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Antidepressant drugs affect dopamine uptake

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The catecholamine hypothesis of depression postulates a deficiency of norepinephrine at functional receptor sites. The evidential basis derives from studies on the turnover and uptake of norepinephrine and the blockade of reuptake by tricyclic antidepressants [1–3]. Serotonin systems may also be involved in antidepressant drug action [4, 5]. Relatively little attention has been paid to the possible role of dopamine in the pharmacotherapy of depression. Although Horn *et al.* [6] included some tricyclic antidepressants in their study on regional catecholamine uptake by rat brain synaptosomes, it is not known whether inhibition of dopamine uptake is a common property of antidepressants currently in clinical use.

Our laboratory has been investigating the mode of action of antidepressant compounds. We have reported time-dependent changes in serotonin metabolism after chlorimipramine: rat brain serotonin increased 30 min after an i.p. injection of the drug and this effect was interpreted as reflecting re-uptake blockade [7]. Since brain dopamine levels are also increased by chlorimipramine (unpublished observation), we investigated the possible effect of this and other compounds from the family of antidepressants on dopamine uptake. We report here that a number of widely used antidepressants inhibit dopamine uptake by rat brain nuclei-free homogenates. For comparative purposes, four stimulants were included in the study.

Male Sprague–Dawley rats (150–200 g, Madison, Wis.) were killed by decapitation and their brains immediately removed. Brains were homogenized (12 strokes, 1 min) in 10 vol. ice-cold 0·32 M sucrose in Tri-R glass homogenizers with Teflon pestles (clearance 0·009 to 0·011 in.), and centrifuged at 1000 *g* for 10 min in a Sorvall RC 2-B centrifuge. The supernatant nuclei-free suspension was separated from the pellet, gently stirred to make a uniform suspension and reserved for uptake and protein assays. We used the method of Snyder and Coyle [8] with minor modifications. The incubation mixture was agitated at 37° in 4·0 ml Krebs–Henseleit bicarbonate buffer (pH 7·4) in 15-ml Corex tubes. The drugs tested (all at 10^{-5} M) were added to the incubation mixture prior to the addition of tritiated dopamine (sp. act. 9·8 Ci/m-mole, New England Nuclear Corp.); preincubation was omitted. At the end of the 8-min incubation period, the samples were placed in ice-water for 2 min. The tubes were centrifuged at 6040 *g* for 20 min in a Sorvall RC 2-B centrifuge to form the tissue pellet. The incubation mixture was aspirated and the pellet rinsed once with 4·0 ml ice-cold saline. Solubilization of the pellets was performed by incubation with 1 ml Soluene-350 (Packard) at 50° for 30 min. The solutions were transferred to glass counting vials, 10 ml toluene fluor was added, and radioactivity was measured in an Isocap/300 scintillation counter (Searle Radiographics, Inc.). The protein content of 0·2 ml aliquots of the synaptosomal suspensions was determined by the method of Lowry *et al.* [9].

The aim in this pilot study was to detect whether inhibition of dopamine uptake is a common property of a number of antidepressants. Preliminary data expressing the variation of uptake of ^{3}H-dopamine with increasing substrate concentrations (0·01 to 1 μM) when graphed revealed two consecutive saturable curves. These data were resolved into two straight lines in Lineweaver–Burk plots, suggesting the existence of two components responsible for the accumulation of ^{3}H-dopamine into synaptosomal suspensions from whole brain. One component operated at low substrate concentrations with a K_m at 1×10^{-7} M and another component at high concentrations of ^{3}H-dopamine with a K_m at $3{\cdot}3 \times 10^{-7}$ M (Fig. 1). Snyder and Coyle [8] have previously reported two affinity systems for dopamine uptake by homogenates from all brain regions except for the striatum which possesses only one uptake system. The value reported by these authors for high affinity uptake was $0{\cdot}8 \times 10^{-7}$ M; the corresponding value for low affinity uptake was $1{\cdot}4 \times 10^{-6}$ M. The different value for low affinity uptake is probably due to the

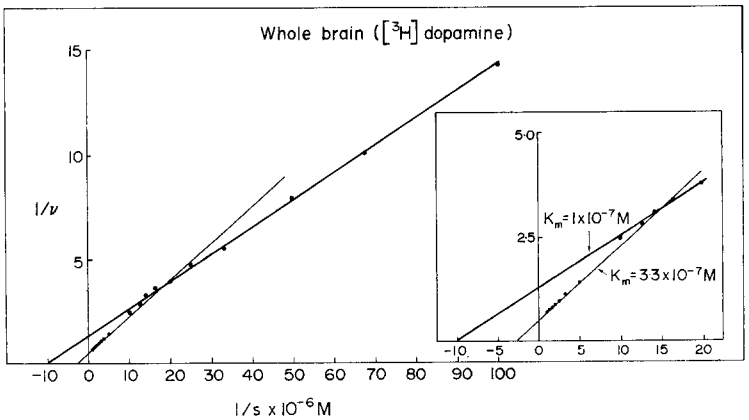

Fig. 1. Double reciprocal plots of low concentrations (0·01 to 0·1 μM) and high concentrations (0·2 to 1 μM) of ^{3}H-dopamine and its accumulation into rat brain nuclei-free homogenates. Amine uptake (v) is expressed as dis./min/mg of protein/8 min $\times 10^{-3}$. Insert illustrates that data are best fitted by two lines.

presence of striatal synaptosomes in our whole brain preparation. Regional analysis of drug effects is now being performed in our laboratory and will be reported elsewhere.

At the concentration (10^{-5} M) tested, all drugs used in the present study inhibit the uptake of ^{3}H-dopamine by rat brain nuclei-free homogenates (Table 1). It is apparent that the drugs tested are not equipotent as inhibitors of ^{3}H-dopamine uptake. For example, chlorimipramine exceeds all other antidepressants in its ability to inhibit uptake, whereas imipramine is the weakest of all. On the basis of these results, it is not possible to detect differences in potency between tertiary and secondary amines, as has been shown for serotonin and nonepinephrine uptake. Nor is it clear at present what accounts for such differences in potency. The absence of a methyl group at the side chain of the secondary amines does not appear to be a crucial factor. The fact that a tetracyclic compound, maprotiline, also inhibits uptake suggests that the 3-ring structure may not be an essential prerequisite. In this context, it is noteworthy that, at 10^{-5} M, maprotiline and desipramine are equipotent. We realize that relative potency is best expressed as the inhibitory concentration that produces 50 per cent reduction of uptake, and these values will be reported in a subsequent publication.

Somewhat surprising was the finding that chlorimipramine, a known potent blocker of serotonin uptake [10–14], was also a potent inhibitor of dopamine uptake among the compounds tested. This drug is of further interest because it is a weak inhibitor of norepinephrine uptake [11], a fact that lends further support to the view that different mechanisms regulate the uptake of the two catecholamines [8]. In addition, the ability of one single compound to affect the uptake of serotonin and dopamine may have worthwhile clinical implications for disease entities, such as Parkinsonism, in which both transmitters have been shown to be deficient.

When compared to the stimulants, antidepressants as a group are weaker in inhibiting ^{3}H-dopamine uptake. For example, chlorimipramine was 2·6 and 1·8 times weaker than *d*-amphetamine and cocaine respectively. This finding is of further interest because chlorimipramine is a potent antidepressant characterized particularly by more rapid onset of clinical effect than other antidepressants. The question arises whether inhibition of dopamine uptake by antidepressants bears any relevance to their clinical efficacy. It is tempting to speculate that psychomotor activation induced in humans by antidepressant medication is related to dopamine uptake inhibition which may potentiate dopaminergic mechanisms at functional receptor sites. In fact, recent evidence suggests that psychomotor retardation might be the symptom most likely linked to a dopamine deficiency [15, 16].

Table 1. Effect of antidepressant and stimulant drugs on ^{3}H-dopamine uptake by rat brain synaptosomal suspensions*

Drug	Uptake of ^{3}H-dopamine†	Per cent of control
Control	160·3 ± 1·9 (15)	
Antidepressants		
Chlorimipramine	67·3 ± 3·1 (9)	42
Nortriptyline	80·2 ± 0·7 (5)	50
Protriptyline	83·4 ± 1·6 (5)	52
Amitriptyline	86·6 ± 1·3 (5)	54
Maprotiline	100·9 ± 0·8 (5)	63
Desipramine	101·0 ± 1·5 (5)	63
Iprindole	104·2 ± 1·6 (8)	65
Doxepin	104·4 ± 3·5 (5)	65
Imipramine	112·2 ± 1·5 (5)	70
Stimulants		
d-Amphetamine	25·6 ± 0·6 (5)	16
Methylphenidate	28·9 ± 0·4 (5)	18
l-Amphetamine	35·5 ± 0·7 (5)	22
Cocaine	36·9 ± 0·6 (5)	23

* The figures represent the mean ^{3}H-dopamine uptake in dis./min/mg of protein $\times 10^3 \pm$ S. E., for the number of determinations in parentheses. The concentration of ^{3}H-dopamine was 0·05 μM. All drugs were tested at 10^{-5} M.

† Statistical analysis was performed by the two-tailed Student's *t*-test. All changes are significant ($P < 0·001$).

The reason why the present findings cannot be associated with certainty to the clinical efficacy of the antidepressants tested relates to our insufficient knowledge of drug levels in discrete brain regions after pretreatment *in vivo*. The drug concentration (10^{-5} M) used in our experiments produced inhibition of uptake between 30 and 60 per cent; this concentration falls within the broad range of concentrations of antidepressants that have been shown to inhibit the uptake of norepinephrine and serotonin [17]. Similarly, relatively high drug levels have been found in the brain of animal and man after administration of imipramine and desipramine *in vivo* [18, 19]. Drug levels reported in these studies would roughly correspond to concentrations between 10^{-5} and 10^{-6} M, assuming a homogeneous distribution of the drug in the brain. Our choice to use a 10^{-5} M concentration was based on these considerations.

To determine whether drug levels attained physiologically produce uptake inhibition comparable to the inhibition obtained under the present experimental conditions, we utilized homogenates from brains of pretreated animals. Since chlorimipramine produced greater inhibition of uptake than the other antidepressants tested, this compound was chosen for further experiments. Rats were given an i.p. injection of 25 mg/kg of chlorimipramine hydrochloride in a volume of 1·3 ml/kg. Animals were sacrificed at various intervals between 5 and 60 min after drug administration and brains were processed as described above except that no drug was added to the incubation mixture. Nuclei-free homogenates from pretreated animals showed inhibition of dopamine uptake at all times within 1 hr after injection. Inhibition was highest (30–35 per cent) between 15 and 30 min and dropped to 15 per cent at 45 and 60 min; these changes were statistically significant ($P < 0·05$). We can infer from these data that, at a dose of 25 mg/kg, drug levels of chlorimipramine in the brain reach a concentration between 10^{-5} and 10^{-6} M, assuming a homogeneous distribution of the drug. It is conceivable that the drug selectively accumulates in specific structures and that the reported effects can be maximized once regional analyses have been performed. The preliminary results indicate that inhibition of dopamine uptake by antidepressants may be of physiological significance.

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Alterations in DNA synthesis in cardiac tissue induced by adriamycin *in vivo*— Relationship to fatal toxicity

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Adriamycin (ADR) is a new antitumor antibiotic isolated from cultures of *Streptomyces peucetius var. caesius* [1]. ADR has rapidly become an important antitumor agent because of its activity against a wide range of solid tumors, and in particular, against a group of otherwise poorly responsive tumors, the soft tissue sarcomas [2] and sarcomas of bone [3, 4]. Enthusiasm for ADR has been tempered, however, as anthracycline antibiotics, such as ADR, possess dose-limiting, fatal, cardiac toxicity. Animal models, while said to be capable of predicting acute bone marrow toxicity [5], failed until recently [6, 7] to demonstrate delayed cardiac toxicity. As ADR has been shown to inhibit DNA as well as RNA synthesis [8], we studied the pattern of suppression and recovery of DNA synthesis, as reflected in the incorporation of tritiated thymidine (^{3}H-TdR) into DNA, in the bone marrow (BM) and gastrointestinal mucosa (GI), and in the important site of unusual toxicity, the cardiac muscle.

Popular conception suggests that little if any DNA synthesis occurs in the adult myocardium. As an example, Zak [9], in a review of cell proliferation during cardiac growth, indicated that heart muscle cells divide only during the first 3 weeks of postnatal life. Claycomb [10] reported that DNA synthesis in rat cardiac muscle is essentially "turned off" by day 17 of postnatal development. Nevertheless, there is considerable information suggesting that, in fact, low levels of DNA synthesis do persist in adult myocardial tissue. Actually, in Claycomb's studies, a basal low level of DNA synthesis in the adult heart did persist, as measured by ^{3}H-TdR incorporation into DNA. Furthermore, studies by Petersen and Baserga [11] have demonstrated a two-phase growth pattern of the ventricular myocardium in mice. The first phase during early postnatal life (0 to 5 weeks old) is associated with an increase in both number and size of myocardial cells, and the second, occurring in adult mice (after 5 weeks of age), is associated with an increase in the size of myocardial cells and a moderate increase in the number of nuclei in the ventricles

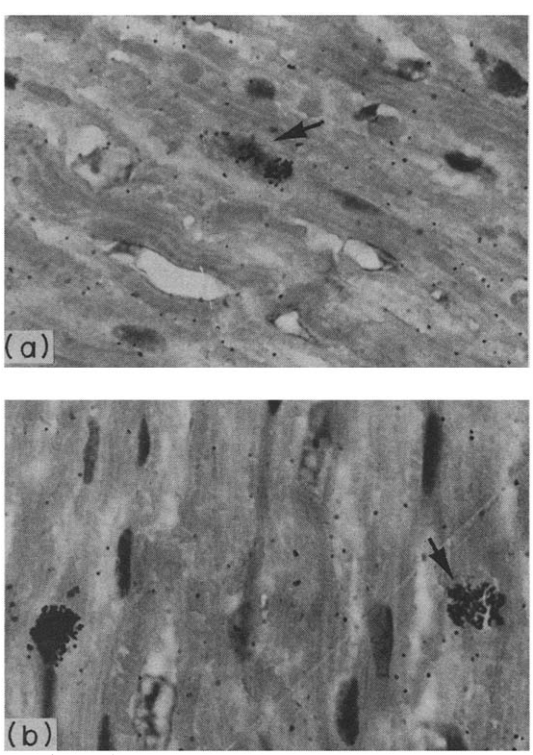

Fig. 1. Autoradiograph of the myocardium of a 10-week-old mouse following 24 hr of pulse labeling *in vivo* with ^{3}H-thymidine showing (A) a labeled cardiac muscle cell nucleus (arrow) and (B) a mitotic figure (arrow) in the background of the cytoplasm of cardiac muscle (H × E) (× 880).