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Desmethylimipramine-induced decrease in β-adrenergic receptor binding in rat cerebral cortex*

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Dibenzazepine derivatives like imipramine and desmethylimipramine are widely used in the treatment of depression. Their mechanism of action remains unknown. In 1964, Glowinski and Axelrod [1] reported that a single injection of these drugs to rats inhibits the uptake of intraventricularly administered radioactive norepinephrine (NE) into brain tissue. Inhibition of NE uptake could account for the reported prolongation and potentiation of adrenergic function produced by the tricyclic antidepressants [2] and is one of the cornerstones of the pharmacologically derived biogenic amine hypotheses of affective disorders [3–5].

Less is known about the effects of the tricyclic drugs when administered chronically. This is important because of the widely held (but not definitely proven) belief that there is a lag period before the tricyclic drugs exert an antidepressant effect [6]. It is of interest, then, that chronic administration of tricyclic drugs reduces some responses elicited by NE. For example, whereas a single injection of imipramine or desmethylimipramine has no effect on the ability of NE to elevate levels of endogenous adenosine 3′,5′-mono-phosphate (cyclic AMP) or [^{3}H]cyclic AMP net synthesis in slices of rat cerebral cortex [7], injection of these compounds for 5 days or longer was associated with a diminished response of the cyclic nucleotide to NE [7–10].

We have speculated previously [8] that the reduction in adrenergic responsiveness owing to repeated tricyclic drug administration may result, in part, from a decrease in adrenergic receptor sensitivity. In the present report, data consistent with this view are presented showing that the administration of desmethylimipramine produces a reduction of the binding of (−)[^{3}H]dihydroalprenolol to brain homogenates. (−)[^{3}H]dihydroalprenolol has been used to study β-adrenergic receptor binding sites in a number of tissues including brain [11–14].

Male Sprague–Dawley rats (225–300 g) were used in these experiments. Drug-treated animals were injected intraperitoneally each time with 10 mg/kg of desmethylimipramine hydrochloride (DMI; obtained as a gift from USV Pharmaceutical Corp.), while control animals received 0.9% NaCl. When two or more injections of DMI were administered, the injections were given twice each day. Except in the experiment in which animals were killed 1 hr after a single drug injection, all animals were killed by decapitation 24 hr after the final drug injection. After decapitation, the cerebral cortex was removed from the rest of the brain. Homogenization of this brain area and estimation of the extent of binding of (−)[^{3}H]dihydroalprenolol (New England Nuclear, Boston, MA; 32.6 Ci/m-mole) were done by modifications of the techniques described for brain by Alexander *et al.* [12] and Bylund and Snyder [14]. Like these authors, we also found that the binding of (−)[^{3}H]dihydroalprenolol to homogenates of cerebral cortex was saturable, reached equilibrium by 10 min, and was readily reversible by the addition of (±)propranolol. Specific binding of (−)[^{3}H]dihydroalprenolol (9 nM) to β-adrenergic receptors was assessed by the addition of (±)propranolol (1 μM) to the incubation media. All incubations, i.e. minus and plus propranolol, were done in quadruplicate. Specific binding averaged 50.0 ± 0.8 per cent ($\bar{x}$ ± S.E.M.). Filter blanks averaged less than 0.1 per cent of added counts.

The following procedure was used to estimate the concentration of DMI in cerebral cortex. After removal of the brain area, the tissue was dipped four times in an isotonic sucrose solution, blotted, and weighed. The tissue was then homogenized in 2 ml NaOH (0.1 M). An aliquot of the homogenate was diluted 1:40 with NaOH (0.1 M) and shaken in a glass-stoppered centrifuge tube with 5 vol of heptane containing isoamyl-alcohol (1.5%). The tubes

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were shaken for 20 min and the suspension was allowed to settle. The bottom layer was frozen in a dry ice–acetone bath and the top heptane layer removed. Succinylation of the heptane layer and determination of the succinylated derivative of DMI were performed by a recently developed radio-immunoassay procedure [15].

Protein was determined by the method of Lowry *et al.* [16]. Statistical evaluations were by Student's *t*-tests [17].

The extent of binding of (−)[^{3}H]dihydroalprenolol to homogenates of the cerebral cortex of rats given a single injection of DMI and killed 24 hr later did not differ from the binding found in the brain of control rats (Fig. 1). In contrast, rats injected two or more times with DMI and decapitated 24 hr later showed reduced binding of (−)[^{3}H]dihydroalprenolol. This effect was apparent after two injections, with the maximum reduction occurring after five injections. The maximum reduction in binding was about 35 per cent, with further reduction of binding not occurring even after twenty injections (Fig. 1).

The reduction in (−)[^{3}H]dihydroalprenolol binding caused by treatment of rats with DMI was not related to the physical presence of the tricyclic drug in the brain tissue. In rats given twenty injections of DMI and killed 24 hr later—and in whom there was a significant reduction in (−)[^{3}H]dihydroalprenolol binding (Fig. 1)—the concentration of DMI in the cortex was 0.8 ± 0.3 μg/g of tissue (N = 6). However, rats killed 60 min after a single injection of DMI had much higher concentrations of the drug in the brain (4.8 ± 0.4 μg/g of tissue, N = 6); yet the binding of (−)[^{3}H]dihydroalprenolol to the cortex of rats killed 1 hr after a single injection of DMI (118 ± 5.8 fmoles/mg of protein, N = 5) was not significantly different than the binding measured in control rat brains (121 ± 3.8 fmoles/mg of protein, N = 16; P > 0.5).

To determine whether the reduced binding of (−)[^{3}H]dihydroalprenolol in DMI-treated rats was due to a decreased number of binding sites or to a change in affinity of the binding sites, tissue samples of rats were incubated with concentrations of (−)[^{3}H]dihydroalprenolol ranging from 1.5 to 11.5 nM. The data obtained were analyzed by the method of Scatchard [18]. A representative analysis of the data obtained is shown in Fig. 2. This type of experiment was done in five control rats and in five rats given five injections of DMI (10 mg/kg, b.i.d.) and killed 24 hr after the last injection. From these experiments it was calculated that there was no significant difference between the dissociation constant for (−)[^{3}H]-dihydroalprenolol in homogenates of cerebral cortex obtained from DMI-treated rats (2.9 ± 0.3 nM) and that for rats given injections of saline (3.1 ± 0.3 nM; P > 0.5). In contrast, the maximum specific binding of (−)[^{3}H]dihydroalprenolol (i.e. the x-intercept on a Scatchard plot) decreased significantly in rats treated with DMI (126 ± 8 fmoles/mg of protein) as compared to the value obtained in control rats (192 ± 14 fmoles/mg of protein; P < 0.005).

The diminished binding of (−)[^{3}H]dihydroalprenolol persisted for several days, in that, compared to control rats, binding remained significantly lower in rats killed 72 hr after the final injection of DMI (10 mg/kg, b.i.d., for a total of five injections).

These studies demonstrate that repeated administration of DMI to rats is associated with reduced binding of the β-adrenergic receptor antagonist (−)[^{3}H]dihydroalprenolol in homogenates of cerebral cortex. The reduction in (−)[^{3}H]dihydroalprenolol binding appears to be due to a decreased number of β-adrenergic receptors in this area rather than to a change in binding affinity. The reduced number of β-adrenergic receptors reflected in the present data may account, in part, for the diminished ability of NE to elevate endogenous cyclic AMP or [^{3}H]cyclic AMP in brain slices from rats treated chroni-

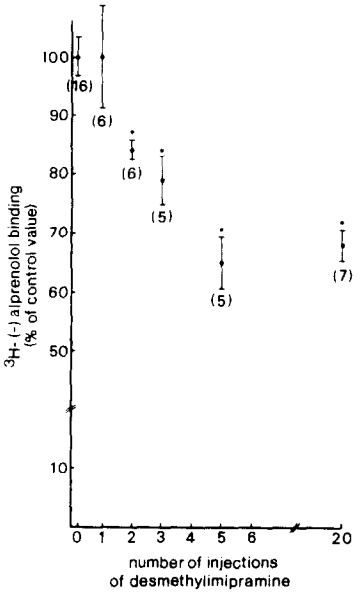

Fig. 1. Inhibition produced by DMI treatment of the specific binding of (−)[^{3}H]dihydroalprenolol to homogenates of cerebral cortex of rats. Incubation of the crude membrane fraction (300–600 μg protein) was carried out for 10 min at 30° in a total volume of 0.5 ml containing (−)[^{3}H]dihydroalprenolol, 1.5 μCi, 9 nM; $MgCl_2$, 25 mM; and Tris buffer, 75 mM, pH 8.0, in the absence and presence of (±)propranolol (1 μM). The reaction was terminated by the addition of 5 ml of cold buffer ($MgCl_2$, 17 mM; Tris, 50 mM, pH 8.0) and by rapid filtration through two Whatman GFC glass fiber filters mounted on a suction apparatus. After six washings with 5 ml of cold buffer, the filters were dried and radioactivity was estimated in a liquid scintillation spectrometer. Rats were injected intraperitoneally with DMI (10 mg/kg). When more than one injection of DMI was given, the injections were done twice each day. All rats were killed 24 hr after the final injection of DMI. Each point and bracket represent the mean value ± S.E.M. The figures in parentheses indicate the number of animals used at each experimental point. The asterisks indicate values significantly different from the control value, P < 0.01. The binding of (−)[^{3}H]dihydroalprenolol to homogenates of cerebral cortex of control rats was 121 ± 3.8 fmoles/mg of protein.

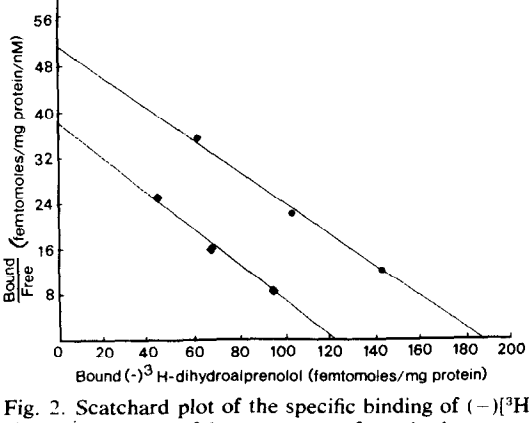

Fig. 2. Scatchard plot of the specific binding of (−)[^{3}H]-dihydroalprenolol of homogenates of cerebral cortex of rats treated either with saline (circles) or DMI (squares). The procedure used to estimate the specific binding is identical to that given in the legend for Fig. 1.

cally with tricyclic drugs [7–10]. The diminished (−)[^{3}H]-dihydroalprenolol binding seen with DMI treatment appears to occur faster than the reduction in catecholamine responsiveness observed after tricyclic drug treament [7–10], although this may be related, in part, to differences in drugs utilized, drug dosage and routes of administration among the different studies. Factors other than diminished receptor sensitivity which might also contribute to the reduced adrenergic responsiveness caused by chronic tricyclic drug treatment, such as changes in phosphodiesterase activity or the generation of an inhibitor of adenylate cyclase, have yet to be explored.

Tricyclic antidepressants produce a variety of pharmacological effects on brain biogenic amine-containing systems. For example, a single injection of these agents produces a highly significant decrease in the uptake of NE into the brain [1, 19] and slows the "turnover" of the catecholamine [see Ref. 20]. With repeated administration of the tricyclic drugs, inhibition of amine uptake persists [20], but other effects may be seen as well: (1) a decrease in the activity of tyrosine hydroxylase in different brain areas [21]; (2) an increase rather than a decrease in the turnover of brain NE [20]; (3) a reduction of the NE-induced elevation of cyclic AMP in brain slices [7–10]; and (4) as indicated in the present report, a decreased number of β-adrenergic receptors in the brain. Some of the effects that develop over time may be viewed as compensatory in that they would be expected to attenuate the potentiation of adrenergic activity produced by tricyclic drug-induced inhibition of NE uptake. The alteration observed in β-adrenergic receptors with repeated tricyclic drug administration may be due to a persistent exposure of the receptors to elevated levels of NE, a consequence of the inhibition by the antidepressants of the neuronal uptake of the amine. Subsensitivity or desensitization of adrenergic receptor binding sites has now been observed in a number of systems after exposure of the receptors to elevated concentrations of agonist [22–26].

A key question is whether the lowered (−)[^{3}H]dihydroalprenolol binding seen with repeated administration of DMI might have some behavioral or clinical significance. The answer remains speculative. Perhaps there is some association between the apparent delay in development of therapeutic response and the development of adrenergic receptor subsensitivity. The fact that both require time to develop may be coincidental or may suggest an important relationship. Alternatively, the changes in adrenergic receptor sensitivity may be deleterious, serving to attenuate the beneficial results that are due to tricyclic drug-induced blockade of NE uptake. The "tolerance" that develops to some of the side effects of these drugs, e.g. drowsiness, may be related to the development of adrenergic receptor subsensitivity. Furthermore, the "tolerance" and "rebound" effects on rapid-eye-movement sleep seen with the tricyclic drugs [27] may result from changes produced in adrenergic receptor sensitivity over time.

These questions remain unanswered. However, theoretical models of the biology of affective disorders should attempt to incorporate the fact that tricyclic drugs produce multiple effects over time on NE-containing neuronal systems in brain.

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Note added in proof.—Recently, Banerjee *et al.* (*Nature* **268,** 455, 1977) reported data similar to that published here. These investigators found that treatment of rats for six weeks either with desmethylimipramine, iprindole, or doxepin lowered significantly the binding of ^{3}H-DHA to homogenates prepared from whole brain.