LOCALIZATION AND CHARACTERIZATION OF [³H]DESMETHYLIMIPRAMINE BINDING SITES IN RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY¹

ANAT BIEGON^{*, 2} AND THOMAS C. RAINBOW[‡]

^{*} Department of Pharmacology II, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110 and [‡] Department of Pharmacology, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104

Received September 28, 1982; Revised December 13, 1982; Accepted December 14, 1982

Abstract

The high affinity binding sites for the antidepressant desmethylinipramine (DMI) have been localized in rat brain by quantitative autoradiography. There are high concentrations of binding sites in the locus ceruleus, the anterior ventral thalamus, the ventral portion of the bed nucleus of the stria terminalis, the paraventricular and the dorsomedial nuclei of the hypothalamus. The distribution of DMI binding sites is in striking accord with the distribution of norepinephrine terminals. Pretreatment of rats with the neurotoxin 6-hydroxydopamine, which causes a selective degeneration of catecholamine terminals, results in 60 to 90% decrease in DMI binding. These data support the idea that high affinity binding sites for DMI are located on presynaptic noradrenergic terminals.

Desmethylinipramine (DMI) is a selective inhibitor of norepinephrine (NE) reuptake (Horn et al., 1971) and a potent antidepressant widely used in clinical practice (Klerman, 1971). Recently, it has been shown that high affinity binding sites exist for DMI in rat brain (Lee and Snyder, 1981; Rehavi et al., 1981). The anatomical distribution of these binding sites is unknown, and the identification of the target sites for DMI might clarify the neural basis for its therapeutic actions. In the present study we have used the recent LKB Ultrofilm-based method of quantitative autoradiography (Palacios et al., 1981; Penney et al., 1981; Quirion et al., 1981; Rainbow et al., 1982) to localize and quantify DMI binding sites in the rat brain. Because there is evidence that DMI binding sites in the rat cortex may be associated with NE nerve terminals (Hrdina et al., 1981; Lee and Snyder, 1981; Rehavi et al., 1982), we have also used the same method to examine the effects of monoaminergic lesions on DMI binding.

Materials and Methods

Male Sprague-Dawley rats were purchased from Charles River Co. and kept under controlled light-dark cycle and temperature until sacrificed by decapitation.

¹ We wish to thank Dr. R. Mangano and Mr. J. D'Amico of Hoffmann-La Roche for their help with the high performance liquid chromatography and lesion work and Ms. Patricia Manion for typing the manuscript. Supported by National Institutes of Health Grant NS17435 and a Sloan fellowship (T. C. R.). A. B. was a recipient of a Chaim Weizman fellowship.

² To whom correspondence should be addressed.

Autoradiographic procedure. Brain sections were labeled *in vitro* with [³H]DMI for quantitative autoradiography with tritium-sensitive sheet film (LKB Ultrofilm, LKB Inc.). Frozen 32- μ thick sections of rat brain were cut with a cryostat at -15° and were thaw mounted onto subbed glass slides. Binding sites for DMI were labeled by placing onto the sections 300 μ l of ice-cold buffer (50 mM Tris-HCl/300 mM NaCl, pH 7.4), containing 1 to 10 nM [³H]DMI (59.6 Ci/mmol, New England Nuclear). The sections were incubated with the ligand for 1 hr at 4° . Nonspecific binding was measured by co-incubation with 100 μ M unlabeled imipramine, nortriptyline, or DMI. The levels of nonspecific binding in the presence of the three displacing drugs were indistinguishable. After incubation, the sections were washed three times for 20 min in slide jars filled with ice-cold buffer. The sections were then either removed with a Whatman filter disk and used for scintillation counting or dried on a hot plate and apposed against LKB Ultrofilm for 1 month as described (Rainbow et al., 1981). The sections used for scintillation counting were taken from König and Klippel (1963) levels A4620 to A2970. We quantified autoradiograms with a densitometer, using the cresyl violet-stained brain sections for reference. The optical density of a brain area was converted into femtomoles of DMI specifically bound per milligram of protein, using tritium standards made from rat brain mash. Tritium standards were made by mixing brain mash with various amounts of [³H]leucine (Reivich et al., 1969). The standards were calibrated in terms of microcuries of ³H per milligram of protein by scintillation counting and were placed against LKB Ul-

trofilm to generate autoradiograms. The optical density (OD) readings from the tritium standards were used to convert OD readings of autoradiograms into equivalents of femtomoles of DMI per milligram of protein. Protein was assayed by the method of Bradford (1976).

Lesion studies. Animals were anesthetized and placed in a stereotaxic apparatus. 6-Hydroxydopamine (6-OHDA, Sigma) was injected into the lateral ventricle. Two hundred micrograms of the toxin were dissolved in 20 μ l of saline containing 0.1% ascorbic acid. Two injections, 72 hr apart, were given, and the animals were sacrificed 9 days after the first injection. Sham controls were injected with vehicle only. A total of four animals was used in the 6-OHDA study. The extent of the lesion was verified by measuring the NE content of a frontal cortex sample in a high performance liquid chromatograph (Waters) with an electrochemical detector. This procedure reliably produces extensive depletion (>70%) of NE throughout the brain (e.g., Malmfors and Tholnen, 1971).

5,7-Dihydroxytryptamine (5,7-DHT, Sigma; 200 μ g in 20 μ l of saline + 0.1% ascorbate) was injected into the lateral ventricle 1 hr after an intraperitoneal injection of 25 mg/kg of DMI (to protect noradrenergic terminals). Animals were sacrificed 2 weeks after a single injection, and the extent and specificity of the lesion were assessed by measuring NE and serotonin content in a frontal cortex sample by high performance liquid chromatography.

Results

Properties of [3 H]DMI binding to brain sections. The binding of [3 H]DMI (1 to 16 nM) to brain sections was saturable, specific, and of a high affinity (Fig. 1). Scatchard analysis (Scatchard, 1949) of the binding to brain sections removed for scintillation counting indicated a single population of sites with a K_d of 6.5 ± 0.5 nM and a capacity of 369 ± 7 fmol/mg of protein ($n = 3$). These values are similar to the properties of the binding site observed in membrane preparations (Hrdina et al., 1981; Lee and Snyder, 1981; Rehavi et al., 1981, 1982). For routine analysis of the binding, we used a concentration of 2 nM [3 H]DMI. At this concentration, specific binding accounted for 70% of the total binding, when sections were removed for scintillation counting. The pharmacological specificity of [3 H]DMI binding to sections also was similar to the binding site in membranes. DMI was 12 times more potent in inhibiting [3 H]DMI binding than imipramine, a tricyclic antidepressant which preferentially blocks serotonin reuptake (Horn et al., 1971). The atypical antidepressant mianserin, which has a weak effect on monamine reuptake, was 1,000 times less potent, and 1-propranolol, an antagonist of β -noradrenergic receptors which has no effect on NE uptake, was 10,000 times less potent than DMI in inhibiting [3 H]DMI binding.

Above 20 nM, [3 H]DMI binds mainly to lower affinity sites which we have described in detail earlier (Yavin et al., 1978, 1979; Biegón and Samuel, 1979a). This is also evident in the displacement curve of [3 H]DMI by cold DMI, which plateaus between 10^{-8} and 10^{-7} M, with a low affinity component displaced with IC_{50} of 2 to 3 μ M (data not shown). These results are in agreement with

our previous description of the low affinity sites and recent reports from other laboratories (Lee and Snyder, 1981). However, at 2 nM [3 H]DMI the low affinity component (based on the displacement curve) accounts for only 30% of the total displaceable binding in cortical areas, probably much less in areas rich in high affinity sites (see "Effect of Noradrenergic and Serotonergic Chemical Lesions on [3 H]DMI High Affinity Binding").

Distribution of [3 H]DMI high affinity sites in the brain. [3 H]DMI exhibits a highly heterogeneous distribution in the rat brain. A small number of regions and nuclei concentrate very high amounts of the ligand, whereas most other brain regions have moderate to low levels and a few are hardly above background or white matter areas (Fig. 2, Table I).

Figure 1. Saturation characteristics of [3 H]DMI in frozen brain sections. The figure illustrates one of three experiments done in triplicate which differed less than 15%. *Bottom*, total and nonspecific binding (in the presence of 100 μ M DMI). *Middle*, specific binding (total minus nonspecific). *Top*, Scatchard analysis of the binding data.

Figure 2. Distribution of $[^3\text{H}]$ DMI binding sites in rat brain. LKB Ultrofilm autoradiograms were made of $[^3\text{H}]$ DMI binding sites as described in the text. *a*, A high concentration of DMI binding is seen in the ventral portion of the bed nucleus of the stria terminalis (v.BN) and the base of the preoptic area. There is a low concentration of $[^3\text{H}]$ DMI binding sites in the caudate putamen, which is not substantially different from the level of nonspecific binding for $[^3\text{H}]$ DMI. This section corresponds to König and Klippel (1963) level A6860. *d.BN*, dorsal aspect, bed nucleus. *b*, level A5780 of König and Klippel. There are high concentrations of DMI binding sites in the anterior ventral nucleus of the thalamus (taV), the paraventricular and periventricular nuclei of the hypothalamus (PV), and in the supraoptic nucleus (SO). *C.Cx*, cingulate cortex. *c*, K-K 4380 μ . The dorsomedial nucleus (dmh) and the zona incerta have high densities of DMI binding sites. There is also substantial binding in the dentate gyrus (DG) and the paraventricular nucleus of the thalamus. *d*, K-K 1760 μ . There is an intermediate density of binding sites in the medial geniculate (mg), the superficial layer of the superior colliculus and in a region posterior and lateral to the central grey, which correspond to a known noradrenergic plexus. *V.hpc*, ventral hippocampus. *e*, Coronal section at the level of the locus ceruleus (LC). There is dense binding in the locus ceruleus.

TABLE I
Regional distribution of [^3H]DMI binding to rat brain

Results are means $\pm$ SE of 6 to 20 determinations from three to five rats. The concentration of [^3H] DMI used was 2 nM. The optical densities of autoradiograms were quantified as described. The OD measurements were converted into femtomoles of DMI/mg of protein using tritium standards. Nonspecific binding was subtracted from all readings.

Region	fmol of DMI/mg of Protein	Region	fmol of DMI/mg of Protein
Accumbens	48 $\pm$ 9	Hippocampus, dorsal CA ₁	120 $\pm$ 40
Bed nucleus of stria		CA ₃	190 $\pm$ 40
Terminalis, ventral aspect	685 $\pm$ 68	Dentate gyrus	288 $\pm$ 30
Dorsal aspect	154 $\pm$ 22	Subiculum	168 $\pm$ 71
Caudate putamen	45 $\pm$ 15	Hypothalamus: anterior	120 $\pm$ 10
Central grey		Dorsomedial nucleus	318 $\pm$ 30
Dorsomedial aspect	126 $\pm$ 30	Ventromedial nucleus	93 $\pm$ 22
Lateral aspect	302 $\pm$ 60	Paraventricular nucleus	489 $\pm$ 73
Cerebellar cortex	172 $\pm$ 36	Periventricular nucleus	360 $\pm$ 50
Colliculi: superior	244 $\pm$ 24	Mamillary nucleus	390 $\pm$ 40
Inferior	66 $\pm$ 22	Interpeduncular nucleus	297 $\pm$ 18
Corpus callosum	30 $\pm$ 25	Locus ceruleus	888 $\pm$ 108
Cortex, frontal, layer I	188 $\pm$ 23	Pineal body	494 $\pm$ 44
Frontal, layer IV	129 $\pm$ 15	Raphe, dorsal nucleus	264 $\pm$ 12
Cingulate	240 $\pm$ 40	Substantia nigra	34 $\pm$ 20
Parietal, layer IV	122 $\pm$ 32	Thalamus, anteroventral nucleus	500 $\pm$ 75
Diagonal band of Broca	284 $\pm$ 30	Dorsal aspect	539 $\pm$ 91
Geniculate: dorsolateral	225 $\pm$ 25	Dorsomedial nucleus	30 $\pm$ 10
Medial	153 $\pm$ 14	Ventral nucleus	92 $\pm$ 18
Globus pallidus	22 $\pm$ 12	Zona incerta	276 $\pm$ 48

Examining the brain from rostral to caudal, we find the *cortex* has moderate to low (200 fmol/mg of protein) levels of [^3H]DMI. The superficial layers of the cortex have more binding sites than deeper layers. The cingulate cortex stands out as the only cortical region with moderately high binding, 240 fmol/mg of protein. The *septum* too, is not a [^3H]DMI concentrating area, with 160 fmol/mg of protein in the lateral portion and 142 in the medial septum. However, within the lateral septum dense labeling appears in a thin line just along the most lateral portion of the lateral septum, and the highest binding within the medial septum is in a thin line along the midline. At the same coronal level, the *diagonal band of Broca* has moderately high [^3H]DMI levels (284 fmol/mg of protein). The *nucleus accumbens*, and more caudally, the *caudate putamen*, have very low DMI binding (40 to 50 fmol/mg of protein)—hardly more than nonspecific or *corpus callosum* (30 $\pm$ 25).

One of the highest concentrations of [^3H]DMI binding sites appears in the ventral portion of the *bed nucleus of the stria terminalis* (685 fmol/mg of protein; Fig. 2a). The dorsal aspect of the same nucleus has only 154 fmol/mg. At the same coronal level, *globus pallidus* has minimal binding (22 $\pm$ 12) undistinguishable from the neighboring caudate putamen, and the *supraoptic nucleus* has moderately high [^3H]DMI binding (318 fmol/mg of protein).

The next coronal level (König and Klippel (1963); 5780, Fig. 2b) contains several of the most dense nuclei in the brain—the *anteroventral thalamus* (500 to 540 fmol/mg) and the *paraventricular nucleus of the hypothalamus* (489 fmol/mg). Somewhat lower levels appear over the *periventricular* hypothalamic nucleus (360 fmol/mg). The neighboring *anterior* and *lateral hypothalamic* nuclei have relatively low (120 fmol/mg) concentrations of DMI binding sites. The dorsal *hippocampus* is hetero-

geneously labeled: low levels of [^3H]DMI appear in CA₁ (120 fmol/mg) and CA₂. The density of labeling is increased over CA_{3c} and CA₄ (>190 fmol/mg of protein) and is moderately high in the dentate gyrus (288 fmol/mg of protein). At the same level (Fig. 2c) the *thalamus*, dorsomedial and ventral nuclei, has low concentrations of binding sites (30 and 90 fmol/mg, respectively). The *dorsomedial nucleus of the hypothalamus* is highly labeled (318 fmol/mg of protein), whereas the ventromedial nucleus is much lower (93 fmol/mg of protein).

The *geniculate body* has a moderate density (225 fmol/mg of protein) of binding sites over the dorsolateral portion. The medial geniculate is lower (153). The *superior colliculus*, too, has moderately high binding (244 fmol/mg of protein), almost 4 times the concentration in the inferior colliculus. The *central grey* has uniformly low (126 fmol/mg of protein) concentration over most of the region. The ventral and lateral aspect of the central grey and an area adjacent and lateral to this portion stand out with a high density of 302 fmol/mg of protein. This is not an anatomically defined nucleus, but it correlates the best with Swanson and Hartman's (1975) description of a dense noradrenergic terminal area at this coronal level.

The *substantia nigra* has very low, almost undetectable levels of [^3H]DMI binding (35 fmol/mg of protein). In contrast to the *mamillary nucleus* (390 fmol/mg of protein) and, more caudally, the interpeduncular nucleus (297 fmol/mg of protein).

Of the non-neuronal elements in the brain, the *pineal gland* has a high density of [^3H]DMI sites (494 fmol/mg of protein), whereas no labeling appears over the ependymal cells in the choroid plexus.

Dorsal raphe nucleus has moderate DMI binding (264 fmol/mg). The cerebellar cortex has uniform, moderately low binding (172 fmol/mg of protein), and the *locus*

ceruleus has the highest concentration of [^3H]DMI binding sites we have observed in the brain—888 fmol/mg of protein (Fig. 2e). The level of the locus ceruleus is the most caudal we have examined.

Effect of noradrenergic and serotonergic chemical lesions on [^3H]DMI high affinity binding. Injection of 5,7-DHT into the ventricle resulted in a significant depletion of serotonin (sham: 2.12 ± 0.06 nmol/gm; 5,7-DHT: 0.6 ± 0.02 nmol/gm, mean $\pm$ SEM of three animals), whereas NE levels were not affected (sham: 2.7 ± 0.1 ; 5, 7-DHT: 2.5 ± 0.15 nmol/gm). This lesion had no effect on [^3H]DMI high affinity binding as determined by autoradiography. Sections from the level of the bed nucleus of the stria terminalis, anteroventral thalamus, and locus ceruleus from three 5,7-DHT and three sham animals had the same levels of [^3H]DMI binding (data not shown). On the other hand, 6-OHDA treatment, which resulted in a significant depletion of NE content (sham: 3.26 ± 0.15 ; 6-OHDA: 1.08 ± 0.15 nmol/gm), also caused a large reduction in the density of [^3H]DMI labeling (Fig. 3). However, the reduction in [^3H]DMI binding following 6-OHDA was not uniform over all brain regions. Those brain regions and nuclei which have the highest NE content and exhibited highest DMI binding in control and sham animals also showed the highest depletion (70 to 95%) of DMI binding following 6-OHDA. These include the anteroventral and anterodorsal thalamic nuclei, paraventricular hypothalamic nucleus, the ventral aspect of the bed nucleus of stria terminalis, and the diagonal band of Broca. Reductions of 35 to 55% occurred in moderately labeled areas, such as hippocampus, cortex, septum, and the dorsal aspect of the bed nucleus of stria terminalis. There was no decrease whatsoever in the caudate putamen and globus pallidus, which exhibited the lowest binding in control animals and are virtually devoid of noradrenergic terminals. In fact, specific binding to these areas seems to increase following the lesion (Fig. 4), but the increase is not statistically significant.

Discussion

In the present study, we have demonstrated that frozen brain sections possess both high affinity and low affinity sites for [^3H]DMI. The high affinity site has a K_d of 6.5 ± 2.5 nM and shows specificity for the tricyclic structure. The saturation characteristics and pharmacology of this site are in very good agreement with those of the high affinity site described in brain membrane preparations by Rehavi et al. (1982). The low affinity component has an affinity (2 to 3 μM) in brain sections which is similar to the low affinity binding we have previously characterized in synaptosomal and membrane preparations (Biegon and Samuel, 1979a) and which has been recently observed by Lee and Snyder (1981). Therefore, we conclude that the binding sites we were studying in brain sections are identical to those described by other workers and ourselves in homogenized brain preparations. The fact that membrane receptors retain their properties when studied in brain section *in vitro* has been documented for a large number of neurotransmitter and receptor types (Young and Kuhar, 1979; Palacios et al., 1981).

Figure 3. Effect of 6-OHDA on [^3H]DMI high affinity binding. *Top panel*, sham-injected animal, level of the bed nucleus of the stria terminalis. *Middle panel*, section from a 6-OHDA-treated animal at the same coronal level. *Bottom panel*, section from a control animal incubated with a large excess of cold DMI (nonspecific binding). Note the large decrease in the density of label over the ventral aspect of the bed nucleus, the visible decrease in the cortex, and the lack of decrease in the caudate putamen.

The distribution of high affinity binding sites for [^3H]DMI in rat brain (Fig. 2, Table I) is remarkably similar to the distribution of NE terminals as determined by anatomical methods (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Swanson and Hartman, 1975) and by direct measurement of NE in punch-out samples (Brownstein et al.,

Figure 4. Effect of 6-OHDA on [^3H]DMI binding in several brain regions. [^3H]DMI binding in 6-OHDA-lesioned animals is expressed as a percentage of control (sham-injected) levels in the same brain regions. Data were collected from a total of four animals. The values in the sham-injected animals were not significantly different from intact controls, e.g., anteroventral nucleus of the thalamus, sham: 390 ± 75 fmol/mg of protein, 6-OHDA: 27 ± 20 fmol/mg; paraventricular nucleus of the hypothalamus, sham: 417 ± 86 , 6-OHDA: 70 ± 14 fmol/mg; frontal cortex (layer I), sham: 207 ± 33 , 6-OHDA: 95 ± 33 fmol/mg; globus pallidus, sham: 27 ± 8 , 6-OHDA: 35 ± 8 (increase not significant, as determined by the Student's t test. All of the decreases were highly significant ($p < 0.01$)). *tad*, anterodorsal thalamus; *tav*, anteroventral thalamus; *dbb*, diagonal band of Broca; *PeV*, periventricular hypothalamus; *V.BN*, ventral aspect, bed nucleus; *L.Sep*, lateral septum; *M.Sep*, medial septum; *dG*, dentate gyrus; *CA₁*, hippocampus, CA₁ field; *P.Cx*, parietal cortex; *CP*, caudate putamen; *GP*, globus pallidus.

1971; Palkovitz et al., 1974; Versteeg et al., 1976). The highest concentrations of binding sites (>300 fmol/mg) were in the locus ceruleus, the nucleus of origin for much of the telencephalic NE innervation (Dahlstrom and Fuxe, 1964), and in several nuclei of the hypothalamus and limbic system: the ventral portion of the bed nucleus of the stria terminalis, the anterior ventral nucleus of the thalamus, the periventricular and paraventricular nuclei of the hypothalamus, and the hypothalamic dorsomedial nucleus. The pineal body also had a high content of [^3H]DMI binding sites. These regions have been described in anatomical studies as having an extremely dense noradrenergic innervation (Dahlstrom and Fuxe, 1964; Fuxe, 1965; Swanson and Hartman, 1975). In biochemical measurements (Brownstein et al., 1971; Palkovitz et al., 1974; Versteeg et al., 1976), the same nuclei have the highest concentrations of NE in the brain (approximately 50 ng/mg of protein).

There were a large number of brain regions that had an intermediate level of [^3H]DMI binding sites (200 to

300 fmol/mg). These regions included the supraoptic and interpeduncular nuclei, the zona incerta, and the diagonal band of Broca (Table I). In previous studies, the NE content of these nuclei ranged from 15 to 30 ng/mg of protein (Brownstein et al., 1971; Palkovitz et al., 1974; Versteeg et al., 1976). A region lateral to the central grey showed an intermediate level of binding sites. This region corresponded to a previously described noradrenergic terminal field (Swanson and Hartman, 1975). Other regions with an intermediate density of [^3H]DMI binding sites included dentate gyrus and the superficial layer of the superior colliculus.

The lowest levels of [^3H]DMI binding were found in brain regions with a low content of NE. There was a low concentration (<200 fmol/mg) of [^3H]DMI binding sites in the CA₁-CA₃ fields of the hippocampus, which receive a sparse innervation of NE fibers (Fuxe, 1965; Swanson and Hartman, 1975). The neocortex of the rat has overall a low content of NE that is greater in the superficial layers of the cortex than in the deeper layers. We observed a similar distribution of [^3H]DMI binding sites in the cortex. Layers I and II of the cerebral cortex had a moderate level of binding (188 fmol/mg), whereas the deeper layers had a lower concentration of DMI binding sites (130 fmol/mg). The lowest level of [^3H]DMI binding sites was in the substantia nigra, caudate putamen, globus pallidus, and accumbens (Table I), regions in rat brain which are practically devoid of NE terminals (Swanson and Hartman, 1975).

The effect of specific chemical lesions further confirms the association of high affinity [^3H]DMI binding with the presynaptic noradrenergic terminal. Although lesions of serotonergic terminals with 5,7-dihydroxytryptamine had no effect on [^3H]DMI high affinity sites, 6-OHDA treatment causes a large decrease in the apparent binding in most brain regions. This finding is in agreement with previous reports (Hrdina et al., 1981; Lee and Snyder, 1981; Rehavi et al., 1982) of a reduction in cortical [^3H]DMI binding following 6-OHDA lesions. In the present study, we show that this depletion is not limited to the cortex; and our examination of the effect of the 6-OHDA lesion in many different brain areas may also explain the disagreement, noted by Rehavi et al. (1982) and Hrdina et al. (1981), between the extent of the 6-OHDA lesion (as expressed by NE uptake or content) and the drop in [^3H]DMI binding. Both of these groups note only a 36% to 50% decline in cortical [^3H]DMI binding, whereas NE uptake and content are more than 90% depleted. As we have noted before, low affinity binding accounts for a certain portion of the total displaceable binding of [^3H]DMI in the brain. The proportion of the low affinity binding is region dependent. Thus, in regions and nuclei with very high density of [^3H]DMI binding sites (and high NE content, as well) we see up to 93% decrease in binding following 6-OHDA treatment. In moderately to sparsely labeled regions, and these include the cortex and the hippocampus, the depletion is 50% or less. In the caudate putamen and globus pallidus, which have the lowest [^3H]DMI binding in the brain, the binding is not decreased following 6-OHDA lesion.

The anatomical information provided in the present study clearly shows that DMI binds with high affinity to

sites different from those labeled with [^3H]imipramine (Raisman et al., 1980; Rehavi et al., 1980), which correlate best with the distribution of serotonin terminals (Palkovitz et al., 1981; Rainbow et al., 1982). The facts that drug efficacies in displacing imipramine and DMI high affinity binding correlate with their affinity toward blocking serotonin and NE reuptake, respectively (Langer et al., 1980; Rehavi et al., 1981), and that lesions of the noradrenergic system decrease DMI binding, whereas only serotonergic lesions affect imipramine binding (Sette et al., 1981) mean that the two sites are both anatomically and biochemically distinct. The most plausible explanation is that imipramine binds with high affinity to the presynaptic serotonin terminals whereas DMI interacts with high affinity with NE terminals, and this interaction underlies the ability of those two drugs to selectively inhibit the reuptake of the respective amines. However, it is not clear whether these binding sites are relevant to the therapeutic action of these drugs. Although imipramine binding has been shown to be lowered in depressive and suicidal patients (Raisman et al., 1981; Stanley et al., 1982) and after chronic treatment in animals (Briley et al., 1982), this situation is not ameliorated following treatment and recovery (Raisman et al., 1981). These observations have been offered as evidence that high affinity imipramine binding may serve as a genetic or biochemical "marker" for susceptibility to depression. No similar information is so far available with regard to [^3H]DMI. Such studies would be most interesting, because subtypes of depression may be distinguishable through different binding patterns of secondary and tertiary amines.

As for the therapeutic action of these drugs, it requires plasma (and brain) concentrations of tricyclics which are several orders of magnitude higher than those required to saturate the high affinity site for imipramine or DMI (see Biegon and Samuel, 1979a, for discussion and references and 1979b). Also, the doses of different tricyclics commonly in use are all within the same order of magnitude (50 to 400 mg/day) and do not correlate with the affinities of tricyclics toward the high affinity site of imipramine or DMI. Because the low affinity sites for DMI (Biegon and Samuel, 1979a) and imipramine (O'Brien et al., 1978) show similar affinities, similar gross distribution in the rat brain, and saturation in the micromolar range (which is the range of therapeutically effective concentrations of tricyclics), further study of these sites may provide additional insights into the mechanism of action of tricyclic antidepressants.

References

- Biegon, A., and D. Samuel (1979a) Binding of labeled antidepressants to rat brain tissue. *Biochem. Pharmacol.* 28: 3361-3363.
- Biegon, A., and D. Samuel (1979b) The *in vivo* distribution of an antidepressant drug (DMI) in male and female rats. *Psychopharmacology* 65: 259-263.
- Bradford, M. M. (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254.
- Briley, M., R. Raisman, S. Arbilla, M. Casademont, and S. Z. Langer (1982) Concomitant decrease in [^3H]imipramine binding in cat brain and platelets after chronic treatment with imipramine. *Eur. J. Pharmacol.* 81: 309-314.
- Brownstein, M., J. M. Saavedra, and M. Palkovits (1971) Norepinephrine and dopamine in the limbic system of the rat. *Brain Res.* 79: 431-436.
- Dahlstrom, A., and K. Fuxe (1964) Evidence for the existence of monoamine containing neurons in the central nervous system: Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol.* 62: 1-55.
- Fuxe, K. (1965) Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine terminal in the central nervous system. *Acta Physiol.* 64 (Suppl. 247): 37-38.
- Horn, A. S., J. T. Coyle, and S. H. Snyder (1971) Catecholamine uptake by synaptosomes from rat brain: Structure activity relationships of drugs with differential effects on dopamine and norepinephrine neurons. *Mol. Pharmacol.* 7: 66-80.
- Hrdina, P. D., K. Elson-Hartman, D. C. S. Roberts, and B. A. Papas (1981) High affinity [^3H]desipramine binding in rat cerebral cortex decreases after selective lesion of noradrenergic neurons with 6-hydroxydopamine. *Eur. J. Pharmacol.* 73: 375-376.
- Klerman, G. L. (1971) In *Brain Chemistry and Mental Disease*, B. T. Ho and W. M. McIsaac, eds., pp. 379-402, Plenum Press, New York.
- König, J. F. R., and R. A. Klippel (1963) *The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem*, Williams & Wilkins, Baltimore.
- Langer, S. Z., C. Moret, R. Raisman, M. L. Dubocovich, and M. Briley (1980) High affinity [^3H]imipramine binding in rat hypothalamus is associated with the uptake of serotonin but not norepinephrine. *Science* 210: 1133-1135.
- Lee, C.-M., and S. H. Snyder (1981) Norepinephrine neuronal uptake binding sites in rat brain membranes labeled with [^3H]desipramine. *Proc. Natl. Acad. Sci. U. S. A.* 78: 5250-5254.
- Malmfors, T., and H. Tholén (1971) *6-Hydroxydopamine and Catecholamine Neurons*, Elsevier-North Holland, New York.
- O'Brien, R. A., N. M. Spirt, and W. D. Horst (1978) Antidepressant receptor binding in brain. *Soc. Neurosci. Abstr.* 4: 1366.
- Palacios, J. M., D. L. Niehoff, and M. J. Kuhar (1981) Receptor autoradiography with tritium sensitive film: Potential for computerized densitometry. *Neurosci. Lett.* 25: 101-105.
- Palkovitz, M., M. Brownstein, J. Saavedra, and J. Acelrod (1974) Norepinephrine and dopamine content of hypothalamic nuclei of the rat. *Brain Res.* 77: 137-149.
- Palkovitz, M., R. Raisman, M. Briley, and S. T. Langer (1981) Regional distribution of [^3H]imipramine binding in rat brain. *Brain Res.* 210: 493-498.
- Penney, J. B., K. Frey, and A. B. Young (1981) Quantitative autoradiography of neurotransmitter receptors using tritium sensitive film. *Eur. J. Pharmacol.* 22: 421-422.
- Quirion, R., R. P. Hammer, Jr., M. Herkenham, and C. B. Pert (1981) The phencyclidine (angel dust)/ σ "opiate" receptor: Its visualization by tritium sensitive film. *Proc. Natl. Acad. Sci. U. S. A.* 78: 5881-5885.
- Rainbow, T. C., W. V. Bleisch, A. Biegon, and B. S. McEwen (1981) Quantitative densitometry of neurotransmitter receptors. *J. Neurosci. Methods* 5: 127-138.
- Rainbow, T. C., A. Biegon, and B. S. McEwen (1982) Autoradiographic localization of imipramine binding in rat brain. *Eur. J. Pharmacol.* 77: 363-364.
- Raisman, R., M. S. Briley, and S. T. Langer (1980) Specific tricyclic antidepressant binding sites in rat brain characterized by high affinity [^3H]imipramine binding. *Eur. J. Pharmacol.* 61: 373-380.
- Raisman, R., D. Sechter, M. S. Briley, E. Zarifian, and S. T. Langer (1981) High affinity [^3H]imipramine binding in plate-

- lets from untreated and treated depressed patients compared to healthy volunteers. *Psychopharmacology* 75: 368-000.
- Rehavi, M., S. M. Paul, P. Skolnick, and F. K. Goodwin (1980) Demonstration of specific high affinity binding sites for [^3H] imipramine on human brain. *Life Sci.* 26: 2273-2279.
- Rehavi, M., P. Skolnick, B. Hulihan, and S. M. Paul (1981) "High affinity" binding of ^3H -desipramine to rat cerebral cortex: Relationship to tricyclic antidepressant-induced inhibition of norepinephrine uptake. *Eur. J. Pharmacol.* 70: 547-599.
- Rehavi, M., P. Skolnick, M. J. Brownstein, and S. M. Paul (1982) High affinity binding of ^3H -desipramine to rat brain. A presynaptic marker for noradrenergic uptake sites. *J. Neurochem.* 38: 889-895.
- Reivich, J., J. W. Lehle, and L. Sokoloff (1969) Measurement of regional cerebral blood flow with [^{14}C]antipyrine in awake cats. *J. Appl. Physiol.* 27: 296-300.
- Scatchard, G. (1949) The attraction of proteins for small ions and molecules. *Ann. N. Y. Acad. Sci.* 57: 660-672.
- Sette, M., R. Raisman, M. Briley, and S. T. Langer (1981) Localization of tricyclic antidepressant binding sites on serotonin nerve terminals. *J. Neurochem.* 37: 40-42.
- Stanley, M., J. Virgilio, and S. Gershon (1982) Tritiated imipramine binding sites are decreased in the frontal cortex of suicides. *Science* 216: 1337-1338.
- Swanson, L. W., and B. K. Hartman (1975) The central adrenergic system: An immunofluorescence study of the location of cell bodies and their different connections in the rat utilizing dopamine- β -hydroxylase as a marker. *J. Comp. Neurol.* 163: 467-505.
- Versteeg, D. H. G., J. Van Der Guyten, W. DeJong, and M. Palkovitz (1976) Regional concentrations of noradrenaline and dopamine in rat brain. *Brain Res.* 113: 563-574.
- Yavin, Z., A. Biegon, M. Segal, and D. Samuel (1978) The *in vivo* binding of ^3H -desipramine and ^3H -chlorpromazine to areas in the rat brain. *Eur. J. Pharmacol.* 51: 121-127.
- Yavin, Z., A. Biegon, R. Hofstein, and D. Samuel (1979) The localization of ^3H -desipramine in central nerve terminals studied with electron microscope autoradiography and subcellular fractionation. *Experientia* 35: 1210-1212.
- Young, W. S., and M. J. Kuhar (1979) A new method for receptor autoradiography: (^3H)-Opioid receptors in rat brain. *Brain Res.* 179: 255-270.