatal 6-hydroxydopamine 6-(OHDA) treatment, the altered development of NE neurons induced through DSP-4 primarily reflects a redistribution of the NE nerve terminal projections of the LC without appreciably affecting the total number of NE terminals (Jonsson et al. 1982; Jonsson and Sachs 1982). Notably, pretreatment with desmethylimipramine (DMI; the NE uptake blocker) or pargyline (non-selective monoamine oxidase inhibitor) effectively counteracts the DSP-4-induced alterations of NE levels in the frontal and occipital cortex, stratum, pons–medulla, cerebellum, and spinal cord, with the exception of the olfactory bulb and hippocampus (only partial protection). Consistent with the results of the NE assay, DMI pretreatment completely abolishes the DSP-4-induced reduction of NE uptake in the cerebral cortex and increase in NE uptake in the cerebellum (Jonsson et al. 1982).

Permanent NE denervation in the cerebral cortex and spinal cord is present at all developmental stages, although this effect is more pronounced in rats treated with

DSP-4 at an early age (up to PND 5). NE reduction is gradually less marked following DSP-4 treatments as late as PND 14, after which the extent of NE depletion is constant into adulthood. Furthermore, NE hyperinnervation in the cerebellum and pons–medulla is only observed when DSP-4 is administered to one- and three-day-old rats, whereas when injected at eight days or older, permanent NE depletion in these structures is observed. Acute NE depletion (one day after DSP-4 treatment) occurs in animals of all ages, and the neurotoxic effect of this compound is rapid, being maximal at 2 h after DSP-4 injection for NE reuptake in vitro and 4–6 h for endogenous NE concentration (Jonsson et al. 1982).

Monoamine neurons respond to lesions with a wide range of compensatory adaptations aimed at preserving functional integrity, ranging from sensitization of the receptor (frequently characterized as the expression of more cell surface receptors or receptor up-regulation) to down-regulation or adaptation of second messengers (Giorgi et al. 2008; Kostrzewa et al. 2008). Basically, this is true when NE denervation is performed in adult rats (Heal et al. 1993; Wolfman et al. 1994; Kreiner et al. 2011) or when other phenotypes, such as dopamine (DA) or 5-hydroxytryptamine (serotonin; 5-HT), are observed (Pranzatelli and Gregory 1993; Sawynok and Reid 1994; Kostrzewa et al. 2011). DSP-4 lesions on PND 3 produce only transient α_2-adrenergic receptor reduction in brain at PND 5. The α_2-adrenergic receptor "recovers" to control levels at PND 15 and PND 25, and no further change in the total receptor density is detected. In addition, on PND 25, there is no alteration in the association of α_2-adrenergic receptors with G proteins (Sanders et al. 2011).

Interestingly, severe damage does not necessarily impair the activity of the NE system in terms of postsynaptic efficacy. There are a number of compensatory mechanisms (increased presynaptic turnover, leading to increased NE synthesis and release per surface unit), which maintain the level of extracellular NE at a sufficient rate to provide appropriate receptor stimulation, at least under basal conditions. This phenomenon is observed either when DSP-4 is administered to neonates (Nowak et al. 2004) or adult rats (Kask et al. 1997), as a consequence of volume transmission and/or reduced clearance due to reductions in NET number, an aftereffect of noradrenergic fiber degeneration (Hughes and Stanford 1998).

3.2 Behavioral and Biological Rhythm Alterations

Learning and memory processes are critically influenced through noradrenergic activity, and numerous studies in adult animals support this hypothesis (Khakpour-Taleghani et al. 2009; Reid and Harley 2010; Gazarini et al. 2013). In neonatal NE-depleted rats, some abnormalities can also be detected. Cornwell-Jones et al. (1990) reported attenuated olfactory learning in adult rats treated at the day of birth (PND 0) with DSP-4 (50 mg/kg), consistent with additional data suggesting that NE depletion impairs the adoption of a new olfactory environment after weaning (Cornwell-Jones et al. 1982). In addition, DSP-4 pretreatment (PND 0) disrupts the acquisition of odor, specifically odor association in the sensory

preconditioning paradigm, in 16-day-old rats, but no effects of DSP-4 are observed on first-order conditioning (Chen et al. 1993). In contrast, DSP-4 at PND 0 does not impair the memory consolidation of emotionally arousing tasks, such as inhibitory avoidance learning, when assessed at 28 days later (Cornwell-Jones et al. 1989). Similar studies yield variable and inconsistent results (Archer 1982; Bennett et al. 1990; Hauser et al. 2012). Interestingly, the slight effects of neonatal DSP-4 treatment on learning and memory processes in rats show close parallels and inconsiderable alterations in the biochemical mechanisms underlying cognitive function, e.g., immediate early gene expression in the brain for the regulation of neuroplasticity. Precisely, neonatal DSP-4 administration leads to only moderate increases or no changes in the levels of Arc, c-fos, and zif268 at PNDs 13, 25, and 60, in marked contrast to the effects of similar lesions in the adult brain (Sanders et al. 2008).

LC, the major noradrenergic nucleus in brain also mediates spontaneous and evoked arousal. The injection of DSP-4 into rats at PND 7 does not significantly affect sleep bout duration, although the wake bout duration and percentage of time awake are significantly increased when tested at PND 21 (Gall et al. 2009).

While basic mechanisms controlling sexual development are complex and controversial, the CNS, including the NE system, is an essential participant in this process (Martins-Afférri et al. 2003; Izvolskaia et al. 2009). A single exposure of five-day-old female rat pups to DSP-4 delays sexual maturation, measured as vaginal opening (VO) (i.e., 34.2 days with saline versus 36.1 days with DSP-4). However, DSP-4 rats showed a concomitant delay in weight gain, and both control and treated animals showed similar VO when adjusted to the same body weight (Jacobson et al. 1988). In conclusion, neonatal DSP-4 treatment marginally influences behavioral and biological rhythms in adulthood.

4 Serotoninergic System

4.1 Biochemical Alterations

Despite being regarded as a highly selective neurotoxin for the noradrenergic system, DSP-4, when administered in the absence of a 5-HT reuptake inhibitor (e.g., zimelidine), induces dose-dependent changes in endogenous 5-HT levels in brain. Neonatal DSP-4 treatment (50 mg/kg) significantly reduces endogenous 5-HT concentrations in the cerebral cortex (∼55 %), hippocampus (∼60 %), spinal cord (∼40 %), and cerebellum (∼30 %), when analyzed at the adult stage. In parallel with these observations, 5-HT reuptake in the occipital cortex, hippocampus, and cerebellum is reduced. No significant effects are observed in other brain regions (Jonsson et al. 1981, 1982). Interestingly, neonatal DSP-4 treatment does not produce any significant change in the endogenous 5-HT concentration and 5-HT reuptake up to one month after drug administration, when whole brain is analyzed (Jonsson et al. 1982).

The SERT blocker, zimelidine (10 mg/kg), abolishes the effects of DSP-4 on 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in frontal cortex, hippocampus, striatum, and hypothalamus without interfering with the action of this neurotoxin on noradrenergic nerve terminals (Jonsson et al. 1981, 1982; Dabrowska et al. 2007). In addition, no significant differences in the 5-HT synthesis rate in the frontal cortex, hippocampus, striatum, hypothalamus (Dabrowska et al. 2007), and cerebellum (Roczniak et al. 2015) between control and DSP-4-pretreated animals are observed. Under these conditions, despite the absence of changes in the content and synthesis rate of 5-HT in the brain, other adaptive alterations in the 5-HT system are detected. These alterations are not noticeable until biochemical responses are "enforced" through the stimulation or blocking of 5-HT receptors. Such alterations are obvious due to a mutual interaction between NE and 5-HT; e.g., numerous brain regions are innervated with both noradrenergic (origin, LC) and serotoninergic neurons originating from the dorsal raphe nuclei (DRN) and median raphe nuclei (MRN). Furthermore, LC, the major NE brain stem nucleus, sends projections to the DRN, while the DRN projects to the LC (Sim and Joseph 1993; Peyron et al. 1996) creating ample opportunity for cross-modulation (Millan et al. 2000a, b; Weikop et al. 2004). The scarcity of studies on this subject in neonatal DSP-4-lesioned rats makes it difficult to predict the net balance between NE vs. 5-HT. Moreover, endogenous NE exerts direct tonic stimulatory control on the release of 5-HT through α_1-adrenoceptors and an indirect tonic inhibitory influence through α_2-adrenoceptors located on noradrenergic nerve terminals within the raphe nuclei, although inhibitory α_2-heteroreceptors are also localized on terminals of serotoninergic neurons in corticolimbic structures (Haddjeri et al. 1995).

The stimulation of the somatodendritic 5-HT_{1A} autoreceptors decreases the cell firing rate, synthesis, turnover, and release of 5-HT within raphe nuclei and subsequently within serotonergic projection areas (Blier et al. 1998). Because NE might have an inhibitory effect on cortical 5-HT release (Haddjeri et al. 1995), noradrenergic denervation results in a moderate increase in basal 5-HT microdialysate content in the medial prefrontal cortex of neonatal DSP-4-treated rats. Also, systemic administration of the 5-HT_{1A} receptor agonist (R-(+)-8-OH-DPAT) induces a long-lasting reduction of extracellular 5-HT content in the medial prefrontal cortex of intact and DSP-4-pretreated rats, but this effect is significantly reduced in noradrenergic lesioned animals, likely reflecting the desensitization of 5-HT_{1A} autoreceptors in the raphe nuclei of such rats (Dabrowska et al. 2008). Conversely, in the same experimental model, the stimulation of 5-HT_{1B} receptors through the systemic administration of a specific agonist (CP 94253) does not evoke significant changes in 5-HT release in the medial prefrontal cortex between control and DSP-4-treated rats (Ferdyn-Drosik et al. 2010).

In the chromatographic assay of the 5-HT synthesis rate, the systemic administration of R-(+)-8-OH-DPAT to control rats inhibits 5-HT synthesis in the prefrontal cortex, hypothalamus, and striatum 42, 20, and 46 %, respectively, and this effect is antagonized through pretreatment with the 5-HT_{1A} receptor antagonist. Interestingly, R-(+)-8-OH-DPAT does not significantly inhibit the 5-HT synthesis rate in all examined brain structures of neonatal DSP-4-treated rats. These results,

together with microdialysis study, suggest the desensitization of 5-HT_{1A} autoreceptors in the raphe nuclei as a result of noradrenergic lesions (Dabrowska et al. 2008). Similar results concerning 5-HT_{1B} receptor stimulation have been reported, i.e., 5-HT_{1B} receptor agonist (CP 94253) reduces the 5-HT synthesis rate in medial prefrontal cortex of control rats (∼33 %), but with no effect on neonatal DSP-treated animals (Ferdyn-Drosik et al. 2010).

4.2 Behavioral Alterations

5-HT_{1A} receptors are located presynaptically on the soma and dendrites of 5-HT neurons of the DRN and MRN as inhibitory autoreceptors, and postsynaptically in the forebrain areas, including the hippocampus, lateral septum, and cortex (Hamon 2000). The stimulation of presynaptic 5-HT_{1A} receptors evokes behavioral responses, such as hyperphagia in satiated rats (Ebenezer 1992) and pigs (Ebenezer et al. 1999) and anxiolytic-like effects in rats (Jolas et al. 1995) and gerbils (File et al. 1996).

Dabrowska et al. (2008) observed that the 5-HT_{1A} receptor agonist (R-(+)-8-OH-DPAT) produces a significant increase in food intake in control rats, but fails to elicit a response in neonatal DSP-4-treated animals. In the anxiolytic-like activity assessment (plus maze test, social interaction test), R-(+)-8-OH-DPAT induces anxiolytic-like activity in control rats; however, this compound does not evoke an effect on DSP-4-lesioned rats. These findings lend support to the hypothesis of 5-HT_{1A} autoreceptor desensitization in neonatal NE-lesioned animals.

Conversely, behavioral responses reflecting postsynaptic 5-HT_{1A} receptor activation include stereotypic behavior in rats (O'Connell and Curzon 1996), an antidepressive effect in mice and rats (Luscombe et al. 1993; Schreiber and De Vry 1993), and hypothermia (Millan et al. 1993). In the forced swimming test and the learned helplessness test, R-(+)-8-OH-DPAT displays antidepressant-like activity to a similar extent in both control and DSP-4-lesioned rats, and this effect is antagonized through the 5-HT_{1A} antagonist, WAY 100635 (Dabrowska et al. 2008). Further studies reveals that R-(+)-8-OH-DPAT induces hypothermia and "5-HT_{1A} syndrome" to a similar extent in both control and NE denervated rats; these effects are completely antagonized by WAY 100635 (Dabrowska et al. 2007). Thus, these data suggest that the reactivity of postsynaptically located 5-HT_{1A} receptors remains unchanged after neonatal DSP-4 lesioning.

Systemic DSP-4 treatment of newborn rats also modulates behavioral responses mediated by the 5-HT_{1B} receptor. Activation of this receptor through a specific agonist (CP 94253) elicits anxiogenic-like effects in intact rats, as demonstrated in the elevated plus maze test (Lin and Parsons 2002). Notably, Ferdyn-Drosik et al. (2008, 2010) also observed that CP 94253 induced anxiogenic-like activity in control rats and depression-like behavior in the Porsolt test. However, no effects are detected in the DSP-4 group, suggesting the existence of regulatory mechanisms in DSP-4-lesioned animals altering the functional efficacy of 5-HT_{1B} receptors and, accordingly, affecting serotoninergic activity, as 5-HT_{1B} receptors are desensitized in these rats.

5 Dopaminergic System

5.1 Biochemical Alterations

Neonatal DSP-4 treatment (50 mg/kg at PND 1 and PND 3) does not acutely (i.e., directly) affect DA and DOPAC levels in the cortex, hippocampus, striatum, mesencephalon, pons–medulla, cerebellum, and spinal cord of adult rats (Jonsson et al. 1981; Nowak et al. 2006). In addition, rats treated with DSP-4 (50 mg/kg) at PND 7 do not exhibit significant changes in the DA or 5-HT concentration in cortical or non-cortical tissues (including medulla, midbrain, and diencephalon, minus the cerebellum) versus saline controls at PND 21 (Gall et al. 2009). In parallel, the DA synthesis rate, assessed as L-DOPA accumulation after aromatic amino acid decarboxylase inhibitor administration (hydroxybenzylhydrazine; NDS-1015), remains unchanged in several tested brain regions (hippocampus, striatum, and cerebellum) (Roczniak et al. 2015). Other studies report an adaptive decrease in the DA content in the occipital cortex (38 %), hippocampus (49 %), and mesencephalon (13 %) (Jonsson et al. 1982) or thalamus (50 %) (Donnerer et al. 1992).

5.2 Behavioral Alterations

The stimulation of specific DA receptors elicits distinct behavioral responses, e.g., acute DA D_1 agonist treatment of rats produces vacuous chewing movements, sniffing, and grooming behavior (Arnt et al. 1987; Hamdi and Kostrzewa 1991; Kostrzewa and Gong 1991). Acute "classical" DA D_2 agonist (e.g., apomorphine, piribedil) administration exerts biphasic effects on behavior, i.e., yawning and hypomotility at low doses and stereotypy and hypermobility at high doses (Butterworth et al. 1993; Eilam and Szechtman 1989; Mogilnicka and Klimek 1997). However, the yawning behavior elicited at low doses of mixed DA D_2/D_3 agonists (e.g., quinpirole) has been associated with DA D_3 receptor stimulation (Kostrzewa and Brus 1991a).

Neonatal DSP-4 treatment does not affect DA D_1 receptor agonist (SKF 38393)-evoked vacuous chewing movements and DA D_2 receptor agonist (quinpirole—high dose)-induced locomotor and exploratory activities in adulthood. Conversely, quinpirole (low dose)-induced yawning behavior is more prominent in the group lesioned with DSP-4 (Nowak et al. 2009).

Notably, repeated treatment with dopaminergic agonists (e.g., amphetamine, apomorphine, and quinpirole) produces an increased response, or sensitization (priming), to the drug-induced effects, referred to as DA receptor supersensitivity (Mattingly and Gotsick 1989; Nowak et al. 2005; Brus et al. 2003). Male rats treated for the first 28 days after birth with the dopamine D_2/D_3 agonist quinpirole develop lifelong sensitization of the central DA D_2/D_3 receptors that manifests as enhanced quinpirole-induced yawning behavior and motor behaviors in adulthood

(Kostrzewa and Brus 1991b). When DSP-4 is administered to rats (PND 1 and PND 3) to largely destroy noradrenergic innervation of the brain, the magnitude of quinpirole-induced action is significantly reduced. This observation suggests that intact central noradrenergic innervation is important for behavioral responses mediated through a variety of DA receptors and the expression of priming phenomenon (Nowak et al. 2006).

6 GABAergic System

6.1 Biochemical Alterations

Neonatal DSP-4 treatment of rats does not alter GABA tissue levels in prefrontal cortex, hippocampus, brain stem, and cerebellum and does not modify phenobarbital- or ethanol-evoked GABA reduction in the aforementioned structures (Bortel et al. 2008c). Similarly, under steady-state conditions, the microdialysate content of GABA in the prefrontal cortex in DSP-4 neonatally lesioned rats does not differ when compared to control rat. However, a twofold greater increase in the extracellular GABA level after vigabatrin (GABA transaminase inhibitor) injection is observed in DSP-4-lesioned rats (Bortel et al. 2008b).

The NE depletion of cerebral cortex and hippocampus produced by DSP-4 treatment is accompanied by a reduction (approximately 20 %) in the number of GABA-A receptors localized on the presynaptic axons and nerve terminals of NE neurons, and a decrease in the Bmax for the low-affinity GABA-B receptor site in cerebral cortex and hippocampus (25 and 28 %, respectively) (Suzdak and Gianutsos 1985). Neonatal DSP-4 treatment also reduces the number of benzodiazepine (BDZ) receptors in the cerebral cortex, while a significant increase in the number of BDZ receptors in the cerebellum occurs (Medina and Novas 1983). These results support the existence of functional coupling between the noradrenergic and GABAergic systems, i.e., decrease or increase in the density of BDZ receptors following the denervation (e.g., cortex) or hyperinnervation of some brain structures (e.g., cerebellum).

6.2 Behavioral Alterations

In the elevated plus maze test, a paradigm based on the conflict between the innate tendencies of rodents to explore novel environments versus an innate tendency to avoid open areas, systemic DSP-4 treatment of newborn rats does not affect spontaneous anxiety-like behavior in adulthood, but diminishes anxiolysis elicited through diazepam administration (Bortel et al. 2007). Neonatal DSP-4 treatment also leads to adulthood changes in the activity of sedative–hypnotic drugs, i.e., extends the time to the loss of righting reflex after phenobarbital and reduces sleep

time after ethanol administration (Bortel et al. 2008c). Moreover, reduced vulnerability of the pro-convulsant activity of bicuculline (GABA-A receptor antagonist) is observed in DSP-4-pretreated rats (Bortel et al. 2008a).

7 Other Neuronal Phenotypic Systems

Alterations of NE exocytosis in the thalamus, brain stem, and other nuclei alter the output of nociceptive information in higher brain centers from projection neurons (Post et al. 1985; Pagano et al. 2012; Suehiro et al. 2013). LC stimulation, which increases NE release in the spinal cord, inhibits nociceptive transmission in the dorsal horn via α_2-adrenergic receptors (Margalit and Segal 1979; Delaney et al. 2007; Jiang et al. 2010). In addition, LC neurons possess a high density of postsynaptic mu-opioid receptors (Van Bockstaele and Commons 2001), and cannabinoids modulate noradrenergic neuronal activity. Scavone et al. (2010) provided evidence for the heterogeneous distribution of CB_1 receptors in the LC and demonstrated that this receptor and mu-opioid receptors coexist in cellular profiles in this region, creating ample opportunity for interactions between cannabinoid and noradrenergic systems. Indeed, the destruction of noradrenergic neurons through neonatal DSP-4 treatment significantly decreases the antinociceptive effects of methanandamide (CB_1 receptor agonist) in the tail-immersion test, hot-plate test, and writhing test, with ambiguous results in paw pressure and formalin hind paw tests. Simultaneously, marked changes in the antinociceptive effects of methanandamide in DSP-4-treated rats are not accompanied by increases in CB_1 receptor density in the rat brain (Korossy-Mruk et al. 2013).

Neonatal DSP-4 treatment (PND 1 and PND 3) produces nearly imperceptible effects on the central histaminergic system in rats. Histamine content remains unchanged in the frontal cortex, hypothalamus, cerebellum, and medulla oblongata, and only a modest but significant reduction (31 %) in the hippocampus is observed in DSP-4 rats. In addition, exploratory activity, irritability, and nociceptive activity are unaltered after histamine receptor antagonist treatment, although locomotor activity and vacuous chewing movements are increased compared with control when histamine H_2 and H_3 antagonists are applied (Nowak et al. 2008).

DSP-4 administered to rat pups (PND 4) reduces [^{3}H]NE uptake in the cortex and hypothalamus approximately 90 and 37 %, respectively, at PND 7. Simultaneously, nicotine-stimulated [^{3}H]NE release from the neonatal hypothalamus is almost completely eliminated after prior lesioning of the LC. Hence, systems controlling critical homeostatic functions (stress, feeding, etc.) under nicotinic acetylcholine receptor (nAChR) influence are disrupted through NE denervation (O'Leary and Leslie 2006).

DSP-4 treatment of rats at PND 2 produces adaptive changes in the neuropeptide innervation in adulthood. Vasoactive intestinal polypeptide (VIP) is markedly elevated in the cortex, hippocampus, striatum, and medulla, whereas substance P (SP), neurokinin-like immunoreactivity (NK-Li), and calcitonin gene-related

peptide (CGRP) remain unchanged (Donnerer et al. 1992). Perhaps, the increased availability of growth factors in the CNS induces denser innervation spared neuron systems (e.g., VIP) after the elimination of noradrenergic innervation, as discussed for peripheral neurons after chemical sympathectomy (Aberdeen et al. 1990). Conversely, the dorsal and spinal cord neuropeptide Y (NPY) concentration does not differ following neonatal DSP-4 treatment (Donnerer et al. 1990).

8 Prenatal DSP-4 Treatment

DSP-4 crosses the blood–placenta barrier and has a potent neurotoxic effect in the prenatal stage. When the pregnant dam is injected with DSP-4 (20 mg/kg) on gestation days 18 and 19, the NE concentration in frontal and occipital cortex of one-day-old pups is reduced, with no change in NE in cerebellum. The NE level in the frontal cortex remains reduced until adulthood (Jaim-Etcheverry and Zieher 1980; König et al. 1985). The absence of changes in the NE concentration in the cerebellum and pons–medulla of treated prenates (observed as adults) contrasts with the effects observed after DSP-4 treatment of newborn animals (Jonsson et al. 1981).

Neonatal DSP-4 administration does not lead to remarkable changes in spontaneous locomotor and exploratory activity. The results obtained from rotating-rod tests (examining motor coordination) are controversial, and subtle differences between male and female rats can be detected. Thus, the absence of distinct changes in locomotor and exploratory activity suggests that the role of NE in these behavioral patterns might be limited (König et al. 1985).

9 Summary

9.1 Noradrenergic System

Neonatal DSP-4 (50 mg/kg s.c.) incurs near complete NE depletion (90–98 %) in the cerebral cortex, hippocampus, and spinal cord. Minor effects are observed in the striatum and hypothalamus, while no significant changes in NE concentration are noted in the thalamus and medulla oblongata. In contrast, a pronounced increase in NE levels is observed in the cerebellum, brain stem, mesencephalon, and pons–medulla (Jonsson et al. 1981, 1982; Dabrowska et al. 2007; Bortel et al. 2008c; Korossy-Mruk et al. 2013). The "picture" of endogenous NE brain alterations suggests that DSP-4 has a preferential, selective neurotoxic effect on nerve terminal projections emanating from noradrenergic perikarya of LC and does not affect NE axons in brain receiving a dense innervation from non-cerulean noradrenergic cells (Jonsson et al. 1981). A near complete elimination of NE in the cerebral cortex and hippocampus by neonatal DSP-4 treatment is accompanied by a parallel reduction in NET (Sanders et al. 2011) and in vitro [^{3}H]NE reuptake in cerebral cortex and spinal

cord with concomitant [^{3}H]NE reuptake increases in the cerebellum and no significant alterations in the striatum (Jonsson et al. 1982; Jonsson and Sachs 1982). Permanent noradrenergic denervation in the cerebral cortex and spinal cord is present at all developmental stages, although this effect is more pronounced in rats treated with DSP-4 at an early age (up to PND 5). NE reduction is gradually less marked following DSP-4 treatments as late as PND 14, after which the extent of NE depletion is constant into adulthood (Jonsson et al. 1982). DSP-4 lesions on PND 3 produce only transient α_2-adrenergic receptor reduction in brain at PND 5. The α_2-adrenergic receptor "recovers" to control levels at PND 15 and PND 25, and no further change in the total receptor density is detected (Sanders et al. 2011). Interestingly, severe damage does not impair the activity of the NE system in terms of postsynaptic efficacy. The compensatory mechanisms maintain the level of extracellular NE at a sufficient rate to provide appropriate receptor stimulation, at least under basal conditions (Nowak et al. 2004). Neonatal DSP-4 treatment marginally influences behavior and biological rhythms in adulthood; some abnormalities in learning and memory processes (Cornwell-Jones et al. 1982, 1989; Chen et al. 1993), spontaneous and evoked arousal (Gall et al. 2009), and sexual development (Jacobson et al. 1988) can be detected.

9.2 Serotoninergic System

DSP-4, when administered in the absence of a 5-HT reuptake inhibitor, reduces (up to 60 %) endogenous 5-HT concentrations in the cerebral cortex, hippocampus, spinal cord, and cerebellum, when analyzed at the adult stage. In parallel with these observations, 5-HT reuptake in the occipital cortex, hippocampus, and cerebellum is reduced (Jonsson et al. 1981, 1982). The SERT blocker, zimelidine, abolishes the effects of DSP-4 on 5-HT and its metabolite 5-HIAA in frontal cortex, hippocampus, striatum, and hypothalamus without interfering with the action of this neurotoxin on noradrenergic nerve terminals (Jonsson et al. 1981, 1982; Dabrowska et al. 2007). Under these conditions, despite the absence of changes in the content and synthesis rate of 5-HT in the brain (Dabrowska et al. 2007; Roczniak et al. 2015), other adaptive alterations in the 5-HT system are detected. These alterations are not noticeable until behavioral or biochemical responses are "enforced" through the stimulation or blocking of 5-HT receptors. Notably, experimental data suggest the existence of regulatory mechanisms in neonatally DSP-4-lesioned animals altering the functional efficacy of 5-HT$_1$ receptors and, accordingly, affecting serotoninergic activity, as 5-HT$_{1A}$ autoreceptors (Dabrowska et al. 2007, 2008) and 5-HT$_{1B}$ receptors (Ferdyn-Drosik et al. 2008, 2010) are desensitized in these rats.

9.3 Dopaminergic System

Neonatal DSP-4 treatment does not affect DA and DOPAC levels in the cortex, hippocampus, striatum, mesencephalon, pons–medulla, cerebellum, and spinal cord of adult rats (Jonsson et al. 1981; Nowak et al. 2006). In parallel, the DA synthesis rate remains unchanged in several tested brain regions (hippocampus, striatum, and cerebellum) (Roczniak et al. 2015). Neonatal DSP-4 treatment does not affect DA D_1 and D_2 receptor agonist-evoked behavioral responses (vacuous chewing movements, locomotor, and exploratory activities), whereas DA D_3 quinpirole-induced yawning behavior as well as lifelong sensitization of the central DA D_2/D_3 receptors are modified. These observations suggest that intact central noradrenergic innervation is important for behavioral responses mediated through a variety of DA receptors and the expression of priming phenomenon (Nowak et al. 2006, 2009).

9.4 GABAergic System

Neonatal DSP-4 treatment of rats does not induce any change in GABA tissue levels (prefrontal cortex, hippocampus, brainstem, cerebellum) and does not modify phenobarbital or ethanol-evoked GABA reduction in the brain (Bortel et al. 2008c). However, DSP-4 treatment increases GABAergic neurotransmission in prefrontal cortex of rats in adulthood (Bortel et al. 2008b). Also, there is a reduction in the number of GABA-A receptors in cerebral cortex and hippocampus (Suzdak and Gianutsos 1985) and BDZ receptors in the cerebral cortex, while a significant increase in the number of BDZ receptors in the cerebellum is noted (Medina and Novas 1983). These results support the existence of functional coupling between the noradrenergic and GABAergic systems, i.e., decrease or increase in the density of BDZ receptors following GABAergic denervation (e.g., cortex) or hyperinnervation of some brain structures (e.g., cerebellum). Moreover, neonatal DSP-4 treatment diminishes anxiolysis elicited through diazepam administration (Bortel et al. 2007), reduces vulnerability of the pro-convulsant activity of bicuculline (Bortel et al. 2008a), and leads to adulthood changes in the activity of sedative–hypnotic drugs (Bortel et al. 2008c).

9.5 Other Neuronal Phenotypic Systems

The destruction of noradrenergic neurons through neonatal DSP-4 treatment significantly decreases the antinociceptive effects of methanandamide (CB_1 receptor agonist) without noticeable changes in CB_1 receptor density in the rat brain (Korossy-Mruk et al. 2013). Conversely, nearly imperceptible effects on the central histaminergic system in rats after DSP-4 treatment are observed. Histamine content

remains unchanged in several tested brain structures, and only a modest reduction in the hippocampus is observed (Nowak et al. 2008). It is noteworthy that neonatal DSP-4 treatment produces some adaptive changes in the neuropeptide innervation in adulthood; VIP is markedly elevated in the cortex, hippocampus, striatum, and medulla, whereas SP, NK-Li, CGRP, and NPY remain unchanged (Donnerer et al. 1990, 1992).

10 Conclusions

Studies by Ross and colleagues in 1973 were a stimulus for investigations of DSP-4 activity, over the next 40 years. DSP-4 has attained pharmacological legitimacy and a "strong" position among the other neurotoxins utilized in the neuroscience field, e.g., 6-OHDA, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and rotenone. This is attributable to the following: (1) DSP-4 appears to be a useful experimental tool in studies of the mechanisms of LC neuron degeneration and recovery. This is based on the findings that DSP-4 interacts with NET, whereby it is then accumulated intraneuronally to trigger the degeneration of noradrenergic terminals in the brain (Lyons et al. 1989). Related DSP-4 effects are observed in the peripheral sympathetic neurons as compared with central noradrenergic phenotypes. However, a complete recovery of peripheral noradrenergic nerves and NE tissue level and reuptake is observed within several weeks (Jaim-Etcheverry et al. 1980; Jonsson et al. 1981); (2) DSP-4 may serve as a useful tool to discriminate between LC- and non-LC-dependent behavioral and biochemical responses. This results from the fact that DSP-4 has been considered a LC-selective noradrenergic neurotoxin based on documented alterations in terminal noradrenergic fibers in brain regions innervated chiefly by the LC without affecting non-cerulean noradrenergic axons (Jaim-Etcheverry and Zieher 1980; Jonsson and Sachs 1982; Cryan et al. 2002); (3) DSP-4 in laboratory animals may serve as a convenient model to study the mechanisms and functional consequences of the compensatory but selective noradrenergic hyperinnervation. Typically, DSP-4 is applied to adult animals but may also be administered during perinatal period. Thus, DSP-4 treatment of newborn rats leads to pronounced denervations of distant nerve terminal projections, while innervation areas close to the cell bodies become hyperinnervated, likely reflecting the collateral sprouting of noradrenergic neurons. Notably, regional hyperinnervation is a hallmark of neonatal DSP-4 treatment, which is not observed after either prenatal or adult DSP-4 application (Jonsson et al. 1982; Bortel et al. 2008c); (4) DSP-4 may be used to investigate synaptic biochemistry including mechanisms of noradrenergic receptor up- and down-regulation, in particular the phenomenon of temporary and reversible receptor changes. Notably, neonatal DSP-4 lesions produce only transient adrenergic receptor reduction in brain with short "recovery" phase resulting finally in no change in the total receptor density (Sanders et al. 2011). This strikingly contrasts with dopaminergic or serotoninergic neonatal lesions (Kostrzewa et al. 2008); and (5) DSP-4 may be helpful in clarifying

the pharmacological bases for the complex interactions between noradrenergic and other neuronal phenotypes. In fact, neonatal DSP-4 treatment produces desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors (Dabrowska et al. 2007, 2008; Ferdyn-Drosik et al. 2010), diminishes GABA-A receptor-mediated behavioral and biochemical responses (Bortel et al. 2007, 2008a, b, c) as well as modifies behavioral responses mediated through a variety of DA receptors and the expression of DA receptor priming phenomenon (Nowak et al. 2006, 2009).

In conclusion, a better understanding of the role of the noradrenergic system in the therapeutic effects of several psychotropic medications as well as introducing new therapies for behavioral and/or psychiatric disorders would not have been possible without apparent (vivid) DSP-4 contribution. Concurrently, it is believed that DSP-4 still possesses the immense pharmacological potential that can be utilized through the next decades in the neuroscience area.

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