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Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test

John F. Cryan [a,1], Michelle E. Page [a,2], Irwin Lucki [a,b,*]

[a] *Department of Psychiatry, University of Pennsylvania, 538A Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104, USA*
[b] *Department of Pharmacology, University of Pennsylvania, 538A Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104, USA*

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Abstract

The novel antidepressant reboxetine is a selective norepinephrine reuptake inhibitor. In this study, the antidepressant-like effects of reboxetine were characterized in a modified rat forced swim test. Further, in order to investigate the role of the locus coeruleus and lateral tegmental noradrenergic systems in the mediation of reboxetine's effects, the impact of different chemical lesions of these two pathways was examined on the behavioral responses induced by reboxetine in the forced swim test. Reboxetine (5–20 mg/kg, s.c.) dose-dependently decreased immobility and swimming behavior in the forced swim test while it simultaneously increased climbing behavior. These effects were similar to those previously demonstrated with tricyclic antidepressants and are indicative of reboxetine's effects on the noradrenergic system. Discrete local injections of the neurotoxin 6-hydroxydopamine were employed to lesion the ventral noradrenergic bundle arising from cells located in the lateral tegmentum. This resulting lesion completely prevented reboxetine (10 mg/kg, s.c.)-induced decreases in immobility and increases in climbing behavior, demonstrating that an intact ventral noradrenergic bundle is required for the manifestation of reboxetine-induced antidepressant-like behavior in the test. In contrast, lesions of the dorsal noradrenergic bundle which consists of neurons arising from the nucleus locus coereleus, were achieved by systemic pretreatment with the selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-2-bromobenzylamine (DSP-4; 50 mg/kg, i.p.). The ability of reboxetine (10 mg/kg, s.c.) to increase climbing and decrease immobility was augmented by DSP-4 pretreatment. Furthermore, neither lesions of the dorsal noradrenergic bundle nor the ventral noradrenergic bundle altered baseline immobility scores in the forced swim test. Taken together, these data suggest that forebrain regions innervated by these two distinct noradrenergic pathways exert opposing influences on the behavioral response to reboxetine in the rat forced swim test.

Keywords: Forced swim test; Reboxetine; DSP-4; Ventral noradrenergic bundle; Antidepressant

1. Introduction

Since the 1960s there has been a strong emphasis on the role of norepinephrine in both the pathogenesis of affective disorders and in the mechanism of action of antidepressant medications (Schildkraut, 1965; Leonard, 1997). This is largely due to the fact that many of the first generation of antidepressants, the tricyclics, increase the synaptic concentrations of norepinephrine. However, many of these compounds also have high affinity for α_1-adrenoceptors, histamine and muscarinic receptors which results in adverse side effects such as weight gain, drowsiness, dry mouth, blurred vision, constipation and decreases in blood pressure (see Stahl, 1997). These side effects could contribute to low compliance rates in patients treated with these compounds. Recently, a new general class of antidepressant compounds, the selective norepinephrine reuptake inhibitor, have been developed (Brunello and Racagni, 1998). Reboxetine is the first of such a class, which selectively inhibits the reuptake of synaptic norepinephrine without any marked affinity for other receptors or transporters (Wong et al., 2000). Reboxetine has been shown to be both clinically effective and well tolerated with a lower side-effect profile than tricyclics (Burrows et al., 1998).

The central noradrenergic neurotransmitter system originates from two distinct groups of cells in the brainstem. The

* Corresponding author. Department of Psychiatry, University of Pennsylvania, 538A Clinical Research Building, 415 Curie Boulevard, Philadelphia, PA 19104-6140 USA. Tel.: +1-215-573-3305; fax:+1-215-573-2149.

E-mail addresses: john_f.cryan@pharma.novartis.com (J.F. Cryan), lucki@pharm.med.upenn.edu (I. Lucki).

[1] Current address: Neuromodulation Unit, Nervous Systems Research, Novartis Pharma AG, Basel, Ch-4002, Switzerland.

[2] Current address: Department of Neurobiology and Anatomy, MCP Hahnemann University, 2900 Queen Lane, Philadelphia, PA 19129, USA.

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nucleus locus coereleus (corresponding to A4 + A6 cell groups as described by Dahlström and Fuxe, 1965) is the site of the majority of noradrenergic projections throughout the neuroaxis innervating areas such as the frontal cortex, hippocampus and amygdala as well as the cerebellum and spinal cord; this ascending projection system is also referred to as the dorsal noradrenergic bundle. Efferents from the lateral tegmentum (corresponding to A1, A2, A5 and A7 cell groups as described by Dahlström and Fuxe, 1965) form the ventral noradrenergic bundle and have less extensive projections. They provide predominant innervation of the hypothalamus and also innervate areas of the septum and the extended amygdala nuclei including the bed nucleus of the stria terminalis (Moore and Card, 1984). The role of norepinephrine in mediating the actions of antidepressants has largely focused on the locus coeruleus system due to its extensive limbic innervation. Indeed, electrophysiological studies have shown that antidepressants from various classes can impact on locus coeruleus discharge rates (Valentino et al., 1990; Curtis and Valentino, 1991). The role of norepinephrine arising from other nuclei in mediating antidepressant action has largely been uninvestigated (for review, see Stanford, 1995).

The forced swim test, as originally described by Porsolt et al. (1977), is the most widely used pharmacological model for assessing antidepressant activity (Weiss and Kilts, 1998). This is largely due to its ease of use, reliability across laboratories and ability to detect a broad spectrum of antidepressants (Borsini and Meli, 1988; Weiss and Kilts, 1998). The development of immobility when rats are placed in an inescapable cylinder of water reflects the cessation of persistent escape-directed behavior, i.e. that serves various adaptive functions in response to stress (Lucki, 1997). Our laboratory has modified the traditional forced swim test (see Lucki, 1997) and demonstrated that the test reveals distinct types of active behaviors, namely swimming, which is sensitive to serotonergic compounds such as the selective serotonin reuptake inhibitors (SSRIs) and 5-HT receptor agonists, and climbing, which is sensitive to tricyclic antidepressants and drugs with selective effects on catecholamine transmission (Detke et al., 1995; Hemby et al., 1998; Cryan and Lucki, 2000). It has also been shown that the distinctive behaviors of pharmacologically selective antidepressants persisted upon chronic treatment (Detke et al., 1997) and that they were superimposable upon the combination of serotonergic and catecholaminergic compounds (Reneric and Lucki, 1998). Furthermore, pretreatment with the tryptophan hydroxylase inhibitor parachlorophenylalanine prevented the swimming behavior produced by the selective serotonin reuptake inhibitor fluoxetine but not the climbing produced by the norepinephrine reuptake inhibitor, desipramine (Page et al., 1999). Nonetheless, it is unclear which noradrenergic circuits are responsible for the mediation of antidepressant-sensitive behaviors in the modified forced swim test.

The intracerebroventricular (i.c.v.) administration of 6-hydroxydopamine, an analogue of norepinephrine (Thoenen and Tranzer, 1968), results in widespread selective destruction of norepinephrine and dopamine neurons. However, discrete injections along the caudal axis of the ventral noradrenergic bundle, can be used to selectively target this noradrenergic system leaving the dorsal noradrenergic bundle relatively intact (Cole and Robbins, 1987; Delfs et al., 2000; Shaham et al., 2000). The neurotoxin *N*-(2-chloroethyl)-*N*-2-bromobenzylamine (DSP-4) (Jonsson et al., 1981) is a selective neurotoxin of noradrenergic neurons (when animals are pretreated with an selective serotonin reuptake inhibitor), and preferentially lesions neurons of the dorsal noradrenergic bundle, leaving the ventral noradrenergic bundle neurons relatively spared (e.g., Fritschy and Grzanna, 1991). The present study examined the impact of selective noradrenergic lesions of the ventral noradrenergic bundle by discrete injections of 6-hydroxydopamine or of the dorsal noradrenergic bundle produced by DSP-4, on the behavioral changes in the rat forced swim test produced by reboxetine.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Wilmington, MA, USA), weighing 300–400 g (at time of behavioral testing), were used in these studies. The animals were housed in pairs in polycarbonate cages and maintained on a 12-h light/dark cycle (lights on at 07:00 h) in a temperature (22 °C)- and humidity-controlled colony. The animals were given free access to food and water. Animals were handled daily for at least 7 days prior to initiation of behavioral testing. Behavioral studies were carried out in the afternoon (12:00–18:00 h) during the months of February–April. All experimental procedures were carried out in accordance with protocols approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

2.2. Ventral noradrenergic bundle 6-hydroxydopamine lesion

Lesions were carried out as previously described (Aston-Jones et al., 1999). Briefly, following acclimation rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic frame (Kopf instruments, Tujunga, CA). Infusion cannulae (28-gauge) were directed toward the VNAB using the following coordinates from bregma, AP: −6.6 mm, ML: ±2.0 mm, DV: −8.2 and −9.2 mm from skull, incisor bar +5.0 mm. Bilateral infusions of 2 μl of 6-hydroxydopamine (3 μg/μl in 0.1% ascorbic acid/0.9% saline) or vehicle (control animals) were made over 4 min at each injection site (0.25 μl/min) using a Hamilton microliter syringe and automated infusion pump (Instech, Plymouth Meeting, PA). The cannulae were kept in place an additional 2–3 min at each site to allow tissue to absorb the

infusion and limit diffusion upward along the cannula tract. Behavioral testing began 8–9 days later. The rats were handled and their bodyweight was monitored daily following the lesion.

2.3. Dorsal noradrenergic bundle DSP-4 lesion

Following at least 1 week of acclimation to the laboratory, rats were given an intraperitoneal injection of DSP-4 (50 mg/kg) or saline (control) 7 days prior to start of the behavioral testing. All rats were pretreated with the selective serotonin reuptake inhibitor paroxetine (10 mg/kg, i.p.) 30 min prior to DSP-4 administration, in order to protect serotonergic nerve terminals (Jonsson et al., 1981). The rats were handled daily following the lesion and their body weight measured.

2.4. Rat forced swim test

The modified rat forced swim test was conducted essentially as described by Detke et al. (1995). Briefly, rats were placed individually in Pyrex cylinders (21 × 46 cm; Fisher Scientific) which were filled with water to a 30-cm depth. The rats were removed 15 min later, dried and placed in their home cage. Following 24 h after their first exposure, the animals were again placed in the swim apparatus for 5 min and behaviors were monitored from above by video camera for subsequent analysis. Animals were randomly assigned to groups that received various drug treatments or 0.9% saline (control). Injections were administered subcutaneously three times at 1, 5 and 23.5 h prior to the test session. The rater of the behavioral patterns was blind with respect to the experimental conditions being scored. A time sampling technique was employed whereby the predominant behavior in each 5-s period of the 300-s test was recorded. Climbing behavior consisted of upward directed movements of the forepaws along the side of the swim chamber. Swimming behavior was defined as movement (usually horizontal) throughout the swim chamber, which also included crossing into another quadrant. Immobility was assigned when no additional activity was observed other than that required to keep the rat's head above the water.

The first study examined the effects of reboxetine within a dose range of 1–20 mg/kg on active behaviors in this modified paradigm. The second study examined the behavioral effects of the 6-hydroxydopamine lesion of the ventral noradrenergic bundle on reboxetine (10 mg/kg)-induced changes in the forced swim test 8–9 days (i.e., pretest swim) following administration of the toxin. The behavioral effects induced by reboxetine (10 mg/kg) 7 days (i.e., pretest swim) following administration of the toxin DSP-4 were examined in the third study. A dose of 10 mg/kg reboxetine was chosen for the lesion studies as it produced robust changes in behavior in the dose-response study.

2.5. Tissue analysis of norepinephrine 3,4-dihydroxyphenylacetic acid (DOPAC)

Following completion of behavioral experiments, animals from the lesion studies were sacrificed by decapitation and their brain quickly dissected on ice. The hypothalamus and frontal cortex of each rat were taken as regions receiving representative ventral noradrenergic bundle and dorsal noradrenergic bundle innervation, respectively (e.g., Shaham et al., 2000; Moore and Card, 1984). DOPAC concentrations were measured as an indicator of the integrity of the dopamine system following 6-hydroxydopamine lesion. Samples were frozen on dry ice and stored at −80 °C until assay. Tissue samples were homogenized in 0.1 N perchloric acid with 100 μM EDTA (15 μl/mg tissue) using a Tissuemizer (Tekmar, Cleveland, OH). Samples were centrifuged at 15,000 rpm (23,143 × *g*) for 15 min at 2–8 °C. The supernatant was filtered through 0.45-μm nylon acrodisk syringe filters. Tissue samples were analyzed for norepinephrine content using high performance liquid chromatography (HPLC) with electrochemical detection. The HPLC system consisted of an ESA solvent delivery system (ESA, Chelmsford, MA) and a Velosep RP-18 column (100 × 3 mm, 3 μm; Varian Chromatography, Walnut Creek, CA) The mobile phase consisted of 60 mM sodium phosphate buffer (pH = 4.2) with 100 μM EDTA, 1.5 mM sodium octyl-sulfate, 3.5% (v/v) methanol. The flow rate through the system was 700 μl/min. The detection system utilized was an ESA 5200 electrochemical detector with three electrodes in series. The conditioning electrode was set at +270 mV. The applied potential of the second electrode was set at −250 mV, and the compound of interest was quantified at a third electrode, which was set at +270 mV. Peak heights for norepinephrine and DOPAC were measured and compared to peak heights of 10^{-8} M standards.

2.6. Drugs

All drugs were prepared freshly prior to use. Reboxetine hydrobromide (Pharmacia & UpJohn, Kalamazoo, MI) was dissolved in distilled water and injected subcutaneously in a volume of 2 ml/kg. Paroxetine hydrochloride (SmithKline Beecham, Harlow, UK) was dissolved in distilled water and sonicated mildly; it was injected intraperitonealy at a volume 1 ml/kg. Drugs were calculated as the base weight. DSP-4 (Sigma, St. Louis, MO) was dissolved in 0.9% saline and injected intraperitonealy in a volume of 1 ml/kg. 6-hydroxydopamine (Sigma) was dissolved in a 0.1% ascorbic acid/0.9% saline solution.

2.7. Statistical analysis

A one way analysis of variance (ANOVA) was carried out in all studies. Any overall statistical differences were analyzed further using Fisher's post-hoc tests.

3. Results

3.1. The effects of reboxetine on active behaviors in the forced swim test

As shown in Fig. 1, reboxetine dose-dependently decreased immobility [$F(5,59) = 13.59$, $P < 0.001$] and swimming [$F(5,59) = 4.42$, $P = 0.002$] behaviors while inducing a corresponding increase in climbing behavior [$F(5,59) = 18.00$, $P < 0.001$]. These effects are representative of antidepressant-like actions in the test and are qualitatively similar to those produced by other antidepressant drugs that enhance catecholamine neurotransmission (Detke et al., 1995; Hemby et al., 1998).

3.2. The effects of 6-hydroxydopamine on reboxetine-induced active behaviors in the forced swim test

Following surgery, both lesion and control animals lost weight (4.3% and 3.3% pre-surgery weight, respectively). Both groups had recovered to their pre-surgery bodyweights prior to testing and their bodyweights were not different from each other (data not shown). Furthermore, the animals showed no observable deficits in sensory or motor function. The effects of the ventral noradrenergic bundle lesion on active behaviors elicited by reboxetine (10 mg/kg) are shown in Fig. 2. ANOVA revealed a significant effect on immobility scores in the test [$F(3,36) = 5.57$, $P < 0.05$]. Post hoc analysis revealed that reboxetine reduced immobility scores in control animals and that this effect was prevented in animals pretreated with 6-hydroxydopamine. There were no differences in behavioral parameters after saline treatment in animals pretreated with vehicle or 6-hydroxydopamine-treated animals. ANOVA also revealed significant differences between groups in climbing behavior in the forced swim test [$F(3,36) = 7.16$, $P < 0.05$]. Post hoc analysis revealed that reboxetine increased climbing scores in control animals and that this effect was absent in animals pretreated with 6-hydroxydopamine. Again, there was no

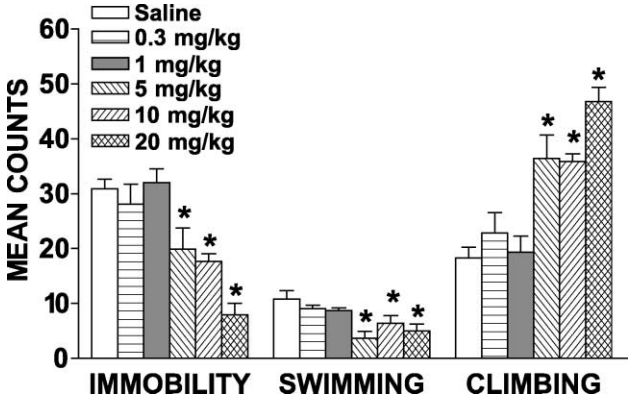

Fig. 1. The effects of the norepinephrine reuptake inhibitor reboxetine on active behaviors in a modified rat forced swim test. Reboxetine [5 mg/kg, $n = 9$; 10 mg/kg $n = 9$; 20 mg/kg, $n = 9$] decreased immobility and swimming behavior with a corresponding increase in climbing behavior compared with saline-treated animals [$n = 19$]. Lower doses of reboxetine [0.3 mg/kg, $n = 9$; 1 mg/kg, $n = 9$] were without effect in the forced swim test. All bars represent mean values with vertical lines indicating 1 S.E.M. Asterisks indicates groups that differed significantly from saline-treated animals: * $P < 0.05$.

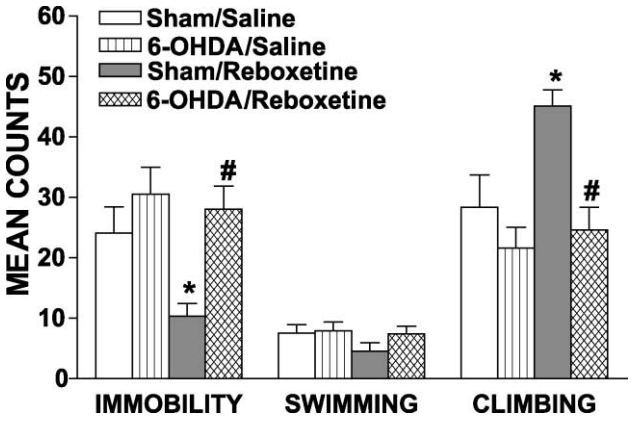

Fig. 2. The effects of a specific 6-hydroxydopamine lesion of the ventral noradrenergic bundle on reboxetine-induced behavioral changes in the forced swim test. Reboxetine [10 mg/kg, $n = 10$] decreased immobility and increased climbing behavior compared with saline-treated animals [$n = 10$] in non-lesioned (sham) animals. However, in animals treated with 6-hydroxydopamine the behavioral effects of reboxetine [10 mg/kg, $n = 10$] on both climbing and immobility were blocked when compared with saline-treated lesioned animals [$n = 10$] and reboxetine-treated sham animals. All bars represent mean values with vertical lines indicating 1 S.E.M. Asterisks indicates groups that differed significantly from saline-treated animals: * $P < 0.05$, whereas # represents groups different from sham control. # $P < 0.05$.

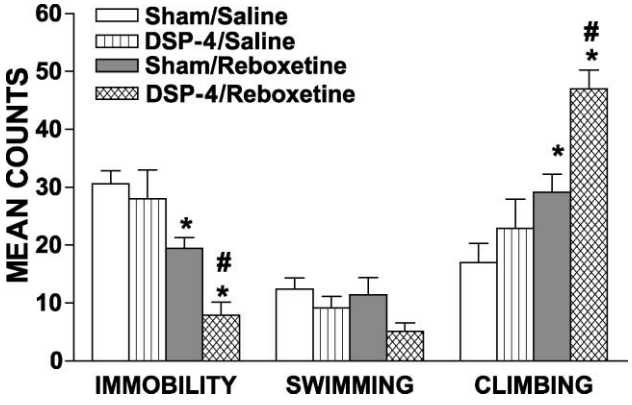

Fig. 3. The effects of a DSP-4 lesion of the dorsal noradrenergic bundle on reboxetine-induced behavioral changes in the forced swim test. Reboxetine [10 mg/kg, $n = 9$] decreased immobility and increased climbing behavior compared with saline-treated animals [$n = 9$] in non-lesioned (sham) animals. However, in animals treated with DSP-4 the behavioral effects of reboxetine [10 mg/kg, $n = 8$] on both climbing and immobility were potentiated when compared with saline-treated lesioned animals [$n = 8$] and reboxetine-treated sham animals. All bars represent mean values with vertical lines indicating 1 S.E.M. Asterisks indicates groups that differed significantly from saline-treated animals: * $P < 0.05$, whereas # represents groups different from sham control. # $P < 0.05$.

difference in climbing behavior between control and 6-hydroxydopamine animals treated with saline. Furthermore, there was no effect of either reboxetine or 6-hydroxydopamine treatments on swimming scores [$F(3,36) = 1.17$, $P = 0.33$].

3.3. The effects of a DSP-4 lesion on reboxetine-induced active behaviors in the forced swim test

Following injection of DSP-4, animals lost weight (-3.1% of pretreatment weight) but had recovered this loss prior to testing ($+5\%$ of pretreatment weight) (data not shown) and showed no observable deficits in sensory or motor function. The effects of pretreatment with DSP-4 on active behaviors elicited by reboxetine (10 mg/kg) are shown in Fig. 3. ANOVA revealed a significant effect of various treatments on immobility scores in the test [$F(3,31) = 10.13$, $P < 0.05$]. Post-hoc analysis revealed that reboxetine reduced immobility scores in control animals and that this effect was significantly potentiated in animals pretreated with DSP-4. There was no difference in immobility scores between saline-treated control or DSP-4-treated animals. ANOVA also revealed a significant effects of the interventions on climbing behavior in the forced swim test [$F(3,31) = 10.90$, $P < 0.05$]. Post hoc analysis revealed that reboxetine increased climbing scores in control animals and that this effect was significantly potentiated in animals pretreated with DSP-4. Again, there was no difference in climbing behavior in sham or DSP-4 animals treated with saline. Furthermore, there was no effect of either reboxetine or DSP-4 treatments on swimming scores [$F(3,31) = 2.12$, $P = 0.12$].

3.4. The effects of DSP-4 dorsal noradrenergic bundle lesions and 6-hydroxydopamine ventral noradrenergic bundle lesions on monoamine content in the frontal cortex and hypothalamus

There were no differences in monoamine concentrations in either brain region analyzed between reboxetine- or saline-treated animals, so that the data shown in Table 1 were the combined data between these two groups. There was a significant depletion of norepinephrine in the cortex of DSP-4-treated animals (69%) [$F(1,33) = 24.20$, $P < 0.001$] but to a much lesser extent (26%) in the hypothalamus [$F(1,32) = 4.67$, $P = 0.038$]. In contrast hypothalamic norepinephrine concentration was greatly reduced (69%) in animals given 6-hydroxydopamine into the ventral noradrenergic bundle [$F(1,37) = 147.09$, $P < 0.001$] with less depletion (42%) in the cortex [$F(1,36) = 15.98$, $P < 0.001$]. DOPAC concentrations did not significantly differ between control and lesion groups in the cortex of both the DSP-4 [$F(1,31) = 0.064$, $P = 0.802$] and 6-hydroxydopamine [$F(1,38) = 0.007$, $P = 0.932$] studies. Likewise, there was no significant difference in hypothalamic DOPAC concentrations between control and lesion groups in both DSP-4 [$F(1,31) = 4.085$, $P = 0.052$] and 6-hydroxydopamine [$F(1,38) = 0.027$, $P = 0.869$] lesioned animals.

Table 1
The effects of DSP-4 dorsal noradrenergic bundle lesions and 6-hydroxydopamine ventral noradrenergic bundle lesions on monoamine content in the frontal cortex and hypothalamus

	Norepinephrine concentration (pg/mg tissue)		DOPAC concentration (pg/mg tissue)	
	Cortex	Hypothalamus	Cortex	Hypothalamus
Dorsal noradrenergic bundle				
Sham	229 + 28	1118 + 82	138 + 31	266 + 31
Lesion	71 + 14*	828 + 109*	126 + 37	185 + 24
% Depletion	69 %	26%	9%	30% ns
Ventral noradrenergic bundle				
Sham	239 + 19	753 + 36	94 + 20	192 + 18
Lesion	139 + 17*	236 + 22*	97 + 23	189 + 13
% Depletion	42%	69%	− 3.1%	2%

A DSP-4 lesion of the dorsal noradrenergic bundle resulted in a significant depletion of cortical norepinephrine levels whereas it had limited effects on hypothalamic norepinephrine concentrations. No significant effects on DOPAC levels were demonstrated. A 6-hydroxydopamine lesion of the ventral noradrenergic bundle system resulted in a significant depletion of hypothalamic norepinephrine, with lesser effects on cortical norepinephrine. No alteration in DOPAC levels in either region was observed. Values represent mean values with 1 S.E.M. ns = not statistically significant.

* Groups that differed significantly from saline-treated animals: * $P < 0.05$.

4. Discussion

The present study characterizes the effects of the novel selective norepinephrine reuptake inhibitor reboxetine in a modified forced swim test model of depression. Our findings using the modified forced swim test are in agreement with recent reports describing the antidepressant-like actions of reboxetine in the traditional forced swim test and other animal models of depression (Connor et al., 1999; Harkin et al., 1999; Wong et al., 2000). Additionally, the pattern of effects that was observed for reboxetine in the modified forced swim test is qualitatively similar to that previously described for the prototypical noradrenergic antidepressant desipramine (Detke et al., 1995). These data coupled with reboxetine's selectivity in vitro for the norepinephrine transporter (Wong et al., 2000) suggest that it is an excellent tool to probe noradrenergic function.

The blockade of the behavioral response in 6-hydroxydopamine ventral noradrenergic bundle lesioned animals coupled with the augmented antidepressant-like response to reboxetine in DSP-4-lesioned animals suggests a complex interaction between ventral and dorsal noradrenergic bundle projecting neurons in modulating the antidepressant-like effects of reboxetine in the test. The role of the ventral noradrenergic bundle in mediating antidepressant action has received little attention to date. Here we show that a substantial lesion of the ventral noradrenergic bundle, as indicated (sp.) by a 68% depletion of hypothalamic norepinephrine

concentration, blocks the antidepressant-like effects of reboxetine on immobility and climbing behavior in the forced swim test. The hypothalamus receives the vast majority of its noradrenergic input from the ventral noradrenergic bundle, although it also is innervated, albeit to a much lesser extent, by neurons originating from the locus coeruleus (see Moore and Card, 1984). This latter fact may explain the small (26%), yet significant depletion of hypothalamic norepinephrine seen in the DSP-4-treated animals. Nonetheless, depletions of hypothalamic norepinephrine concentrations are used routinely to quantify the extent of ventral noradrenergic bundle lesions (e.g., Shaham et al., 2000). Furthermore, we cannot rule out that the substantial cortical depletion observed subsequent to the 6-hydroxydopamine lesion may be responsible at least in part, for the dampening of reboxetine's effects in the test. Nonetheless, the potentiated behavioral response following a much greater cortical depletion with DSP-4 indicates that this is unlikely to be the case. The lack of effect of the ventral noradrenergic bundle lesion on cortical DOPAC levels strengthens the case that the effects of the toxin are not due to non-specific targeting, as 6-hydroxydopamine in the ventricular system has been shown to cause dramatic reductions in cortical DOPAC concentrations (e.g., Gandolfi and Dall'Olio, 1996).

The consequences of a DSP-4 lesion on reboxetine-induced behavioral changes are somewhat counterintuitive given the large body of studies that demonstrate antidepressant drugs impact on locus coeruleus function and that locus coeruleus noradrenergic neurons are critical for integrating the physiological response to stressors (Valentino et al., 1990, 1998; Stanford, 1995). However, recent in vivo microdialysis studies (Hughes and Stanford, 1998 but see Kask et al., 1997) have shown that pretreatment with DSP-4 (40 mg/kg) actually leads to an increase in the basal extracellular concentration of norepinephrine in rat frontal cortex despite causing a 75% lesion of cortical norepinephrine content which is further potentiated following challenge with the norepinephrine reuptake inhibitor desipramine. These studies offers an appealing explanation for the augmented behavioral response seen in the present study after DSP-4 treatment although an underlying mechanism remains to be elucidated. The potentiation may be due to an increase in the release of norepinephrine by surviving terminals although reduced clearance is also possible. Furthermore, we cannot rule out a reciprocal compensation mechanism in the ventral noradrenergic bundle neurons following locus coeruleus denervation which may influence the augmented response of reboxetine in the forced swim test.

Many other studies have shown that DSP-4 pretreatment results in widespread morphological and neurochemical alterations to locus coeruleus noradrenergic cells and almost exclusively locus coeruleus innervated forebrain areas. Locus coeruleus cells that survive the injury demonstrate stronger dopamine-β-hydroxylase immunohistochemistry and enlarged immunoreactive fibers have been found following DSP-4 treatment (Booze et al., 1988). Electrophysiological studies have shown that the firing rate of the remaining neurons originating from the locus coeruleus are decreased compared to sham operated animals (Magnuson et al., 1993). It is of interest also that stimulation of cortical β-adrenoceptors has been shown to facilitate a positive feedback mechanism regulating release of norepinephrine (Murugaiah and O'Donnell, 1995) and the density of these receptors is increased after DSP-4 treatment (Jonsson et al., 1981; Dooley et al., 1983a). Kask et al. (1997) demonstrated a blunted response to α_2-adrenoceptor antagonism by atipamezole on norepinephrine concentration in the frontal cortex following DSP-4 which correlates with previous binding data that suggested a decrease in affinity for and density of presynaptic cortical α_2-adrenoceptors (Dooley et al., 1983a; Heal et al., 1993). Furthermore, behavioral data suggest that DSP-4-treated rats are more sensitive to the suppressive effects of the α_2-adrenoceptor agonist clonidine in the hole-board test which could be indicative of a supersensitivity of postsynaptic α_2-adrenoceptors (see Dooley et al., 1983b). Thus, it is possible that a reduced number of presynaptic autoreceptors which restrain norepinephrine release and an increased sensitivity of postsynaptic α_2-adrenoceptors may influence the potentiation of the antidepressant-like effects of reboxetine following DSP-4.

Previous studies by Kostowski and colleagues (Kostowski et al., 1984a; Danysz et al., 1985) showed that DSP-4 pretreatment prevented the anti-immobility effects of a single injection of the tricyclic antidepressant desipramine but not imipramine. However, of perhaps more relevance to the present study, they also showed that neither electrolytic nor DSP-4-induced dorsal noradrenergic bundle lesions altered the antidepressant-like effects of desipramine following repeated administration (14–21 days) (Kostowski et al., 1984a; Danysz et al., 1985). Similarly, Esposito et al. (1987) demonstrated that DSP-4 treatment failed to alter the anti-immobility effects of chronic (7-day) desipramine in the classic forced swim test. Furthermore, none of these studies investigated the effects of dorsal noradrenergic bundle norepinephrine depletion under the subchronic injection protocol routinely employed in forced swim test procedures i.e., three injections given 23.5, 5 and 1 h prior to the test session. In addition, there are other methodological differences between these studies and the present one. Our test uses a greater water depth (30 vs. 15–18 cm) in order to prevent rats from developing immobile adaptations when they touch the bottom of the tank (Detke and Lucki, 1996). The greater water depth produces lower baseline values while behavioral responses to both serotonergic and noradrenergic antidepressants are augmented (Detke and Lucki, 1996). Therefore, it is conceivable that there are differential changes to the noradrenergic system following exposure to water at this increased depth compared with the traditional test, which may translate into augmented alterations in behavioral responses consequent to subchronic administration of reboxetine in DSP-4 lesioned animals. Of further

interest is additional studies carried out by Kostowski and colleagues in which they have demonstrated that other manipulations of the noradrenergic systems, either by direct injection of norepinephrine into the dorsal hippocampus (Kostowski et al., 1986; Plaznik and Kostowski, 1985) or into the nucleus accumbens (Plaznik et al., 1985), or by electrical stimulation of locus coeruleus (Kostowski et al., 1984a,b) reduced immobility time in the traditional test. Whether such strategies would impact immobility in our modified paradigm remains to be assessed.

There is a paucity of data examining reciprocal compensatory interactions between either the dorsal noradrenergic bundle and ventral noradrenergic bundle systems following the compromization of the other system. Indeed the neurochemical and morphological consequences of ventral noradrenergic bundle lesions on the remaining norepinephrine cells of the ventral noradrenergic bundle, in dorsal noradrenergic bundle innervated areas, in other brain regions and on other neurotransmitter systems is largely uninvestigated. The influence of such potential compensatory changes on the blockade of reboxetine's behavioral effects in the forced swim test also remains to be examined. Nonetheless, there has been renewed interest in psychopharmacological effects mediated by the ventral noradrenergic bundle, with a definite role for this pathway in opiate withdrawal and drug-reinstatement behavior (Delfs et al., 2000; Shaham et al., 2000). Which forebrain projection region(s)of the ventral noradrenergic bundle mediates the antidepressant-like effects of reboxetine in the forced swim test remains to be determined. Furthermore, it is of interest that Kostowski et al. (1978) proposed that the dorsal noradrenergic bundle and ventral noradrenergic bundle confer opposing effects on the actions of various psychotropic drugs in behavioral paradigms such as locomotor activity and neuroleptic-induced catalepsy, which subsequently has been shown to be the case for other behaviors such as aggression (Kostowski et al., 1980), amphetamine-induced hyperthermia (Kostowski et al., 1982), and acquisition, performance, and extinction of aversive conditioning (Cole and Robbins, 1987). Kostowski and colleagues claimed that such a hypothesis also translates to the effects of antidepressants in the traditional forced swim test. Our data support this postulation but only in the opposite direction to that which they originally predicted.

The lack of baseline difference between lesion and sham operated controls, suggests that endogenous norepinephrine may not be essential for mediation of the various behaviors in the forced swim test or alternatively, that the remaining terminals compensate for the loss. Of interest, we also have shown that mice with targeted disruption of the dopamine-β-hydroxylase gene, and therefore fail to synthesize endogenous norepinephrine, do not show any baseline differences in immobility scores in the mouse version of the forced swim test or in the closely related tail suspension test (Cryan et al., 2001a,b). A similar lack of baseline difference in the forced swim test was seen in rats following administration of the serotonin-depleting agent parachlorophenylalanine (Page et al., 1999) however, the antidepressant-like effects of fluoxetine were prevented. On the other hand, 6-hydroxydopamine injections into the prefrontal cortex have been shown to induce a significant reduction of immobility scores in rat forced swim test (Espejo and Minano, 1999) indicating that particular dopamine pathways may play a modulatory role in the production of immobility behavior in the forced swim test.

In conclusion, we have shown that the novel antidepressant reboxetine, demonstrates antidepressant-like behavior in the modified forced swim test in a pattern characteristic of tricyclics. Furthermore, we have shown that these effects are blocked by a lesion of the ventral noradrenergic bundle using 6-hydroxydopamine and are potentiated by pretreatment with the dorsal noradrenergic bundle selective norepinephrine neurotoxin DSP-4. Taken together they support a complex interaction between these two noradrenergic systems in mediating norepinephrine reuptake inhibitor-induced antidepressant-like effects on active behaviors in the forced swim test.

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References

Aston-Jones, G., Delfs, J.M., Druhan, J., Zhu, Y., 1999. The bed nucleus of the stria terminalis. A target site for noradrenergic actions in opiate withdrawal. Ann. N. Y. Acad. Sci. 877, 486–498.

Booze, R.M., Hall, J.A., Cress, N.M., Miller, G.D., Davis, J.N., 1988. DSP-4 treatment produces abnormal tyrosine hydroxylase immunoreactive fibers in rat hippocampus. Exp. Neurol. 101, 75–86.

Borsini, F., Meli, A., 1988. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology 94, 147–160.

Brunello, N., Racagni, G., 1998. Rationale for the development of noradrenaline reuptake inhibitors. Hum. Psychopharmacol. 13, S13–S19.

Burrows, G.D., Maguire, K.P., Norman, T.R., 1998. Antidepressant efficacy and tolerability of the selective norepinephrine reuptake inhibitor reboxetine: a review. J. Clin. Psychiatry 59 (Suppl. 14), 4–7.

Cole, B.J., Robbins, T.W., 1987. Dissociable effects of lesions to the dorsal or ventral noradrenergic bundle on the acquisition, performance, and extinction of aversive conditioning. Behav. Neurosci. 101, 476–488.

Connor, T.J., Kelliher, P., Harkin, A., Kelly, J.P., Leonard, B.E., 1999. Reboxetine attenuates forced swim test-induced behavioural and neurochemical alterations in the rat. Eur. J. Pharmacol. 379, 125–133.

Cryan, J.F., Lucki, I., 2000. Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine$_{2C}$ receptors. J. Pharmacol. Exp. Ther. 295, 1120–1126.

Cryan, J.F., Dalvi, A., Hirsch, B.R., Jin, S.-H., Lucki, I., Thomas, S.A., 2001a. Use of dopamine-β-hydroxylase deficient mice to determine the role of norepinephrine in the mechanism of action of antidepressant drugs. J. Pharmacol. Exp. Ther. 298, 651–657.

Cryan, J.F., Hirsch, B.R., Jin, S.-H., Lucki, I., Thomas, S.A., 2001b. Re-

duced sensitivity to the antidepressant-like effects of SSRIs in dopamine-β-hydroxylase deficient mice. Soc. Neurosci. Abstr. 27 Program No. 665.9.

Curtis, A.L., Valentino, R.J., 1991. Acute and chronic effects of the atypical antidepressant, mianserin on brain noradrenergic neurons. Psychopharmacology 103, 330–338.

Dahlström, A., Fuxe, K., 1965. Evidence for the existence of monoamine neurons in the central nervous system: I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol. Scand. 64 (Suppl. 232), 1–55.

Danysz, W., Kostowski, W., Kozak, W., Hauptmann, M., 1985. On the role of noradrenergic neurotransmission in the action of desipramine and amitriptyline in animal models of depression. Pol. J. Pharmacol. Pharm. 38, 285–298.

Delfs, J.M., Zhu, Y., Druhan, J.P., Aston-Jones, G., 2000. Noradrenaline in the ventral forebrain is critical for opiate withdrawal-induced aversion. Nature 403, 430–434.

Detke, M.J., Lucki, I., 1996. Detection of serotonergic and noradrenergic antidepressants in the rat forced swimming test: the effects of water depth. Behav. Brain Res. 73, 43–46.

Detke, M.J., Rickels, M., Lucki, I., 1995. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology 121, 66–72.

Detke, M.J., Johnson, M.J., Lucki, I., 1997. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Exp. Clin. Psychopharmacol. 5, 107–112.

Dooley, D.J., Mogilnicka, M., Delini-Stula, A., Waechter, F., Truog, A., Wood, J., 1983a. Functional supersensitivity to adrenergic agonists in the rat after DSP-4, a selective noradrenergic neurotoxin. Psychopharmacology 81, 1–5.

Dooley, D.J., Bittiger, H., Hauser, K.L., Bischoff, S.F., Waldmeier, P.C., 1983b. Alteration of central alpha 2- and beta-adrenergic receptors in the rat after DSP-4, a selective noradrenergic neurotoxin. Neuroscience 9, 889–898.

Espejo, E.F., Minano, F., 1999. Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test. Neuroscience 88, 609–615.

Esposito, E., Ossowska, G., Samanin, R., 1987. Further evidence that noradrenaline is not involved in the anti-immobility activity of chronic desipramine in the rat. Eur. J. Pharmacol. 136, 429–432.

Fritschy, J.M., Grzanna, R., 1991. Selective effects of DSP-4 on locus coeruleus axons: are there pharmacologically different types of noradrenergic axons in the central nervous system? Prog. Brain Res. 88, 257–268.

Gandolfi, O., Dall'Olio, R., 1996. Modulatory role of dopamine on excitatory amino acid receptors. Prog. Neuropsychopharmacol. Biol. Psychiatry 20, 659–671.

Harkin, A., Kelly, J.P., McNamara, M., Connor, T.J., Dredge, K., Redmond, A., Leonard, B.E., 1999. Activity and onset of action of reboxetine and effect of combination with sertraline in an animal model of depression. Eur. J. Pharmacol. 364, 123–132.

Heal, D.J., Butler, S.A., Prow, M.R., Buckett, W.R., 1993. Quantification of presynaptic alpha 2-adrenoceptors in rat brain after short-term DSP-4 lesioning. Eur. J. Pharmacol. 249, 37–41.

Hemby, S.E., Lucki, I., Gatto, G., Singh, A., Thornley, C., Matasi, J., Kong, N., Smith, J.E., Davies, H.M., Dworkin, S.I., 1998. Potential antidepressant effects of novel tropane compounds, selective for serotonin or dopamine transporters. J. Pharmacol. Exp. Ther. 282, 727–733.

Hughes, Z.A., Stanford, S.C., 1998. A partial noradrenergic lesion induced by DSP-4 increases extracellular noradrenaline concentration in rat frontal cortex: a microdialysis study in vivo. Psychopharmacology 136, 299–303.

Jonsson, G., Hallman, H., Ponzio, F., Ross, S., 1981. DSP-4 (*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine)-a useful denervation tool for central and peripheral noradrenaline neurons. Eur. J. Pharmacol. 72, 173–188.

Kask, A., Harro, J., Tuomaine, P., Rago, L., Mannisto, P.T., 1997. Overflow of noradrenaline and dopamine in frontal cortex after [*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine] (DSP-4) treatment: in vivo microdialysis study in anaesthetized rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 355, 267–272.

Kostowski, W., Jerlicz, M., Bidzinski, A., Hauptmann, M., 1978. Evidence for existence of two opposite noradrenergic brain systems controlling behavior. Psychopharmacology 59, 311–312.

Kostowski, W., Trzaskowska, E., Jerlicz, M., Bidzinski, A., Hauptmann, M., 1980. Effects of lesions of the ventral noradrenergic bundle on aggressive behavior in rats. Physiol. Behav. 24, 429–433.

Kostowski, W., Plaznik, A., Pucilowski, O., Malatynska, E., 1982. Effect of lesions of the brain noradrenergic systems on amphetamine-induced hyperthermia and locomotor stimulation. Acta Physiol. Pol. 33, 383–387.

Kostowski, W., Danysz, W., Nowakowska, E., 1984a. Studies on brain noradrenergic neurons in animal model for antidepressive activity. Psychopharmacol. Bull. 20, 320–322.

Kostowski, W., Danysz, W., Plaznik, A., Nowakowska, E., 1984b. Studies on the locus coeruleus system in an animal model for antidepressive activity. Pol. J. Pharmacol. Pharm. 36, 523–530.

Kostowski, W., Malatynska, E., Plaznik, A., Dyr, W., Danysz, W., 1986. Comparative studies on antidepressant action of alprazolam in different animal models. Pol. J. Pharmacol. Pharm. 38, 471–481.

Leonard, B.E., 1997. The role of noradrenaline in depression: a review. J. Psychopharmacol. 11, S39–S47.

Lucki, I., 1997. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav. Pharmacol. 8, 523–532.

Magnuson, D.S., Staines, W.A., Marshall, K.C., 1993. Electrophysiological changes accompanying DSP-4 lesions of rat locus coeruleus neurons. Brain Res. 628, 317–320.

Moore, R.Y., Card, J.P., 1984. Noradrenaline-containing neuron systems. In: Bjorklund, A., Hokfelt, T. (Eds.), Handbook of Chemical Neuroanatomy. Vol. 2: Classical Transmitters in the CNS Part 1. Elsevier, Amsterdam, pp. 123–156.

Murugaiah, K.D., O'Donnell, J.M., 1995. Facilitation of norepinephrine release from cerebral cortex is mediated by beta 2-adrenergic receptors. Life Sci. 57, PL327–PL332.

Page, M.E., Detke, M.J., Dalvi, A., Kirby, L.G., Lucki, I., 1999. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology 147, 162–167.

Plaznik, A., Kostowski, W., 1985. Modification of behavioral response to intra-hippocampal injections of noradrenaline and adrenoceptor agonists by chronic treatment with desipramine and citalopram: functional aspects of adaptive receptor changes. Eur. J. Pharmacol. 117, 245–252.

Plaznik, A., Danysz, W., Kostowski, W., 1985. A stimulatory effect of intraaccumbens injections of noradrenaline on the behavior of rats in the forced swim test. Psychopharmacology (Berlin) 87, 119–123.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatments. Nature 266, 730–732.

Reneric, J.P., Lucki, I., 1998. Antidepressant behavioral effects by dual inhibition of monoaminereuptake in the rat forced swimming test. Psychopharmacology 136, 190–197.

Schildkraut, J.J., 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry 122, 509–522.

Shaham, Y., Highfield, D., Delfs, J., Leung, S., Stewart, J., 2000. Clonidine blocks stress-induced reinstatement of heroin seeking in rats: an effect independent of locus coeruleus noradrenergic neurons. Eur. J. Neurosci. 12, 292–302.

Stahl, S.M., 1997. Psychopharmacology of Antidepressants. Martin Dunitz, London.

Stanford, S.C., 1995. Central noradrenergic neurones and stress. Pharmacol. Ther. 68, 297–342.

Thoenen, H., Tranzer, J.P., 1968. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-Hydroxydopamine. Naunyn-Schmiedeberg's Arch. Exp. Pathol. 261, 271–288.

Valentino, R.J., Curtis, A.L., Parris, D.G., Wehby, R.G., 1990. Antidepres-

sant actions on brain noradrenergic neurons. J. Pharmacol. Exp. Ther. 253, 833–840.

Valentino, R.J., Curtis, A.L., Page, M.E., Pavcovich, L.A., Florin-Lechner, S.M., 1998. Activation of the locus coeruleus brain noradrenergic system during stress: circuitry, consequences, and regulation. Adv. Pharmacol. 42, 781–784.

Weiss, J.M., Kilts, C.D., 1998. Animal models of depression and schizophrenia. In: Nemeroff, C.B., Schatzberg, A.F. (Eds.), Textbook of Psychopharmacology. American Psychiatric Association Press, New York, pp. 88–123.

Wong, E.H., Sonders, M.S., Amara, S.G., Tinholt, P.M., Piercey, M.F., Hoffmann, W.P., Hyslop, D.K., Franklin, S., Porsolt, R.D., Bonsignori, A., Carfagna, N., McArthur, R.A., 2000. Reboxetine: a pharmacologically potent, selective, and specific norepinephrine reuptake inhibitor. Biol. Psychiatry 47, 818–829.