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Simultaneous analyses of the neurochemical and behavioral effects of the norepinephrine reuptake inhibitor reboxetine in a rat model of antidepressant action

Received: 17 June 2002 / Accepted: 4 September 2002 / Published online: 9 November 2002 © Springer-Verlag 2002

Abstract
Rationale: The forced swimming test (FST) is a rodent behavioral assay widely used to predict clinical efficacy of putative antidepressants. Few studies have examined the effects of the FST on neurotransmitter levels and how antidepressant drug treatment may alter neurotransmitter levels and behavior simultaneously during the performance of a stressful task. Objectives: The present study examined the role of norepinephrine in mediating active behaviors in the FST after treatment with reboxetine, a selective norepinephrine reuptake inhibitor. Methods: High-pressure liquid chromatography was used to analyze microdialysis samples collected from awake, freely moving rats before, during and after exposure to the FST. Reboxetine (10 mg/kg) was given three times over a 24-h period prior to the test swim. Behavioral responses, including immobility, swimming and climbing, were counted during the 5-min test on day 1 and day 2. Results: The first exposure to swim stress elicited a 65% increase in extracellular norepinephrine (NE). A second exposure on day 2 elicited a 52% increase of NE and a behavioral profile characterized by increased immobility and a reduction of active behaviors. A subchronic course (three injections over 24 h) of treatment with reboxetine between the two swim exposures resulted in antidepressant-like activity, i.e., decreased immobility and increased climbing behavior on day 2. A significantly greater increase in extracellular NE (112%) was observed in the group of animals that received reboxetine injections. Conclusions: Treatment with reboxetine in a schedule commonly used in the FST resulted in a potentiated noradrenergic response to the swim challenge concomitant with behavioral alterations consistent with antidepressant-like activity.

Keywords Forced swimming test · Antidepressant · Norepinephrine · Behavior

Introduction
Stress has been shown to activate monoamine systems and may be associated with the precipitation of depressive symptomatology (Anisman and Zacharko 1982; McEwen 2000). The locus coeruleus (LC) noradrenergic system is a vital component of an individual’s response to stressors or challenges and provides the majority of the noradrenergic innervation of the forebrain (Grzanna and Fritschy 1991; Valentino et al. 1997). LC discharge properties have been well characterized in awake and anesthetized animals and can be described as being sensitive to changes in the internal and external environment (Aston-Jones and Bloom 1981a, 1981b; Morilak et al. 1987a, 1987b). Thus, it is generally believed that dysregulation of this system may be an important factor in the onset of symptoms of anxiety and depression (Stanford 1995). Furthermore, treatment of depressive disorders with noradrenergic modulating compounds has met with reasonable success and points, at least, to its involvement in the therapeutic response (Heninger et al. 1996).

The forced swimming test (FST) is a behavioral assay that has been shown to reliably predict the efficacy of potential antidepressant compounds in both rats and mice (Porsolt et al. 1977, 1978; Borsini and Meli 1988; Cryan et al. 2002a). The rat FST is usually conducted with two tests separated by 24 h, a 15-min pretest on day 1 and a test session for 5 min on day 2, and is dependent on an animal’s reaction to the inability to escape from a
stressful environment. Exposure to the pretest decreases
the latency to the induction of behavioral immobility
upon the second test exposure. Antidepressant compounds
given between the first and second test sessions effect-
ively attenuate the duration of immobility and elicit
active behaviors consistent with escape-directed activity.
We have previously described modifications to the
traditional FST procedure that provide more detailed
information upon subsequent analysis (Detke et al. 1995b;
Lucki 1997).

A continuing effort to identify more efficacious
antidepressant compounds with lower side-effect profiles
has led to the emergence of the selective norepinephrine
(NE) reuptake inhibitors (Brunello and Racagni 1998).
The selective NE reuptake inhibitor reboxetine is a
potentially effective antidepressant compound based on
a number of clinical trials as well as experimental data
gathered from animal studies (Connor et al. 1999; Harkin
et al. 1999, 2000; Cryan et al. 2002b). Like other NE
reuptake inhibitors (Detke et al. 1995a), reboxetine
reduces immobility and increases climbing behavior in
the modified rat FST. The effects of reboxetine in the FST
are under the complex control of NE systems arising from
both the LC and other brainstem nuclei that send
projections through the ventral noradrenergic bundle
(Cryan et al. 2002b). Reboxetine selectively inhibits the
reuptake of synaptic NE without any marked affinity for
other receptors or transporters (Wong et al. 2000).
Additionally, reboxetine has been shown to be both
clinically effective and well tolerated with a lower side-
effect profile than tricyclic antidepressants (Burrows et al.
1998).

The present study sought to examine the role of NE in
mediating the antidepressant-like actions of reboxetine in
the FST. The combination of microdialysis with the FST
is a useful experimental approach as it allows one to
assess the relationship between neurotransmitter output
and behavior on effectively the same time scale in the
same animal under the same environmental conditions.
Previous reports from this lab have described the activity
of the serotonergic system during the FST (Kirby et al.
1995, 1997; Price and Lucki 2002). However, there has
been less focus to date on the contribution of other
neurotransmitter systems to the antidepressant-like be-
havioral effects in the FST. Jordan and colleagues
previously described alterations in monoamine neuro-
transmitters associated with the FST on two consecutive
days but did not report changes associated with antide-
pressant treatment (Jordan et al. 1994). In the present
studies, we examined alterations of extracellular NE in
animals exposed to two sessions of the FST and in
response to reboxetine treatment. Using in vivo micro-
dialysis, NE output in the medial prefrontal cortex was
measured in response to the initial exposure to the swim
challenge (i.e., pretest), in response to repeated injections
of reboxetine (three injections in intervening 24 h) and in
response to the test swim on day 2. The experimental
protocol followed was identical to that previously
described for assessment of behavioral changes in the
FST (Detke et al. 1995a). Finally, NE output elicited by
the FST following reboxetine treatment was correlated
with changes in behavioral response in the same animals.

Materials and methods

Animals

Male, Sprague-Dawley rats (Charles River Laboratories, Wilming-
ton, Mass.) weighing 200–250 g at the start of the experiment were
housted two per cage and maintained under conditions of constant
temperature (22°C) on a 12-h/12-h light/dark cycle (0700 hours on
and 1900 hours off) with free access to food and water. Animal
procedures were conducted in accordance with the guidelines
published in the NIH Guide for Care and Use of Laboratory
Animals, and all protocols were approved by the University of
Pennsylvania Institutional Animal Care and Use Committee and the
Drexel University College of Medicine Institutional Animal Care
and Use Committee.

Dialysis probe construction and calibration

Vertical concentric microdialysis probes were utilized (Abercrom-
bie et al. 1988). Briefly, a piece of fused silica (Polymicro
Technologies, Phoenix, Ariz.) was inserted through PE10 tubing
(Clay Adams, Parsippany, N.J.) and a semipermeable membrane of
hollow cuprammonium rayon fibers with a 224-μm o.d. and 35,000-
MW cutoff (C series; Terumo Corp., Somersett, N.J.) was fixed over
the fused silica into the PE10 tubing with epoxy. The open end of
the dialysis fiber was sealed with a 0.5-mm epoxy plug and a region
was coated with epoxy, leaving an active area of 3 mm for
exchange across the membrane. The in vitro recovery rate was
determined by placing the probe in a beaker of artificial cerebro-
spinal fluid (aCSF) containing a known concentration of NE
standard. The concentration of NE in the perfusate was compared
with the amount in the bath. Probes that did not correspond to the
typical range of recovery (16–24%) were identified and eliminated.
Because the diffusion properties of neurochemicals in brain tissue
are likely different from in vitro conditions, dialysate values were
not corrected for the recovery of the probe.

Surgical procedures

Acclimation to the animal facility was allowed for at least 1 week
prior to surgical manipulation. Rats were anesthetized with Na-
pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus
with the skull flat. Probe placements were alternated between the
right and left frontal cortex (FC) from one rat to the next. A small
burr hole was made in the skull centered 3.2 mm anterior and
±0.7 mm lateral to bregma. The microdialysis probe was slowly
lowered 5.0 mm from dura into the FC and secured with skull
screws and dental acrylic. The inlet of the probe was connected to a
fluid swivel (Instech Laboratories, Plymouth Meeting, Pa.) and the
rat was placed into an awake animal apparatus (Instech Labora-
tories), a cylindrical plexiglass container (31x38 cm) with the floor
covered with bedding. A spring counterbalanced lever arm mounted
on a swivel at the top of the cage allowed a free range of motion.
Food pellets and a water bottle were easily accessible. aCSF
(147 mM NaCl, 1.7 mM CaCl₂, 0.9 mM MgCl₂ and 4 mM KCl)
was continuously perfused through the probe at a rate of 1.5 μl/min
via a microliter infusion pump (Instech Laboratories) and the rat
was allowed to recover overnight. Approximately 18 h following
surgery, dialysate sample collection began and continued at 20-min
intervals for the duration of the experiment. At the conclusion of
the experiment, rats were deeply anesthetized, and green dye was
infused through the probe to mark its location. The rats were
decapitated and the brains removed for subsequent histological