Effect of Chronic Administration of Antidepressants on α_2-Adrenoceptors in the Locus Coeruleus and Its Projection Fields in Rat Brain Determined by Quantitative Autoradiography

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The density of α_2-adrenoceptors, using ^{3}H-idazoxan as the radioligand, was determined by quantitative autoradiography in the locus coeruleus and in 13 noradrenergic projection fields following chronic administration of drugs acting on noradrenergic and/or serotonergic neurons. Protriptyline, an inhibitor of the uptake of norepinephrine, and mianserin, an α_2-adrenoceptor antagonist, reduced the binding of ^{3}H-idazoxan only in the locus coeruleus. Phenelzine, an inhibitor of both type A and type B monoamine oxidase (MAO), reduced the binding of ^{3}H-idazoxan in the locus coeruleus and in several areas with noradrenergic innervation from tegmental cell bodies. Clorgyline, a selective inhibitor of type A MAO, had no effect. Of the two selective inhibitors of serotonin uptake, citalopram caused a modest increase in binding only in one terminal field area, whereas sertraline had no effect. Although these antidepressants did not produce consistent effects on α_2-adrenoceptors, protriptyline, mianserin, and phenelzine were similar in that they all decreased the binding of ^{3}H-idazoxan in the locus coeruleus without widely affecting its binding in the coerulean terminal fields. Deprenyl, a selective inhibitor of type B MAO, the only drug in this study without proven antidepressant efficacy, differed from all other drugs in that it decreased the binding of ^{3}H-idazoxan both in the locus coeruleus as well as in most terminal fields with primarily coerulean noradrenergic innervation. ***[Neuropsychopharmacology 8:57–65, 1993]***

KEY WORDS: *α_2-adrenoceptors; ^{3}H-Idazoxan; Locus coeruleus: Antidepressants*

When given repeatedly, antidepressant drugs produce multiple effects on noradrenergic and serotonergic neurons and receptors (see Heninger and Charney 1987; Frazer et al. 1988). Recent autoradiographic studies identified specific regions of the brain where effects on β-adrenergic receptors by these drugs are most prominent (Ordway et al. 1991). There has been considerable interest in the response of α-adrenoceptors to chronic administration of antidepressants. Some α_2-adrenoceptors in the brain are situated in the projection fields of noradrenergic neurons, where they modulate the release of norepinephrine as presynaptic autoreceptors (Langer 1977; Starke 1981), whereas others act as postsynaptic receptors (U'Prichard et al. 1977). A number

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of studies found a decrease in the density of these receptors following chronic administration of antidepressant drugs, but other studies found no effect (Pilc 1987).

Alpha$_2$-adrenoceptors are also found in the locus coeruleus. These somatodendritic adrenoceptors exert an inhibitory effect on the firing of the noradrenergic neurons of the locus coeruleus (Svensson et al. 1975; Cedarbaum and Aghajanian 1976), which have widespread axonal projections throughout the brain. To the best of our knowledge no study has dealt with the effect of different types of antidepressant drugs on the density of α_2-adrenoceptors in this cell body area. However, a number of studies have dealt with the effect of repeated administration of antidepressant drugs on the spontaneous and evoked electrical activity of the locus coeruleus (Scuvee-Moreau and Svensson 1982; Blier and de Montigny 1985; Campbell et al. 1985; Curtis and Valentino 1991). These studies showed that whereas some antidepressants do cause changes in locus coeruleus firing or the response of these cells to α_2-adrenoceptor agonists, others do not produce such effects.

The present study examined the effect of repeated administration of antidepressant drugs on the binding of ^{3}H-idazoxan, a highly selective α_2-adrenoceptor antagonist (Chapleo et al. 1981; Dettmar et al. 1983), to α_2-adrenoceptors in the locus coeruleus and in the terminal fields of coerulean neurons. The measurements were obtained using the technique of quantitative receptor autoradiography, the best available method for measuring receptors in discrete regions of the brain (Kuhar 1985).

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (weighing between 200 and 225 g) were purchased from Ace Animals (Boyertown, PA) and housed in clear polycarbonate cages in groups that did not exceed four animals per cage. The rats received Purina Rodent Lab Chow #5001 (Purina Corp., St. Louis, Missouri) and tap water ad libitum. Light in the animal quarters was regulated on a 12-hour light/dark cycle with the room being dark from 1800 hours until 0600 hours.

Administration of Drugs

Table 1 shows the dose, route, and schedule of administration for each drug used in this study. The drugs, whose concentrations are expressed as the free base, were dissolved in distilled water so as to permit injection of a volume of 0.1 ml/100 g of body weight for all drugs except for mianserin and sertraline, which were injected in a volume of 0.2 ml/100 g of body weight. Animals treated once a day were injected between 0900 and 0930 hours, whereas those treated twice a day received their injections at 0900 to 0930 and at 1600 to 1630 hours. Dose schedules were selected either because they caused alterations in β-adrenoceptors (Ordway et al. 1991), or produced serotonergic autoreceptor subsensitivity (Blier and de Montigny 1985; Chaput et al. 1986). The regimens for clorgyline and deprenyl are selective for type A and type B monoamine oxidase (MAO), respectively (Johnstone 1968; Campbell et al. 1979; Eckstedt et al. 1979). The doses of sertraline or protriptyline have been shown to block in vivo the uptake of serotonin (5-HT) (Wolfe et al. 1987) or norepinephrine (Schildkraut et al. 1971), respectively.

Preparation of Tissue Sections

Rats were euthanized by decapitation between 1000 and 1100 hours the morning after receiving their last injection of drug or saline (i.e., 18 to 25 hours after the final injection). The brains were removed, rinsed in ice-cold saline, and frozen on powdered dry ice. The frozen tissue was stored at −80°C until sectioning. From each brain, two sets of coronal sections were cut 20 μm in thickness in a cryostat at −15°C. One set of sections was cut at the level of the dorsomedial hypothalamic nucleus and the other was cut in the mid-level of the locus coeruleus. The sections were thaw mounted onto gelatin-coated frozen microscope slides and dehydrated overnight at 4°C. Several sections from each serially cut set were stained with thionin and examined under a light microscope to establish their position along the rostral-caudal axis, using the stereotaxic atlas by Paxinos and Watson (1986) as reference. With the aid of these stained sections the rostral and caudal limits of Figure 29 of the atlas were identified. By a similar process, the rostral and caudal limits of Figure 58 of the atlas, showing the most densely packed cells of the locus coeruleus, were bracketed. Two to four sections at each level were incubated.

Incubation Procedure

In preparation for incubation, the sections were transferred from a freezer at −80°C to a desiccator at 4°C for temperature equilibration. The incubation procedure was performed essentially as described by Boyajian et al. (1987) and Chamba et al. (1991). First, the sections were preincubated at room temperature in buffer (58 mmol/L Na_2HPO_4, 8.5 mmol/L KH_2PO_4, pH 7.4) for 15 minutes. Incubation was carried out at 4°C for 2 hours in buffer containing 3.0 nmol/L ^{3}H-idazoxan (42–53 Ci/mmol, Amersham Corp., Arlington Heights, IL). A maximum of five slides, containing two sections each, were placed in 30 ml of incubation medium in polyethylene Coplin jars. Upon completion of incubation, the

Table 1. Treatment Regimen For Drugs[a]

Drug	Dose	Route	No. Administrations	No. Rats
Saline	1 ml/kg	IP	once daily	14
Citalopram	20 mg/kg	IP	once daily	5
Clorgyline	1 mg/kg	SC	once daily	7
Deprenyl	0.25 mg/kg	SC	once daily	5
Mianserin	15 mg/kg	IP	twice daily	7
Phenelzine	5 mg/kg	IP	once daily	8
Protriptyline	10 mg/kg	IP	twice daily	6
Sertraline	5 mg/kg	IP	twice daily	5

[a] Treatments were for 21 days, except for citalopram, which was administered for 14 days.

sections were washed twice for 1 minute in 50 mmol/L Tris buffer, pH 7.6, at 4°C, dipped into deionized water at room temperature, and then air-dried on a slide warmer. Nonspecific binding was defined using 10 µmol/L phentolamine as a displacing agent, based on the results of displacement experiments (see Results). Nonspecific binding was approximately 15% of total binding in the locus coeruleus and 20% to 25% in noradrenergic terminal fields. Total binding and nonspecific binding were determined on adjacent sections.

Quantitative Autoradiography

The dried, slide mounted sections were placed into spring-loaded metal x-ray cassettes along with plastic embedded tritium standards (American Radiolabelled Chemicals, St. Louis, MO), previously calibrated against brain mash standards according to the method described earlier (Geary and Wooten 1983; Geary et al. 1985), and apposed at room temperature to tritium sensitive film (Ultrofilm ^{3}H, Cambridge Instruments, Gaithersburg, MD). Sections containing the locus coeruleus were exposed for 28 days and those showing terminal field areas were exposed for 49 days. The exposed films were processed using Kodak GBX developer and fixer. The autoradiograms were analyzed on a DUMAS (Drexel Unix based Microcomputer Image Analysis System) densitometer using the BRAIN software package (Feingold et al. 1986). The incubated sections were subsequently stained with thionin to facilitate identification of neuroanatomic structures that were defined and named according to the atlas of Paxinos and Watson (1986).

Statistical Methods

The samples were analyzed blind and identified by randomly chosen code numbers. The data were examined by analysis of variance (Sokal and Rohlf 1969), and individual groups were compared to the control group by Dunnett's test (Zar 1984). P values of less than 0.05 were considered significant. In competition experiments the data were analyzed using a nonlinear least-squares parametric curve fitting program (Sigmaplot 4.0, Jandel Scientific, Corte Madera, CA). One-site and two-site models were compared using a Fisher ratio (F) computed as follows:

$$F = (SS_1 - SS_2)/dfss_1 - ss_2/SS_2/dfss_2,$$

where SS_1 and SS_2 are the error sum of squares for the one-site model and two-site model, respectively; $dfss_1$ and $dfss_2$ are the degrees of freedom for the one-site and two-site models. The two-site model is accepted as a significantly better fit than the one-site model when the value of F is greater than or equal to the tabulated value at the given degrees of freedom and the probability of a type I error of 0.05.

Drugs

The antidepressants used in this study were citalopram (H. Lundbeck, Copenhagen, Denmark), clorgyline (May and Baker Ltd., London, England), deprenyl (Somerset Pharmaceutical, Inc., Denville, N.J.), mianserin (Organon, Int., Oss, The Netherlands), phenelzine (Sigma Chemical Co., St. Louis, MO), protriptyline (Merck, Sharp and Dohme Research Labs, West Point, PA), and sertraline (Pfizer Inc., Groton, CT).

RESULTS

The radioligand ^{3}H-idazoxan is a highly selective α_2-adrenoceptor antagonist (Chapleo et al. 1981; Dettmar et al. 1983); however, it binds to sites other than α_2-adrenoceptors and a portion of this non-α_2 adrenoceptor binding is displacable by certain α_2-adrenoceptor antagonists (Michel et al. 1989; Brown et al. 1990; Wikberg and Uhlen 1990). Consequently, we carried out experiments to ascertain if ^{3}H-idazoxan was binding to non-α_2-adrenoceptors under our experimental conditions. Experiments were also carried out to determine what nonradioactive drug would best define the binding of ^{3}H-idazoxan to α_2-adrenoceptors, i.e., which drug should be used to define specific binding.

Competition experiments were carried out to ascer-

tain the extent of displacement of total binding of ^{3}H-idazoxan. In these experiments coronal sections were used either from the level of the midbrain, where the terminal fields were analyzed, or from the level of the locus coeruleus; binding of ^{3}H-idazoxan was measured either by total radioactivity per section determined by liquid scintillation spectroscopy or by quantitative autoradiographic analysis of the locus coeruleus, respectively. The concentration of ^{3}H-idazoxan used was 3 nmol/L, the same as that in the subsequent experiments. Figure 1A shows the results of competition experiments with the adrenergic antagonists phentolamine, prazosin, SKF-104375, and the physiologic neurotransmitter (−)norepinephrine in the locus coeruleus. These drugs were selected as they do differentiate α_2-adrenergic from non-α_2-adrenergic binding sites of ^{3}H-idazoxan (Michel et al. 1989). It is evident from the illustration that each drug displaced approximately 80% to 90% of total binding of ^{3}H-idazoxan. Nonlinear regression analysis indicated that the displacement curves were adequately fit by a one-site model for all of the drugs. The K_i values were in agreement with those obtained by Boyajian and Leslie (1987). Similar results were obtained when samples from the midbrain were incubated and radioactivity per section was determined by liquid scintillation spectroscopy (results not shown). From such data, it was inferred that only a small portion of the total binding of ^{3}H-idazoxan was to non-α_2-adrenoceptors. Further, these results indicated that 10 μmol/L phentolamine could be used to define the specific binding of ^{3}H-idazoxan to α_2-adrenoceptors.

To characterize further the binding of ^{3}H-idazoxan, saturation experiments were carried out. In these experiments randomized coronal sections were used from the level of the caudate-putamen and total radioactivity of sections was determined by liquid scintillation spectroscopy. The results, shown in Figure 1B, indicate that ^{3}H-idazoxan bound to a single class of binding sites with a K_d of 0.99 ± 0.23 nmol/L (mean ± SD, n = 3), in agreement with the value reported by Boyajian et al. (1987). In subsequent experiments, a concentration of 3 nmol/L ^{3}H-idazoxan was used, three times its K_d value. This concentration of ^{3}H-idazoxan occupies 75% of α_2-adrenoceptors, which increases the likelihood that a recorded change in the binding of ^{3}H-idazoxan reflects an alteration in the density of binding sites, rather than a change in the affinity of the binding site for the ligand.

The effect of chronic administration of drugs on the binding of ^{3}H-idazoxan in the locus coeruleus is shown in Figure 2. Four of the drugs, namely protriptyline, mianserin, phenelzine, and deprenyl, caused modest (13% to 18%) but significant ($p < 0.01$) decreases in the binding of ^{3}H-idazoxan in the locus coeruleus. Treatment with clorgyline, sertraline, and citalopram had no effect.

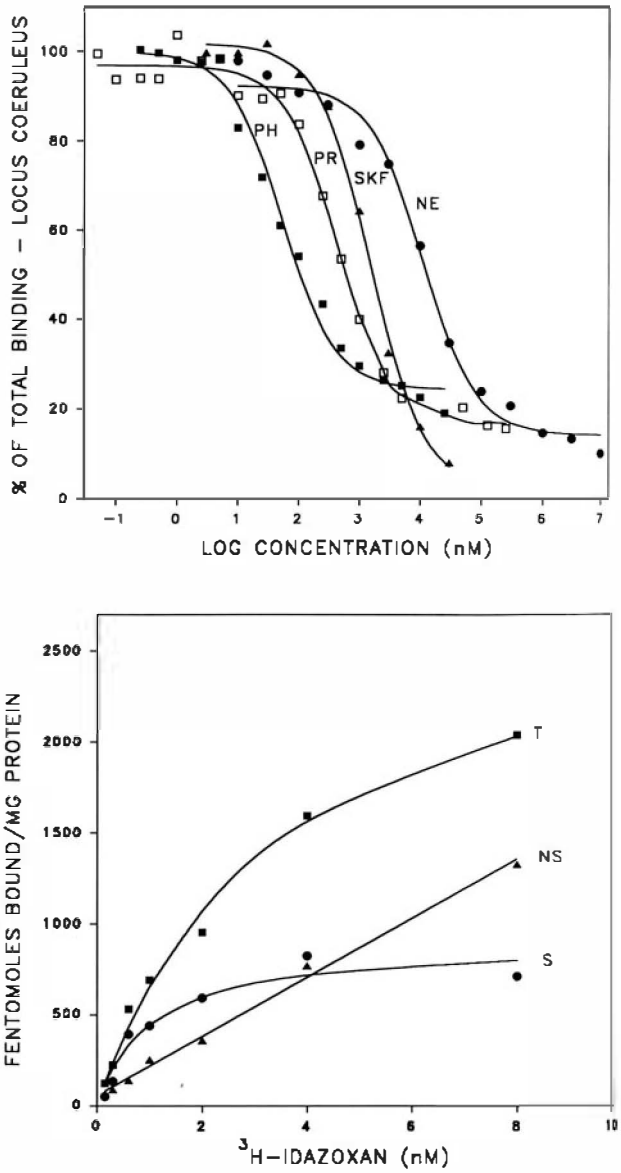

Figure 1 **A:** Competition with binding of ^{3}H-idazoxan (3nmol/L) in the locus coeruleus by adrenergic antagonists and (−)norepinephrine. The drugs used are phentolamine (PH), prazosin (PR), SKF-104078 (SKF), and (−)norepinephrine (NE). The curves for PH, PR, and NE represent the mean of three experiments, the curve for SKF is the mean of five experiments. **B:** Saturation isotherm for ^{3}H-idazoxan. Randomized coronal sections were used from the level of the caudate-putamen. T = total binding; S = specific binding; NS = nonspecific binding. Radioactivity per section was determined by liquid scintillation counting. Nonspecific binding was defined by 10 μmol/L phentolamine. This figure shows one of three experiments.

The effect of chronic administration of these drugs on the binding of ^{3}H-idazoxan in terminal field areas is summarized in Table 2. In these structures, only deprenyl and phenelzine altered binding significantly. Both drugs reduced the binding of ^{3}H-idazoxan in the basomedial amygdaloid nucleus to approximately the same degree (about 13%). Phenelzine also reduced

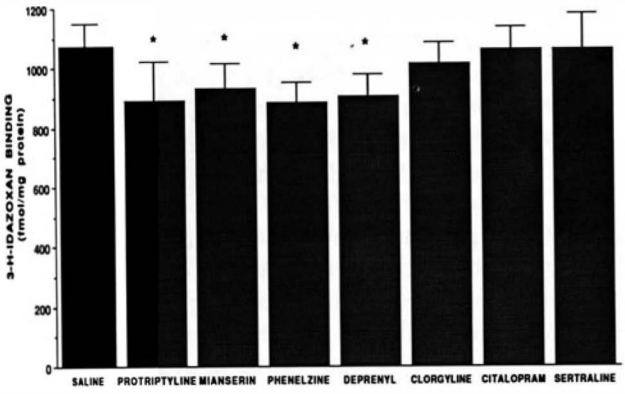

Figure 2 The effect of antidepressants (3 nmol/L on the specific binding of ^{3}H-idazoxan in the locus coeruleus. Data shown are mean ± SD; n = 14 for control samples and 6–8 for treated samples. * = $p < 0.01$.

binding significantly in the dorsal hypothalamic area (18%), in the central amygdaloid nucleus (16%), and in the paraventricular thalamic nucleus (13%). Deprenyl reduced binding in all the cortical areas analyzed. The greatest effects were produced in Areas 1 and 2 of the frontal cortex (22%), in the hindlimb area of the cortex (18%), and in the retrosplenial cortex (17%). More modest reductions in binding were found in the parietal cortex, in Layers 2 through 6 (12%). Layer 1 was not analyzed because this narrow superficial structure often did not remain intact.

In contrast to the ability of these inhibitors of MAO to decrease the binding of ^{3}H-idazoxan both in the locus coeruleus and in some terminal field areas, mianserin and protriptyline had no effect on the binding of this radioligand in projection fields (Table 2). Finally, citalopram produced a modest (8%), but statistically significant increase in binding of ^{3}H-idazoxan in the dorsal hypothalamic area, whereas sertraline and clorgyline had no statistically significant effect in any of the terminal fields examined (results not shown).

DISCUSSION

The radioligand ^{3}H-idazoxan, with 10 μmol/L phentolamine as the displacing agent to define specific binding, has been used for quantitative autoradiographic measurement of α_2-adrenoceptors prior to this study (Boyajian et al. 1987; Boyajian and Leslie 1987; Chamba et al. 1991). Others speculated that, due to the presence of a nonstereoselective adrenergic binding site, the density of α_2-adrenoceptors may be overestimated by this method (Convents et al. 1989; Lehmann et al. 1989). This speculation was based on measurements performed on homogenates of human and rabbit cortex (Convents et al. 1989) in the presence of a buffer considerably different and at a concentration of ^{3}H-idazoxan significantly higher than that used in the autoradiographic procedure. The displacement curve produced by phentolamine under those conditions was shallow and biphasic, and 10 μmol/L phentolamine only displaced approximately 40% of the total binding. This

Table 2. The Effect of Antidepressants on the Specific Binding of ^{3}H-Idazoxan (3 nmol/L) in Noradrenergic Terminal Field Areas[a]

Noradrenergic Innervation Primarily Coerulean					
Area	**Saline**	**Protrip[b]**	**Mianserin**	**Phenelzine**	**Deprenyl**
Hindlimb area of cortex	349 ± 5	339 ± 6	365 ± 5	341 ± 9	288 ± 8[d]
Retrosplenial Cortex	399 ± 10	373 ± 9	400 ± 12	363 ± 12	331 ± 16[d]
Frontal Cortex Area 1 & 2	354 ± 9	340 ± 16	366 ± 15	334 ± 19	275 ± 18[d]
Parietal Cortex Layers 2, 3, 4	455 ± 10	412 ± 23	470 ± 8	416 ± 11	401 ± 18[c]
Parietal Cortex Layer 5	356 ± 10	330 ± 14	357 ± 13	324 ± 8	312 ± 15[c]
Parietal Cortex Layer 6	325 ± 10	305 ± 18	331 ± 12	298 ± 8	285 ± 14[c]
Basomedial Amygd. N.	1129 ± 30	1067 ± 64	1114 ± 35	975 ± 54[c]	986 ± 26[c]
Laterodorsal Thalamic N.	784 ± 37	724 ± 73	897 ± 14	797 ± 35	863 ± 46
Noradrenergic Innervation Primarily Noncoerulean					
Area	**Saline**	**Protrip[b]**	**Mianserin**	**Phenelzine**	**Deprenyl**
Paraventr. Thalamic N.	943 ± 16	919 ± 43	1003 ± 31	819 ± 25[c]	886 ± 31
Ventromed. Hypoth. N.	741 ± 21	667 ± 92	772 ± 31	676 ± 25	655 ± 39
Lateral Hypoth. Area	657 ± 17	549 ± 73	643 ± 37	586 ± 23	604 ± 40
Dorsal Hypoth. Area	927 ± 12	894 ± 25	933 ± 35	759 ± 27[c]	860 ± 31
Central Amygd. N.	1022 ± 41	955 ± 35	1011 ± 29	855 ± 22[c]	946 ± 55

[a] Data are shown as mean ± SEM (fmole/mg protein); n = 14 for control and 5–8 for treated samples.
[b] protriptyline.
[c] $p < 0.05$.
[d] $p < 0.01$.

result is markedly different from what we observed under our assay conditions, using slide-mounted sections of rat brain and 3 nmol/L ^{3}H-idazoxan. Both (−)norepinephrine, the physiologically active neurotransmitter, and phentolamine produced steep monophasic displacement curves and displaced ^{3}H-idazoxan to the same extent: approximately 80% of the total binding in the locus coeruleus (Fig. 1A), and 70% to 80% of the total binding in the terminal fields (data not shown). Also, ^{3}H-idazoxan binds to a nonadrenergic (idazoxan) receptor, in addition to α_2-adrenoceptors, and in some tissues as much as 50% of the total binding by ^{3}H-idazoxan is to this nonadrenergic site (Michel et al. 1989; Brown et al. 1990; Wikberg and Uhlen 1990). Certain adrenergic antagonists, such as phentolamine and prazosin, displace ^{3}H-idazoxan only from α_2-adrenoceptors, whereas others, such as SKF-104078 and oxymetazoline, are capable of displacing ^{3}H-idazoxan from both the α_2-adrenoceptors as well as from the nonadrenergic (idazoxan) receptor (Michel et al. 1989). Figure 1A shows that in the locus coeruleus the binding of ^{3}H-idazoxan is displaced by SKF-104078 to a slightly greater extent than by either phentolamine or prazosin. Similar results were obtained in the terminal fields (results not shown). These results indicate that although a minor portion (perhaps 10%) of the total binding by ^{3}H-idazoxan may be to the idazoxan receptor in our samples, nevertheless, phentolamine does not recognize this site, in agreement with the findings of Michel et al. (1989). Taken together, these findings demonstrate that our assay conditions, using ^{3}H-idazoxan and 10 μmol/L phentolamine to define specific binding, are appropriate for measurement of α_2-adrenoceptors in the locus coeruleus and in the terminal fields at the level of the midbrain using slide-mounted sections.

Chronic administration of some of the antidepressant drugs studied decreased the density of α_2-adrenoceptors in the locus coeruleus. These drugs were protriptyline, an inhibitor of the uptake of norepinephrine (Schildkraut et al. 1971; Richelson and Pfenning 1984), phenelzine, an inhibitor of both type A and type B MAO (Robinson et al. 1979), and mianserin, an α_2-adrenoceptor antagonist (Baumann and Maitre 1977; Engberg and Svensson 1980). It is unlikely that the observed decrease in binding after treatment with mianserin is due to the presence of residual drug. All samples were preincubated for 15 minutes (350 ml buffer per 25 sections) prior to incubation with ^{3}H-idazoxan; some samples from mianserin-treated rats were preincubated for 45 minutes and they produced results similar to those that were preincubated for only 15 minutes. Deprenyl, an inhibitor of type B MAO (Knoll and Magyar 1972), a drug best known for potentiating L-DOPA treatment in parkinsonian patients (Birkmayer et al. 1983), also reduced the binding of ^{3}H-idazoxan. Although the data are not yet conclusive, it appears that deprenyl is not an effective antidepressant medication (Mendlewicz and Youdim 1983; Murphy et al. 1987). The other antidepressants, namely clorgyline, an inhibitor of type A MAO (Johnston 1968; Hall et al. 1969), sertraline and citalopram, selective inhibitors in vivo of the uptake of 5-HT (Koe et al. 1983; Hyttel and Larsen 1985), did not have this effect.

Antidepressant drugs have dissimilar effects on the firing activity of the locus coeruleus. For example, the spontaneous firing of the locus coeruleus shows a sustained decrease during chronic administration of either clorgyline or phenelzine (Blier and de Montigny 1985), but it is not affected by repeated administration of mianserin (Curtis and Valentino 1991). On the other hand, acute administration of imipramine and desipramine markedly inhibits the firing of the locus coeruleus, but during repeated administration of these drugs their inhibitory effect on the electrical activity of the locus coeruleus becomes less pronounced (Svensson and Usdin 1978; McMillen et al. 1980; Scuvee-Moreau and Svensson 1982). It was proposed that the gradual dissipation of the inhibitory effect of imipramine and desipramine was due to the development of subsensitive α_2-adrenoceptor responsiveness, since microiontophoretically applied clonidine, an α_2-adrenoceptor agonist, produced a diminished inhibitory effect on the firing of the locus coeruleus after continued administration of those drugs (McMillen et al. 1980).

Our autoradiographic data are consistent with the electrophysiologic measurements in that chronic administration of protriptyline, which like imipramine and desipramine inhibits the uptake of norepinephrine, decreased the density of α_2-adrenoceptors in the locus coeruleus. Furthermore, chronic administration of clorgyline had no effect on either the sensitivity of locus coeruleus cells to clonidine (Blier and de Montigny 1985; Campbell et al. 1985) or on the binding of ^{3}H-idazoxan in the locus coeruleus. On the other hand, whereas we found a modest decrease in the density of α_2-adrenoceptors in the locus coeruleus after repeated administration of phenelzine or deprenyl, similar treatments produced no changes in the response of the locus coeruleus to clonidine (Blier and de Montigny 1985). The daily dose of phenelzine in the latter study, however, was considerably lower than that used in our experiments. Both the electrophysiologic and the autoradiographic measurements indicate, nevertheless, that the effects by different types of antidepressant drugs on the responsivity or density of α_2-adrenoceptors in the locus coeruleus are not uniform.

Only two of the drugs, deprenyl and phenelzine, produced substantial reductions in binding of ^{3}H-idazoxan in noradrenergic terminal fields. Some of the terminal fields examined in this study receive all of their noradrenergic fibers from the locus coeruleus, namely the parietal cortex, frontal cortex, retrosplenial cortex

hindlimb area of the cortex, laterodorsal thalamic nucleus, and basomedial amygdaloid nucleus. Other brain areas receive either mixed coerulean and noncoerulean (i.e., tegmental) noradrenergic innervation, or receive only noncoerulean noradrenergic innervation, namely the paraventricular thalamic nucleus, ventromedial hypothalamic nucleus, dorsal hypothalamic area, lateral hypothalamic area, and central amygdaloid nucleus (Lindvall and Bjorklund 1978; Moore and Card 1984; Aston-Jones et al. 1984).

Deprenyl, an inhibitor of type B MAO, produced a distinctive pattern of effect in the noradrenergic terminal fields (Table 2): it reduced the binding of ^{3}H-idazoxan in seven of the eight areas of the brain with noradrenergic projections originating only from the locus coeruleus, whereas its effects were consistently smaller and were statistically not significant in brain areas that receive noradrenergic innervation, either exclusively or partially, from tegmental cell bodies. Phenelzine, an inhibitor of type A and type B MAO, decreased the binding of ^{3}H-idazoxan primarily in those terminal field areas where deprenyl had no effect, namely those that receive noradrenergic projections, either exclusively or partially, from tegmental cell bodies (Table 2). Given this, together with the fact that clorgyline, a type A MAO, had no effect, it is not clear how the effects on α_2-adrenoceptors by deprenyl or phenelzine are related to inhibition of MAO.

Interestingly, phenelzine produced similar regional effects on β_2-, but not on β_1-adrenoceptors (Ordway et al. 1991), as on α_2-adrenoceptors. Phenelzine decreased the binding of ^{125}I-iodopindolol to β_2-adrenoceptors in the habenular and paraventricular thalamic nuclei, which receive primarily non-coerulean innervation, whereas it had no effect on β_2-adrenoceptors in seven thalamic nuclei that receive only coerulean noradrenergic fibers according to Lindvall and Bjorklund (1978). Phenelzine also caused a marked reduction in binding to β_2-adrenoceptors in all three hypothalamic areas examined, the lateral hypothalamic area, the ventromedial, and dorsomedial hypothalamic nuclei, all of which receive predominantly tegmental noradrenergic fibers (Moore and Card 1984), but produced no effect on β_2-adrenoceptors in cortical and hippocampal areas, which receive only coerulean noradrenergic fibers (Loy et al. 1980; Moore and Card 1984). There was only one exception to this pattern, and, as in our study, it occurred in the amygdala: phenelzine reduced the binding to β_2-adrenoceptors in the medial amygdaloid nucleus, which receives coerulean noradrenergic innervation according to Moore and Card (1984).

Since there appears to be no evidence to the contrary, it is assumed that ^{3}H-idazoxan binds to both presynaptic and postsynaptic α_2-adrenoceptors. Since postsynaptic α_2-adrenoceptors, in general, significantly outnumber the presynaptic ones (Greenberg et al. 1976; U'Prichard et al. 1977), it is likely that our results with ^{3}H-idazoxan in terminal field areas represent binding predominantly to postsynaptic α_2-adrenoceptors. The α_2-adrenoceptor population has been divided into at least two subtypes, α_{2A}- and α_{2B}-adrenoceptors, based on their pharmacologic properties (Bylund et al. 1988). Our competition experiments, involving the locus coeruleus (Fig. 1A) and terminal fields at the level corresponding to Figure 29 of the atlas by Paxinos and Watson (1986), indicate that ^{3}H-idazoxan binds to a single population of α_2-adrenoceptors. Further studies are needed to determine whether these adrenoceptors are of the α_{2A} or α_{2B} subtypes.

In conclusion, whereas some antidepressants in this study showed no effect, others produced modest decreases in α_2-adrenoceptors in distinctive regional patterns. Further studies are needed to examine the significance of these findings with regard to the mechanism of action of antidepressant drugs.

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