concentration of 30 mg original wet tissue weight/ml with [^{3}H]-imipramine (29.8 Ci/mmole N.E.N. Chemicals) for 60 min at 0°C. After this time 100 µl of the incubation medium was rapidly diluted into 5 ml of ice-cold buffer and filtered through Whatman GF/F glassfibre filters. The filters were washed with 3 × 5 ml ice-cold buffer, dried and the radioactivity counted by liquid scintillation spectrometry. Specific binding was defined as that inhibited by the presence of desipramine (10 µM) and represented 55% of the total binding at [^{3}H]-imipramine (5 nM).

The specific binding of [^{3}H]-imipramine was saturable, giving a linear Scatchard plot. The mean dissociation affinity constant, Kd, calculated from 13 such Scatchard plots, using membranes prepared from cerebral cortex, was 4.04 ± 0.52 nM (mean + s.e. mean). The maximal binding, Bmax, was 13.82 ± 1.29 pmoles/g original wet tissue weight. The binding rapidly reached equilibrium, t being 5 min at 0°C with [^{3}H]-imipramine (2.5 nM). The binding was unevenly distributed in the brain with a maximum difference of nearly 8 fold between the richest region, the hypothalamus, (Kd, 5.21 ± 3.4 nM; Bmax, 15.97 ± 2.71 pmoles/g original wet tissue weight, n = 4), and the poorest, the cerebellum (Kd, 8.0 ± 3.1 nM; Bmax, 2.20 ± 0.39 pmoles/g original wet tissue weight, n = 3). No specific [^{3}H]-imipramine binding could be detected in the heart.

The binding is potently inhibited by tricyclic antidepressants such as amitriptyline (IC_{50}, 25 nM) and protriptyline (IC_{50}, 20 nM), and less strongly by atypical antidepressants such as iprindol (IC_{50}, 5.5 µM) and mianserine (IC_{50}, 20 µM). Some monoamine uptake inhibitors and certain other compounds also weakly inhibit the binding but there does not appear to be any obvious relationship between the inhibition of [^{3}H]-imipramine binding and any of the major pharmacological activities so far investigated.

Thus the specific binding of [^{3}H]-imipramine is of high-affinity, rapid and reversible. It is assymetrically distributed in the brain and absent in the heart. In addition we have observed that chronic treatment with desipramine (10 mg kg^{-1} day^{-1}) for three weeks reduced the binding of [^{3}H]-imipramine in the cortex by about 42% (Control, Kd, 3.95 + 1.15 nM; Bmax, 11.84 + 1.11 pmoles/g original wet tissue weight, n = 6: desipramine-treated, Kd, 3.52 ± 0.46 nM; Bmax, 6.87 ± 0.64 pmoles/g original wet tissue weight, n = 7; difference of Bmax 41.97% $P < 0.005$). It is concluded that this binding of imipramine may be related to the site of action of tricyclic antidepressant drugs and as such could open up a new approach to the study of the biochemical basis of depression and of the mechanism of action of antidepressant drugs.

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Effects of chronic antidepressant administration on the synthesis of monoamines in rat brain

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A characteristic feature of tricyclic antidepressants is their ability to inhibit the uptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT). Secondary tricyclics, such as desipramine, have a greater inhibitory effect on NA uptake whereas tertiary tricyclics, for example chlorimipramine, preferentially block 5-HT uptake. Carlsson & Lindqvist (1978) have found a correlation between blockade of transmitter uptake and transmitter synthesis following the acute administration of a number of established and potential antidepressants. To be clinically effective, antidepressants must be administered chronically. Hence the synthesis

of NA, dopamine (DA) and 5-HT in rat brain was studied during the chronic administration of agents which differ in their patterns of monoamine uptake inhibition. Compounds investigated were desipramine, chlorimipramine, maprotiline, a specific inhibitor of NA uptake (Waldmeier, Baumann, Greengrass & Maitre, 1976), Org 6582 (±-8-chloro-11-anti-aminobenzo-(b)-bicyclo-[3.3.1]-nona-3,6a-(10a)-diene hydrochloride), a specific inhibitor of 5-HT uptake (Sugrue, Goodlet & Mireylees, 1976) and mianserin, an atypical antidepressant devoid of effect on monoamine uptake *in vivo* (Leonard, 1974; Goodlet, Mireylees & Sugrue, 1977).

Male Sprague-Dawley rats weighing 120–150 g at the start of chronic drug administration were used. Drugs (10 mg/kg, i.p.) were injected once daily at noon for 14 days. Twenty-four h after the last injection the probenecid-induced increase in brain 5-hydroxyindoleacetic acid (5-HIAA) concentration and the α-methyl-*p*-tyrosine-induced fall in brain NA and DA contents were measured. In other experiments levels of brain (minus striatum) 3-methoxy-4-hydroxyphenyl-glycol (MHPG), striatal DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and whole brain NA, DA, 5-HT and 5-HIAA were determined using conventional fluorimetric procedures.

The chronic administration of desipramine resulted in a significant increase in both MHPG concentration and in the α-methyl-*p*-tyrosine-induced fall in brain NA content. Chronic mianserin also caused a small, but significant, increase in MHPG levels. Neither desipramine nor mianserin altered synthesis of DA or 5-HT or steady levels of 5-HIAA, DOPAC or HVA. Chronically administered Org 6582 decreased by approx. 40% brain 5-HIAA content and significantly antagonized the probenecid-induced increase in brain 5-HIAA levels. Chronic Org 6582 had no effect on synthesis of NA or DA and on levels of MHPG, DOPAC or HVA. Neither maprotiline nor chloripramin at the dosage regimen employed, altered synthesis of NA, DA or 5-HT or steady state levels of metabolites.

These findings reveal that, in contrast to the acute situation, chronically administered desipramine increases the synthesis of NA in rat brain. Brain 5-HT synthesis is decreased by the chronic administration of the specific 5-HT uptake inhibitor Org 6582. Similar results are seen following the acute administration of the compound. These observations suggest that rat brain 5-hydroxytryptaminergic systems are more resistant than noradrenergic systems to adaptive changes following a prolonged inhibition of monoamine uptake.

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Investigation of food consumption using a dietary self-selection procedure: effects of pharmacological manipulation and feeding schedules

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It is known that pharmacological manipulation of catecholamine (CA) (Leibowitz, 1976) and serotonin (5-HT) systems (Blundell, 1977) may bring about a depression of food intake. These effects have usually been examined by measuring the weight of food consumed by deprived rats allowed access to a single diet. When rats are given the opportunity to self-select particular proportions of protein and carbohydrate from separate diets, it has been reported that drugs known to increase 5-HT metabolism (fenfluramine and fluoxetine) spare protein intake while suppressing total intake in the weanling rat (e.g. Wurtman & Wurtman, 1977). On the other hand amphetamine, which is believed to exert its anorexic actions via CA mechanisms (e.g. Blundell & Latham, 1978), suppressed both protein intake and total food intake. However, it is known that the age of animals and deprivation regimens may affect protein requirements (NAS-NRC, 1978), 5-HT metabolism (Perez-Cruet, *et al.*, 1972) and feeding behaviour (Levitsky, 1970). Accordingly, we have further investigated the effects of various doses of (i) amphetamine and fenfluramine