Proc. Natl. Acad. Sci. USA
Vol. 78, No. 8, pp. 5250–5254, August 1981
Neurobiology

Norepinephrine neuronal uptake binding sites in rat brain membranes labeled with [^{3}H]desipramine

(imipramine/tricyclic antidepressants/serotonin/sodium and neurotransmitter transport)

CHI-MING LEE AND SOLOMON H. SNYDER*

Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

Contributed by Solomon H. Snyder, May 7, 1981

ABSTRACT **Neuronal uptake recognition sites in norepinephrine neurons have been labeled with the antidepressant [^{3}H]desipramine. A high-affinity component of [^{3}H]desipramine binding to rat cerebral cortex membranes is abolished selectively by 6-hydroxydopamine lesions, which destroy central catecholamine neurons. The high-affinity [^{3}H]desipramine binding has a nanomolar affinity constant for desipramine and is inhibited by tricyclic antidepressants with potencies that correlate with their ability to inhibit the neuronal uptake of norepinephrine. [^{3}H]Desipramine binding to the uptake sites is markedly stimulated by sodium, with potassium, lithium, and choline being much less effective. The ability to monitor norepinephrine neuronal uptake "receptors" in simple binding studies should permit a differential analysis of drug influences on the norepinephrine recognition site and the translocation mechanism. The regulation of [^{3}H]desipramine binding by sodium may help clarify how sodium influences neurotransmitter uptake.**

After its synaptic release the neurotransmitter norepinephrine (NE) is reaccumulated into nerve terminals by a specific uptake system, which serves to terminate its synaptic actions (1–3). The tricyclic antidepressants are potent inhibitors of the neuronal uptake of both NE and serotonin, effects which are thought to explain their clinical antidepressant actions (4). Among the tricyclic antidepressants, the tertiary amine forms tend to be relatively more potent competitive inhibitors of serotonin than of NE uptake, whereas the reverse holds true for secondary amine antidepressants such as desipramine (DMI) (5, 6).

Molecular studies of biogenic amine neuronal uptake in the brain have usually involved measurements of the accumulation of radiolabeled amines by slices or pinched-off nerve terminals, called "synaptosomes" (3, 5). The NE uptake recognition binding site on NE nerve terminals has not been studied biochemically separately from the Na^+,K^+-ATPase-dependent amine accumulating system. For neurotransmitter postsynaptic receptors, recognition sites can be labeled by the direct binding of radioactive forms of the transmitter or related drugs (7). Recently, [^{3}H]imipramine binding to brain (8) and platelet (9) membranes has been described; the binding properties suggest that the binding sites represent serotonin neuronal uptake recognition sites. Relative drug potencies in competing for [^{3}H]imipramine binding parallel affinities for the serotonin uptake system, and selective destruction of serotonin neuronal systems reduces [^{3}]imipramine binding (10–12).

We now report a high-affinity binding of [^{3}H]DMI to rat brain membranes that appears to label recognition sites for the neuronal uptake of NE.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

DMI was from Merrell–National Laboratory (Cincinnati, OH), imipramine was from Geigy Pharmaceuticals (Ardsley, NY), (+)- and (−)-amphetamine, chlorpheniramine, and chlorpromazine were generous gifts from Smith, Kline and French Laboratories, mianserin was from Organon, benztropine, cyproheptidine, and amitriptyline were from Merck Sharp and Dohme, (+)-epinephrine and phenylephrine were from Sterling–Winthrop Research Institute (Rensselaer, NY), chlorimipramine was from CIBA Pharmaceutical, iprindole was from Wyeth, clonidine was from Boehringer Ingelheim (Elmsford, NY), prazosin and doxepin were from Pfizer, 6-hydroxydopamine (6-OH-dopamine) and NE were from Sigma.

(±)-[^{3}H]NE (7.5 Ci/mmol), [^{3}H]DMI (57.0 Ci/mmol), and [^{3}H]imipramine (74.3 Ci/mmol) were obtained from New England Nuclear (1 Ci = 3.7×10^{10} becquerels).

For [^{3}H]DMI binding, male Sprague–Dawley rats weighing 150–200 g were killed by decapitation and each cerebral cortex was homogenized in 30 vol of ice-cold assay buffer (50 mM Tris·HCl/120 mM NaCl/5 mM KCl, pH 7.4) with a Brinkmann Polytron PT-10 at a setting of 5 for 10 sec. The homogenate was centrifuged at 50,000 × *g* for 10 min. The pellet was resuspended in 30 vol of the assay buffer by a Polytron at setting 5 for 5 sec and centrifuged again. This washing procedure was repeated twice and the final pellet was resuspended in 30 vol of assay buffer.

The binding of [^{3}H]DMI was routinely measured in triplicate at 0°C for 1 hr, using 200 μl of the membrane preparation with 0.2–10 nM [^{3}H]DMI in the presence or absence of various tested drugs in a final volume of 250 μl. At the end of the incubation period, 5 ml of ice-cold assay buffer was added to each tube and its content was filtered immediately under reduced pressure through Millipore glass fiber filters. Each of the filters was then washed three times with 5 ml of ice-cold buffer and radioactivity was measured by liquid scintillation spectrometry, using Formula 947 scintillation mixture (New England Nuclear). Nonspecific binding, unless otherwise stated, was defined as binding in the presence of 100 μM DMI. Specific binding of [^{3}H]DMI was calculated by subtracting the nonspecific from total binding and expressed as fmol/mg of protein.

To study the effect of various monovalent cations on [^{3}H]DMI binding, rat cortical membranes were prepared in 50 mM Tris·HCl buffer (pH 7.4). Specific binding was measured at 2 nM [^{3}H]DMI in the presence of various concentrations of sodium, potassium, lithium, or choline chloride.

Abbreviations: DMI, desipramine; NE, norepinephrine; 6-OH-dopamine, 6-hydroxydopamine.

* To whom reprint requests should be addressed.

For 6-OH-dopamine injections, male adult Sprague–Dawley rats weighing 150–200 g were anesthetized with Equithesin. 6-OH-dopamine (200 μg/20 μl) was dissolved in 0.9% NaCl containing 1% ascorbic acid and delivered into the left lateral ventricle over a period of 2 min under stereotaxic control (13). For some animals, a second dose of 6-OH-dopamine (200 μg/20 μl) was given 24 hr later. Sham-operated animals received the same volume of vehicle. Animals were decapitated 3 or 7 days after the second injection and each cerebral cortex was homogenized in 10 vol of ice-cold 0.3 M sucrose. The homogenate was centrifuged at 50,000 × g for 10 min, the pellet was resuspended in 10 vol of 0.3 M sucrose, and aliquots were saved for [^{3}H]NE uptake studies. The rest of the homogenate was diluted with the binding assay buffer, homogenized with a Polytron, and centrifuged at 50,000 × g for 10 min. The membranes were washed twice with 30 vol of the assay buffer, and the final pellet was resuspended in 30 vol of the assay buffer by Polytron treatment. Binding of [^{3}H]DMI was performed as described in the previous section, using 2 nM [^{3}H]DMI.

RESULTS

General Properties of [^{3}H]DMI Binding. Initial experiments at 37°C showed little difference between total binding of [^{3}H]DMI and nonspecific binding measured with either 10 μM or 100 μM unlabeled DMI. However, at 0°C reproducible specific binding could be obtained with several regions of rat brain. With washed rat cerebral cortical membranes incubated with 2 nM [^{3}H]DMI, total binding was about 3200 cpm and nonspecific binding in the presence of 100 μM DMI or 10 μM DMI, respectively, was 900 and 1200 cpm. Under standard binding conditions radioactivity in the medium represented unmetabolized [^{3}H]DMI when assayed by silica gel thin-layer chromatography (ethanol/ammonium hydroxide, 29:1, vol/vol).

Regional dissection experiments indicated that most brain regions displayed substantial levels of specific [^{3}H]DMI binding except for the corpus striatum and cerebellum, areas with lowest endogenous NE content, whose levels were substantially less than those of the cerebral cortex.

The [^{3}H]DMI binding site appears to be labile. Storing washed membranes overnight at −20°C produced a 20–30% loss of specific [^{3}H]DMI binding. Preincubation of membranes at 37°C for 1 hr followed by binding assays at 0°C resulted in a 40% decrease in binding.

Cation Dependence of Specific [^{3}H]DMI Binding. The NE neuronal uptake system is absolutely dependent upon sodium (2). We found sodium to be important for specific [^{3}H]DMI binding (Fig. 1). At 100 mM NaCl, specific [^{3}H]DMI binding was increased 5-fold, and a maximal enhancement of almost 7-fold occurred at 300 mM NaCl. This effect was highly specific for sodium, because potassium concentrations examined between 10 and 300 mM failed to influence [^{3}H]DMI binding, and both lithium and choline at 150 mM also did not stimulate binding.

Drug Specificity of [^{3}H]DMI Binding. Displacement curves showed a multiphasic reduction in [^{3}H]DMI binding by unlabeled DMI (Fig. 2). Complete displacement of specific [^{3}H]DMI binding by unlabeled DMI took place over 5 orders of magnitude of the unlabeled drug and displayed a Hill coefficient of 0.37. With cortical membranes prepared by Polytron treatment it was difficult to discriminate distinct components visually in displacement curves. About 50% reduction of specific [^{3}H]DMI binding (using 100 μM DMI to define nonspecific binding levels) occurred at approximately 0.03–0.1 μM. Under these incubation conditions nonspecific binding was only 1/4th of total

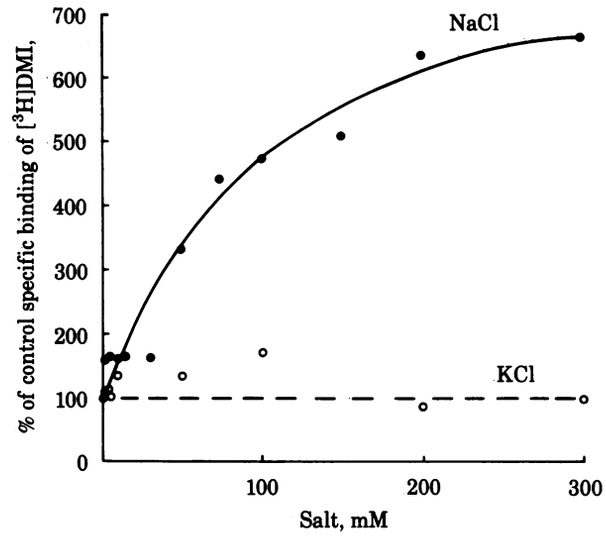

FIG. 1. Sodium dependency of [^{3}H]DMI binding to rat cerebral cortical membranes. Specific binding of [^{3}H]DMI was measured at 2 nM [^{3}H]DMI in the presence of various concentrations of sodium (●) or potassium (○) chloride. Specific binding of [^{3}H]DMI in the absence of sodium was 19.2 ± 2.7 fmol/mg of protein (mean ± SEM). Results are expressed as percent of binding relative to that of the no-sodium control and are the means of three experiments.

[^{3}H]DMI binding. When membranes were prepared after initial homogenization in sucrose under conditions to produce synaptosomes, nonspecific binding measured with 100 μM DMI was about 40% of total binding. In the sucrose-prepared membranes, nonspecific binding measured with 10 μM DMI was 65–70% of total binding, whereas with membranes homogenized with a Polytron in Tris buffer, nonspecific binding with 10 μM DMI was about 35% of total binding. Because the total

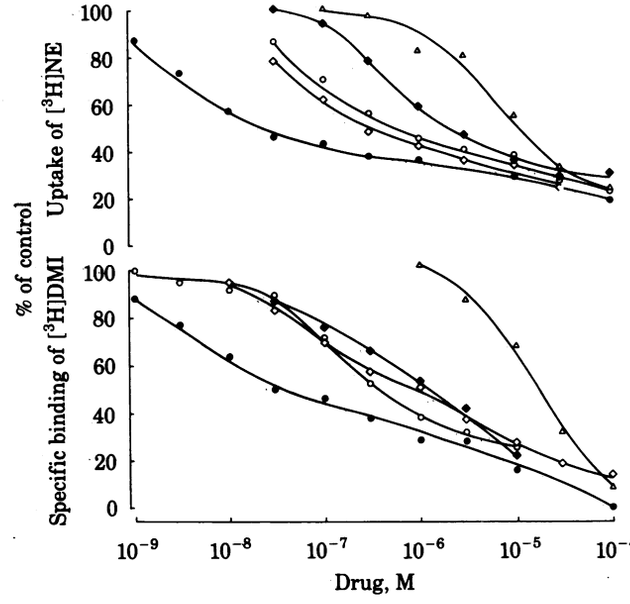

FIG. 2. Drug specificity of [^{3}H]NE uptake and [^{3}H]DMI binding. Inhibition of uptake of [^{3}H]NE into rat cortical synaptosomes and inhibition of specific binding of [^{3}H]DMI to rat cortical membranes by desipramine (●), imipramine (○), chlorimipramine (◆), doxepin (◇), and iprindole (△) were determined. Results are the mean of three or four separate experiments performed in triplicate determinations. The concentration of [^{3}H]NE and [^{3}H]DMI were 50 and 2 nM, respectively.

Table 1. Drug inhibitory potencies on [^{3}H]NE uptake and [^{3}H]DMI binding in the rat cerebral cortex

Drugs	IC_{50},* μM	
	[^{3}H]NE uptake	[^{3}H]DMI binding
Tricyclic antidepressants		
Desipramine	0.02	0.03
Imipramine	0.79	0.36
Doxepin	0.31	1.0
Chlorimipramine	1.8	1.4
Atypical antidepressants		
Mianserin	0.88	2.25
Iprindole	12.0	17.0
Others		
Chlorpromazine	1.8	0.13
Benztropine	2.7†	4.0
(+)-Amphetamine	0.07	12.0
(−)-Amphetamine	0.12	10.0
Cyproheptidine	2.7	3.0
Atropine	—	195.0
Neurotransmitters		
NE	0.4‡	≥1000
Dopamine	—	≥1000
Serotonin	—	>1000

γ-Aminobutyric acid had no effect on [^{3}H]DMI binding even when tested at 1 mM. The following drugs inhibited [^{3}H]DMI binding less than 50% when tested at 1 mM: yohimbine, phenylephrine, clonidine, and naloxone. [^{3}H]NE uptake was assayed in rat cerebral cortical homogenates by minor modifications of the techniques of Coyle and Snyder (14).

* Values are means of three independent determinations, which varied less than 15%.

† From Horn *et al.* (5).

‡ From Coyle and Snyder (14).

binding per mg of protein in sucrose-prepared membranes was 40–50% higher than binding in Tris-prepared membranes, the actual specific binding was fairly similar in the two preparations. Displacement curves in sucrose-prepared membranes showed clearly biphasic displacements, with a plateau between 0.01 and 0.3 μM at 60% of specific binding (using nonspecific binding defined with 10 μM DMI). The concentration for 50% inhibition (IC_{50}) of DMI for the high-affinity component of this displacement was about 2 nM, whereas the IC_{50} value for the low-affinity component was about 2000 nM (Fig. 3).

For all the antidepressants evaluated except for iprindole, displacement curves were quite shallow, with Hill coefficients substantially less than 1.0. The displacement curves for inhibition of [^{3}H]NE uptake into cerebral cortical synaptosome preparations were quite similar to displacements of specific [^{3}H]DMI binding (Fig. 2). For DMI itself the absolute potencies at different concentrations and the overall shapes of displacement curves were quite similar for the two processes. Imipramine, doxepin, and chlorimipramine also showed shallow displacement curves, with similar potencies both for [^{3}H]NE uptake and [^{3}H]DMI binding, whereas a substantially less shallow displacement curve was observed with iprindole for both [^{3}H]NE uptake and [^{3}H]DMI binding.

For most of the tricyclic antidepressants evaluated, absolute potencies in inhibiting [^{3}H]NE uptake and [^{3}H]DMI binding were quite similar (Table 1). The neuroleptic chlorpromazine was substantially more potent in inhibiting [^{3}H]DMI binding than [^{3}H]NE uptake. Of the "atypical" antidepressants, mianserin and iprindole had fairly similar potencies at [^{3}H]DMI and [^{3}H]NE sites. Both (+)- and (−)-amphetamine were about 100 times more potent inhibitors of [^{3}H]NE uptake than of [^{3}H]DMI binding.

NE itself was also substantially weaker in inhibiting [^{3}H]DMI binding than [^{3}H]NE uptake. However, a higher-affinity component of NE displacement could be detected with a 20% reduction in [^{3}H]DMI binding between 1 and 10 μM and then a plateau with no further reduction till 1 mM. The IC_{50} for this high-affinity component of NE displacement was about 3 μM. Dopamine was similar in potency to NE both in inhibiting [^{3}H]DMI binding and in inhibiting [^{3}H]NE uptake, whereas serotonin was weaker in influencing both processes.

Several drugs that influence receptors unrelated to adrenergic systems were weak at both [^{3}H]NE uptake and [^{3}H]DMI-binding sites.

Destruction of NE Neurons Reduces [^{3}H]DMI Binding. If [^{3}H]DMI labels NE neuronal uptake sites, destruction of NE-containing neurons should reduce [^{3}H]DMI binding. Accordingly, we administered 6-OH-dopamine intraventricularly under experimental conditions that produced various degrees of destruction of NE neuronal systems as monitored by [^{3}H]NE uptake (Table 2). In these experiments we measured nonspecific binding in two ways, with 0.1 μM and 100 μM unlabeled DMI. The very shallow displacement curves for DMI indicate the presence of distinct components of [^{3}H]DMI binding. If the highest-affinity component of such binding reflects binding sites to NE nerve terminals, while lower-affinity binding involves other sites, then monitoring the higher-affinity sites with 0.1 μM DMI for measuring nonspecific binding should indicate a greater depletion of [^{3}H]DMI binding sites when NE neurons are destroyed.

Destruction of NE neurons with 6-OH-dopamine did reduce [^{3}H]DMI binding (Table 2). Reduction in binding was greater with 6-OH-dopamine lesions that produced proportionally greater reduction in [^{3}H]NE uptake. Also, a greater degree of

Table 2. Effect of 6-OH-dopamine lesions on rat cerebral cortical [^{3}H]NE uptake and [^{3}H]DMI and [^{3}H]imipramine binding

Animals	[^{3}H]NE uptake, pmol/mg protein per 5 min	Specific [^{3}H]DMI binding, fmol/mg protein		Specific [^{3}H]imipramine binding, fmol/mg protein
		Total	High-affinity	
Control (n = 9)	1.94 ± 0.14 (100%)	149.9 ± 8.5 (100%)	39.4 ± 2.5 (100%)	86.6 ± 4.8 (100%)
6-OH-Dopamine lesion				
One dose, 3 days (n = 4)	1.62 ± 0.09 (84%)	138.9 ± 21.4 (93%)	27.4 ± 6.0 (70%)	—
Two doses, 3 days (n = 4)	1.01 ± 0.05 (52%)*	128.8 ± 3.1 (86%)	18.5 ± 2.8 (47%)*	75.5 ± 2.3 (87%)
Two doses, 7 days (n = 4)	0.20 ± 0.02 (10%)†	121.1 ± 5.8 (81%)	4.7 ± 3.4 (12%)†	79.4 ± 2.1 (92%)

6-OH-Dopamine (200 μg/20 μl, intraventricular) was administered under stereotaxic control. To some animals, a second dose of 6-OH-dopamine was administered 24 hr later. Cortical [^{3}H]NE uptake and specific [^{3}H]DMI and [^{3}H]imipramine binding were examined in sham-operated animals and animals with lesions at 3 and 7 days after the second injection. The data represent the mean ± SEM of n separate determinations, each performed in triplicate. Specific binding of [^{3}H]imipramine measured at 2 nM [^{3}H]imipramine was calculated by subtracting the nonspecific binding in the presence of 100 μM DMI from the total binding. Total and high-affinity specific binding of [^{3}H]DMI measured at 2 nM [^{3}H]DMI were calculated by subtracting the nonspecific binding in the presence of 100 μM and 0.1 μM DMI, respectively, from the total binding.

*, $P < 0.01$; †, $P < 0.005$ compared to control.

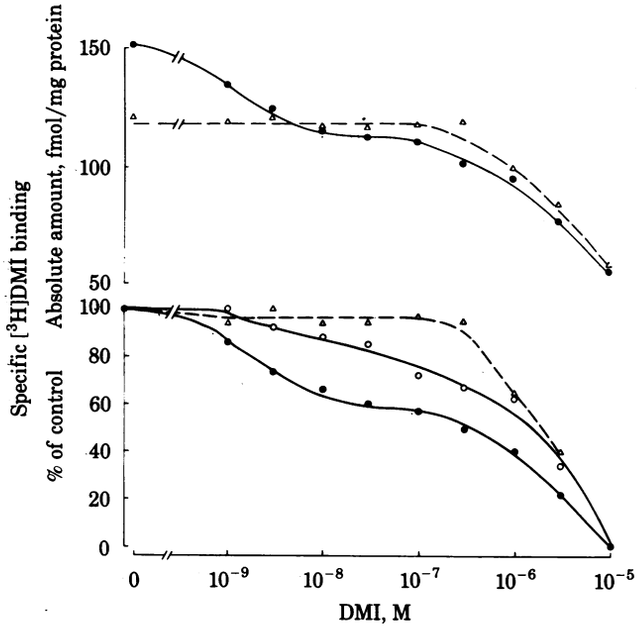

FIG. 3. Reduction of high-affinity [^{3}H]DMI binding by 6-OH-dopamine lesion. Displacement of [^{3}H]DMI binding in cerebral cortical membranes by unlabeled DMI was measured at 2 nM [^{3}H]DMI. Specific binding was calculated by subtracting the nonspecific binding in the presence of 10 μM DMI from the total binding and is expressed as fmol/mg of protein (*Upper*) or as percent of control binding in the absence of DMI (*Lower*). Results are the mean of four or six determinations performed in triplicate. ●, Control (n = 6); ○, 6-OH-dopamine, 3 days (n = 4); △, 6-OH-dopamine, 7 days (n = 4).

reduction of high-affinity than of total specific [^{3}H]DMI binding was obtained. Thus, lesions that reduced [^{3}H]NE uptake by 50% and 90% reduced high-affinity [^{3}H]DMI binding also by 50% and 90%, respectively, whereas only about 15% and 20% reduction in total specific [^{3}H]DMI binding was obtained.

As a control we measured [^{3}H]imipramine binding, which is thought to label primarily serotonin nerve terminal uptake sites (9–12). Very little reduction of [^{3}H]imipramine binding occurred with any of the 6-OH-dopamine lesions.

Displacement curves of [^{3}H]DMI binding revealed differences between control and 6-OH-dopamine-treated animals (Fig. 3). The high-affinity component of DMI displacement of [^{3}H]DMI binding was reduced in animals with lesions, while the low-affinity component was unaffected. In animals with 90% lesion of their NE uptake activity, an essentially monophasic displacement curve of [^{3}H]DMI binding by unlabeled DMI with a Hill coefficient of 0.9 and IC_{50} of 2 μM was obtained. NE also became substantially less effective in reducing [^{3}H]DMI binding after lesions (Table 3). These results support the notion that only the higher-affinity [^{3}H]DMI binding selectively labels NE neuronal uptake sites.

Table 3. Effect of 6-OH-dopamine lesion on NE displacement of [^{3}H]DMI binding from rat cerebral cortical membranes

Animals	Specific [^{3}H]DMI binding displaced by NE (1 mM), fmol/mg protein
Control (n = 6)	42.8 ± 2.9 (100%)
6-OH-Dopamine lesion	
Two doses, 3 days (n = 4)	19.5 ± 4.4* (46%)
Two doses, 7 days (n = 4)	10.2 ± 4.2* (24%)

The results represent the mean ± SEM of n separate determinations, each performed in triplicate. Specific binding of [^{3}H]DMI displaceable with NE measured at 2 nM [^{3}H]DMI was calculated by subtracting the binding in the presence of 1 mM NE from the total binding. *, $P < 0.05$ compared to control.

DISCUSSION

The major finding of this study is that [^{3}H]DMI appears to label NE neuronal uptake sites. Evidence to support this conclusion includes the fact that the drug specificity for the binding sites in general parallels the influence of these drugs upon NE uptake. Sodium, which selectively stimulates [^{3}H]DMI binding, is selectively required for NE uptake. Selective destruction of NE neurons with 6-OH-dopamine provides parallel reductions in [^{3}H]NE uptake and [^{3}H]DMI binding.

The absolute requirement of sodium for NE uptake may indicate a linkage with the Na^+,K^+-ATPase, which serves as a driving force for NE transport (2). The selective requirement of sodium for [^{3}H]DMI binding might reflect a property of the NE recognition site itself. Alternatively, an allosteric linkage of the NE recognition site with the ion transport system might explain the influence of sodium on [^{3}H]DMI binding. A sodium-dependent binding of ^{3}H-labeled γ-aminobutyric acid is thought to involve neuronal uptake sites (15), as is the sodium-dependent binding of [^{3}H]glutamate (16, 17). Influences of ions on ligand bindings to neurotransmitter receptors are thought to reflect interactions between the recognition sites for the transmitters and the ion conductance channels. Such examples include the regulation of glycine (18) and benzodiazepine (19) receptor binding by chloride ion and the influence of sodium on opiate (20) and α-adrenergic (21, 22) receptor binding.

Displacement experiments revealed multiple components of [^{3}H]DMI binding of different affinities. Because 6-OH-dopamine lesions reduce high-affinity but not low-affinity binding it appears clear that the higher-affinity binding is more selectively associated with NE neuronal uptake sites.

There are certain interesting differences in drug specificity between NE uptake and [^{3}H]DMI binding. Amphetamine is substantially weaker in reducing [^{3}H]DMI binding than in reducing [^{3}H]NE uptake. Perhaps amphetamine inhibits [^{3}H]NE uptake secondarily to a release of NE and so is not a truly potent inhibitor of the NE uptake process (23). Because amphetamine possesses only a single benzene ring, in contrast to the three phenyl groups of tricyclic antidepressants, it is possible that a major portion of [^{3}H]DMI binding involves parts of the tricyclic ring structure that amphetamine lacks. Though NE is weak in reducing total [^{3}H]DMI binding, it has micromolar potency at the high-affinity binding sites. On the other hand, several tricyclic drugs are potent at both high- and low-affinity DMI sites. The identity of the low-affinity sites is unclear. Though tricyclic antidepressants have potent actions at α-adrenergic, muscarinic cholinergic, and histamine H_1 receptors, the drug specificity of [^{3}H]DMI binding does not fit any of these sites. The drug specificity and response to 6-OH-dopamine lesions indicates that, unlike [^{3}H]imipramine, [^{3}H]DMI does not label serotonin uptake sites.

We thank Marla Bowman for superb technical assistance and Dawn C. Hanks for manuscript preparation. This work was supported by U.S. Public Health Service Grants MH-18501 and NS-16375, Research Scientist Award DA-00074 to S.H.S., a National Huntington's Disease Association fellowship to C.-M.L., and a grant from the McKnight Foundation.

1. Axelrod, J. (1965) *Recent Prog. Hormone Res.* 21, 597–619.
2. Iversen, L. L. (1967) *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge Univ. Press, New York).
3. Snyder, S. H. (1970) *Biol. Psychiatry* 2, 367–389.

4. Schildkraut, J. J. & Kety, S. S. (1967) *Science* **156,** 21–30.
5. Horn, A. S., Coyle, J. T. & Snyder, S. H. (1971) *Mol. Pharmacol.* **7,** 66–80.
6. Ross, S. B. & Renyi, A. L. (1975) *Acta Pharmacol. Toxicol.* **36,** 382–408.
7. Snyder, S. H. & Goodman, R. R. (1980) *J. Neurochem.* **35,** 5–15.
8. Langer, S. Z. & Briley, M. S. (1981) *Trends Neurosci.* **4,** 28–31.
9. Talvenheimo, J., Nelson, P. J. & Rudnick, G. (1979) *J. Biol. Chem.* **254,** 4631–4635.
10. Langer, S. Z., Moret, C., Raisman, R., Dubocovich, M. L. & Briley, M. (1980) *Science* **210,** 1133–1135.
11. Rehavi, M., Paul, S. M., Skolnick, P. & Goodwin, F. K. (1980) *Life Sci.* **26,** 2273–2279.
12. Palkovits, M., Raisman, R., Briley, M. & Langer, S. Z. (1981) *Brain Res.* **210,** 493–498.
13. Hedreen, J. (1975) in *Chemical Tools in Catecholamine Research*, eds. Jonsson, G., Malmfors, T. & Sachs, C. (North-Holland, New York), Vol. 1, pp. 91–100.
14. Coyle, J. T. & Snyder, S. H. (1969) *J. Pharmacol. Exp. Ther.* **170,** 221–231.
15. Enna, S. J. & Snyder, S. H. (1975) *Brain Res.* **100,** 81–97.
16. Bandry, M. & Lynch, G. (1979) *Eur. J. Pharmacol.* **57,** 283–285.
17. Vincent, S. R. & McGeer, E. (1980) *Brain Res.* **184,** 99–108.
18. Young, A. B. & Snyder, S. H. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4002–4005.
19. Costa, T., Rodbard, D. & Pert, C. B. (1979) *Nature (London)* **277,** 315–317.
20. Pert, C. B. & Snyder, S. H. (1974) *Mol. Pharmacol.* **10,** 868–879.
21. Greenberg, D. A., U'Prichard, D. C., Sheehan, P. & Snyder, S. H. (1978) *Brain Res.* **140,** 378–384.
22. Glossman, H. & Presek, P. (1979) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **306,** 67–73.
23. Heikkila, R. E., Orlansky, H., Mytilineou, C. & Cohen, G. (1975) *J. Pharmacol. Exp. Ther.* **194,** 47–56.