Norepinephrine Uptake Inhibitors as Biochemically and Behaviorally Selective Antagonists of the Locomotor Stimulation Induced by Indirectly Acting Sympathomimetic Amines in Mice

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Abstract. Pretreatment with the selective noradrenergic uptake inhibitors nisoxetine and desipramine antagonized the locomotor stimulant effect of d-amphetamine without reducing the drug's stereotypy-inducing action. A similar antagonism was observed with imipramine but not with fluoxetine, a selective serotonin uptake inhibitor and structural analog of nisoxetine. The order of potency of antagonism was desipramine > nisoxetine > imipramine. Nisoxetine also selectively reduced the locomotor activity induced by maximally effective doses of cocaine, d-N-ethylamphetamine, and methylphenidate, but not that induced by morphine. Biochemically, nisoxetine blocked the selective reduction in cerebral cortical endogenous and \(^{3}\)H-norepinephrine produced by amphetamine without itself significantly altering either measure. These data support the involvement of norepinephrine in the locomotor stimulant action of indirectly acting sympathomimetic amines.

Key words: Psychomotor stimulants — Uptake inhibitors — Catecholamines — Locomotor activity — Stereotypy

Moderate doses of amphetamine stimulate large amplitude movements, such as locomotion in rodents, while higher doses are associated with a reduction in locomotion and a concomitant increase in the frequency of small amplitude movements, such as licking, chewing, and grooming behaviors (i.e., stereotypy) (Lewander, 1977; Cole, 1978; Tyler and Tessel, 1979a). Amphetamine also releases central norepinephrine (NE) and dopamine (DA) (Azzaro and Rutledge, 1973; Chiuah and Moore, 1974) and, although it is generally agreed that amphetamine-induced stereotypy is due to the drug's ability to release central DA with amphetamine-induced NE release possibly having an inhibitory role (Antelman and Caggiula, 1977), the neurohumoral substrate of amphetamine's locomotor stimulant action is not clearly determined. Most evidence supports the contention that the latter behavioral action of amphetamine, like the former, is mediated by the release of DA (Hollister et al., 1974; Pijnenburg et al., 1975; Kelly and Iversen, 1976). However, Segal and his colleagues (Geyer and Segal, 1973; Segal et al., 1974) have found that infusion of NE and amphetamine into rat cerebral ventricles also result in increased locomotor activity, while DA infusions increase activity but appear to depend upon conversion to NE for this effect (Geyer et al., 1972).

An alternative approach in examining a possible role of NE in the locomotor stimulant action of amphetamine utilizes the observation that inhibitors of NE uptake can antagonize the amphetamine-induced release of this neurotransmitter in vitro (Azzaro et al., 1974; Paton, 1976). Results of most previous attempts to use this procedure have not been clearly interpretable due either to the use of a species (rat) in which the simultaneous presence of an uptake inhibitor (i.e., desipramine) and amphetamine result in markedly increased brain levels of the latter drug (Sulser et al., 1966), or a behavioral measurement procedure which cannot discriminate reduction in locomotor stimulation from a general sedative effect (Lew et al., 1971). However, new procedure has been developed (Tyler and Tessel, 1979a) that allows simultaneous and objective measurement of locomotion and stereotypy in individual mice, a species in which inhibitors of NE uptake, such as desipramine and imipramine, do not appear to augment brain levels of amphetamine (Lew et al., 1971). Tyler and Tessel (1979b), using this procedure, reported that another potent inhibitor of NE uptake, nisoxetine (Wong and Bymaster, 1976a, b), selectively antagonized the locomotor stimulant action...
of amphetamine and, in vitro, inhibited the amphetamine-induced release of $^3$H-NE from mouse cerebral cortex, but not that of $^3$H-DA or $^3$H-serotonin ($^3$H-5-HT) from mouse corpus striatum.

The purpose of the present study was to further investigate the hypothesis that inhibition of NE uptake selectively antagonizes the locomotor stimulant action of indirectly acting sympathomimetic amines by antagonizing the drug-induced release of that neurotransmitter.

**Materials and Methods**

**Animals.** Male ICR mice (Spartan Labs, Haslett, Michigan) weighing 25 – 30 g were used. Mice were maintained on a 12-h light-dark cycle and allowed continuous access to food and water. Mice were acclimatized after arrival in group cages for at least 4 days prior to use. Mice were tested only between 8 a.m. and 6 p.m. to reduce the possible influence of diurnal variation.

**Behavioral Measurements.** The procedure of Tyler and Tessel (1979a, b) was used to allow simultaneous and objective measurement of both locomotor activity and stereotypy in individual mice. Briefly, a Varimex dual channel activity meter (Columbus Instruments, Columbus, Ohio) with two capacitative sensors each tuned to 0.7 was used. One sensor (low sensitivity for locomotor activity detection) was placed directly on top of a black-painted polystyrene rat cage (activity chamber) and another sensor (high sensitivity for total behavioral activity) was placed immediately below the activity chamber. The low sensitivity channel was calibrated to measure locomotion as follows: Mice were injected IP with 3 mg/kg d-amphetamine and observed visually through a slight gap between the upper rim of the cage and the upper sensor. The counting threshold of the low sensitivity channel was raised until only running movements were counted (setting 3.8). A similar procedure was used to determine the counting threshold of the alternate channel except that 10 mg/kg d-amphetamine was used and the behaviors under observation were intense sniffing, grooming, and chewing (setting 10.0). Animal movement associated with respiration was not recorded. The difference between high sensitivity counts (total activity) and low sensitivity counts (locomotion) gave small-movement counts, presumably due to stereotyped behavior (Tyler and Tessel, 1979a, b). An individual mouse was injected IP with saline (0.01 ml/g) or a dose of one of the following drugs: d-amphetamine; cocaine; methylphenidate; d-N-ethylamphetamine; or morphine. The animal was then placed in the activity chamber and its activity monitored for 1 h. In some experiments, the above injections were preceded by IP saline (0.5 or 1 h), various doses of nisoxetine, desipramine, or imipramine (0.5 h), or the potent and selective serotonin uptake inhibitor fluoxetine (1 h) (Wong et al., 1975). The pretreatment times and dose ranges were selected due to previous reports concerning the ability of nisoxetine, desipramine, and imipramine to antagonize 6-hydroxydopamine-induced reductions in rat brain NE or the in vitro uptake of $^3$H-NE into rat brain homogenates or synaptosomes after IP administration of the drugs (Wong and Bymaster, 1976a; Sugrue and Mireylees, 1978). Similar reports involving p-chloroamphetamine-induced 5-hydroxytryptamine (5-HT) depletion and $^3$H-5-HT uptake were used to select the time and dose of fluoxetine pretreatment (Fuller et al., 1975; Wong and Bymaster, 1976a).

**Endogenous Catecholamine Assay.** The liquid chromatographic procedure with electrochemical detection (Keller et al., 1976) was used with certain modifications. Briefly, an individual mouse was killed by cervical dislocation, the brain immediately removed and chilled on glass over ice. The cerebral cortex, hypothalamus, corpus striatum, and pons medulla were dissected out following the procedure of Glowinski and Iversen (1966) as modified here for mice. Each brain region was then immediately frozen on glass over dry ice, weighed, and placed in a 1.5 ml polypropylene tube.

For simultaneous NE and DA measurement (all regions except pons medulla and corpus striatum, since these regions have very low levels of DA and NE, respectively), 20 µl 0.1 M HClO₄ containing the internal standard DHBA (3,4-dihydroxybenzylamine hydrobromide) was added to each tube and the tube was ruptured by sonication (Biosonic, WWR Scientific, San Francisco, California). One milliliter of 0.5 M TRIS buffer (pH 8.6) was added to each tube followed by addition of 15 – 20 mg acid-washed alumina (Anton and Sayre, 1962). The tubes were capped and contents mixed by continuously inverting the tubes for 15 min. The solution was then aspirated off and the alumina washed three times with a wash solution (100 ml H₂O, 100 µl 1.0 M NaHSO₄, and 1.0 ml 0.5 TRIS buffer at pH 8.6). HClO₄ (50 µl, 0.1 M) was added to desorb the catecholamines and DHBA from the alumina. An aliquot (20 µl) of each sample was injected into the liquid chromatographic column (see below).

**Preparation of standards involved dissolving known amounts of NE or DA in 0.1 M HClO₄ with DHBA and omitting sonication. Calculations were based on peak height ratios of NE to DA in standards versus brain samples using this equation for NE content:**

$$\text{NE (ng/g)} = \left( \frac{\text{NE}}{\text{DHBA}} \right)_{\text{samples}} - \left( \frac{\text{ngNE}}{\text{DHBA}} \right)_{\text{standards}} \times \text{brain region weight (g)}.$$  

Sample preparation for DA assay only (corpus striatum) was the same as above, except that 100 µl 0.1 M HClO₄ without DHBA was added to the frozen tissue and the tissue was then sonicated. The tissue was then separated from acid by centrifugation. The acid supernatant containing DA was injected in a 40 µl volume directly into the liquid chromatograph. Appropriate external standards were used to quantify the DA concentration in samples tested.

For simultaneous NE and DA determination, the cation exchange column was packed with Zipax SCX strong cation exchange resin (E. K. DuPont De Nemours, Wilmington, Delaware). The column was 2 mm inner diameter and 100 cm in length, eluant was an acetate citrate buffer (pH 5.2). Column pressure was approximately 450 – 500 psi and the working electrode potential was set at (+)0.55 V versus the reference electrode.

For DA measurement only (corpus striatum), to reduce the very long retention time (approximately 30 min) of DA on the above Zipax column, the cation exchange column was packed with Vydac CX cation exchange resin (The Separation Group, Heperin, California). The column was 2 mm inner diameter and 40 cm in length. All other conditions were as described above.

$^3$H-Norepinephrine Assay. Unanesthetized mice were hand held and injected (lateral cerebral ventricle) with 2.5 µCi $^3$H-d-NE (13 Ci/mmol) in 5 µl 0.9% saline using a modification of procedures previously reported (Clark et al., 1968). Verification of the accuracy of this injection technique was obtained in preliminary studies by injecting India ink in physiological saline into the lateral ventricle and the distribution throughout the brain regions studied. Absence of radioactivity in any or all of the brain regions studied suggested an improper injection and none of the data from that mouse was used. One and one-half hour after intracerebroventricular (ICV) injection, drug treatment was begun and, 3 h after ICV injection, the animal was killed by cervical dislocation, and the brain removed and chilled on glass over ice. Appropriate individual brain regions were dissected as described earlier and placed in 1 ml chilled 2 N HCl. The tissue was then disrupted by sonication and separated from the acid by centrifugation. The tissue pellet was resuspended with 1 ml 2 N HCl and after centrifugation, the acid supernatants from each centrifugation were combined. $^3$H-NE and $^3$H-O-methylated amines