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Effects of acute and chronic administration of desmethylimipramine on the content of norepinephrine and other monoamines in the rat brain

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ACUTE ADMINISTRATION of tricyclic antidepressant drugs does not affect the content of norepinephrine in the brain.[1,2] In contrast, the concentration of endogenous norepinephrine in whole rat brain has been found to decrease during chronic administration of the tricyclic antidepressants imipramine and protriptyline.[3,4] In the present study, we compared the effects of acute and chronic administration of another tricyclic antidepressant, desmethylimipramine (DMI), on the content of norepinephrine, dopamine and serotonin in rat brain. We also examined the content of norepinephrine in various brain regions to see if regional differences occur in the effects of acutely and chronically administered DMI.

Adult male Sprague–Dawley rats received intraperitoneal injections of desmethylimipramine hydrochloride (DMI) (10 mg/kg) or saline either as a single acute injection or twice daily for 3 weeks. In other experiments, rats were treated chronically for 3 weeks with DMI dissolved in their drinking water. Each animal drank about 25 ml of water per day and the concentration of DMI in the drinking water was adjusted to provide a dose of approximately 20 mg/kg/day. Control animals for these studies of orally administered DMI were given tap water and their cages were kept in the same rack as the DMI-treated animals throughout the period of drug administration.

The animals were killed 6 hr after the last injection of DMI or approximately 3 hr after the drinking water containing DMI had been removed from the cages unless it is stated otherwise in the tables. The brains were removed and, in some experiments, were divided into six regions using a modification of the procedure described by Glowinski and Iversen.[5] The six regions were: cortex, cerebellum, hypothalamus, pons and medulla, corpus striatum and "rest of brain" (which included midbrain, thalamus and limbic areas). In other experiments the brains were divided into corpus striatum and the remainder of the brain.

Norepinephrine was assayed according to a modification of the procedure by Anton and Sayre.[6] Large amounts of DMI (up to 10 mg) added to homogenates of rat brain did not interfere with the assay for norepinephrine. The serotonin assay used was a modification of the procedure described by Snyder *et al.*[7] Dopamine was assayed fluorometrically using a modification of the procedure of Carlsson and Waldeck[8] in which the samples, after oxidation, were placed in a boiling water bath for 5 min.

A group of matched control animals was run with each experiment. In order to combine the results from various separate experiments, which examined the effects of DMI on norepinephrine in brain regions, these results were expressed as per cents of the matched control mean values. [The ranges of control mean values of norepinephrine (expressed in ng/g of brain part) from six separate experiments were: pons–medulla, 279–444; cortex, 149–269; cerebellum, 122–222; hypothalamus, 1097–1982; and rest of brain, 322–530. This range of control values, which may reflect differences in previous social and environmental experiences as well as differences in ages of the animals used in various experiments, emphasizes the need for running matched control groups with each separate experiment.]

TABLE 1. EFFECTS OF CHRONIC ADMINISTRATION OF DESMETHYLIMIPRAMINE (DMI) ON CONTENT OF NOREPINEPHRINE, DOPAMINE AND SEROTONIN IN RAT BRAIN*

Treatment	Whole brain minus corpus striatum		Corpus striatum
	Norepinephrine (ng/g tissue)	Serotonin (ng/g tissue)	Dopamine (ng/g tissue)
Saline	319 ± 14 (23)	662 ± 45 (12)	10,824 ± 759 (11)
DMI	256 ± 14† (23)	747 ± 58 (12)	11,511 ± 1,048 (11)

* Animals received DMI (10 mg/kg) or saline twice daily for 3 weeks by intraperitoneal injection. Data are expressed as means ± standard errors of the means. In parentheses are the number of determinations, i.e. animals comprising each mean.

† $P < 0.005$ when compared with control values.

TABLE 2. NOREPINEPHRINE IN VARIOUS REGIONS OF RAT BRAIN AFTER DESMETHYLIMIPRAMINE (DMI)*

	Norepinephrine (% of control)				
	Pons + medulla	Cortex	Cerebellum	Hypothalamus	"Rest of brain"
Acute DMI	100 ± 4	100 ± 2	97 ± 3	96 ± 3	101 ± 4
Chronic DMI	78 ± 3†	81 ± 3†	72 ± 2†	80 ± 3†	78 ± 3†

* In the acute experiments, animals received a single intraperitoneal injection of DMI (10 mg/kg); matched controls received isotonic saline. In the chronic experiments animals received two daily intraperitoneal injections of DMI (10 mg/kg) for 3 weeks; matched controls received isotonic saline. Data from several experiments were combined and expressed as per cents of the matched control mean values (100 %) ± standard errors of the means. Each value represents the mean of 17–27 observations.

† $P < 0.001$ when compared to control values.

In a number of independent experiments, we found that the concentration of norepinephrine in whole rat brain or in whole brain minus corpus striatum was decreased after chronic, but not after acute, administration of DMI. The data from one of these experiments are summarized in Table 1. After chronic administration of DMI we did not observe a comparable decrease of dopamine in the corpus striatum or serotonin in the remainder of the brain (excluding corpus striatum) (Table 1).

Table 2 shows a comparison of the effect of acute and chronic treatment with DMI in five different brain regions. After chronic but not acute administration of DMI, the content of norepinephrine was lowered to about the same extent (19–28 per cent) in all five brain areas examined: pons–medulla, cortex, cerebellum, hypothalamus and the rest of the brain (Table 2). Since the noradrenergic cell bodies of the brain are located in the pons-medulla region,[9] the fact that norepinephrine was reduced in that region to approximately the same extent as in other brain regions may indicate that chronic administration of DMI decreases norepinephrine in both nerve cell bodies and endings. However, the effects of DMI administration are not in all ways uniform throughout the brain, since we have found that the effects of both acutely and chronically administered DMI on the rate of disappearance of intracisternally injected norepinephrine-^{3}H are different in the pons–medulla compared to other brain regions.[10]

The content of norepinephrine in brain which was reduced by chronic administration of DMI returned to normal values within 1 week after drug treatment ceased (Table 3). Thus, it seems unlikely that this effect is the result of irreversible damage to noradrenergic neurons.

TABLE 3. BODY WEIGHT, BRAIN WEIGHT AND BRAIN NOREPINEPHRINE AFTER CESSATION OF CHRONIC DESMETHYLIMIPRAMINE (DMI) TREATMENT*

Weeks after treatment	Body wt (g)		Brain wt (g)		Norepinephrine (ng/g brain)	
	Control	DMI	Control	DMI	Control	DMI
0	273 ± 4	241 ± 5† (88 ± 2)	1·853 ± 0·024	1·841 ± 0·014 (99 ± 1)	363 ± 10	262 ± 8† (72 ± 2)
1	305 ± 14	303 ± 12 (99 ± 4)	1·912 ± 0·025	1·850 ± 0·022 (97 ± 1)	365 ± 20	376 ± 24 (103 ± 7)
3	378 ± 12	366 ± 7 (97 ± 2)	1·926 ± 0·047	1·956 ± 0·034 (102 ± 2)	340 ± 11	335 ± 15 (99 ± 4)

* As described in the text, DMI (20 mg/kg/day) was administered for 3 weeks in drinking water; control animals received tap water. Animals were killed at varying times after cessation of the DMI administration. Data are expressed as means ± standard errors of the means. In parentheses are the values for DMI-treated animals expressed as per cents of matched control means (100 %) ± standard errors of the means. Each value represents the mean of 6–10 animals.

† $P < 0.001$ when compared with control values.

In all of our experiments, the animals which received DMI for 3 weeks by either injection or ingestion gained less body weight during the period of treatment than did control animals, and in some but not all experiments brain weight was slightly decreased. The effect of DMI on weight gain of the treated rats was also reversed within 1 week after the end of DMI administration at which time weights of treated rats were not significantly different from controls (Table 3). We do not know if the failure to gain weight normally during the period of DMI treatment was due to an anorectic effect of the drug, which may even be related in some way to the decreased concentration of norepinephrine in brain, or if it is due to a non-specific toxic effect of DMI. It is unlikely that the decreased concentration of norepinephrine in brain is simply a consequence of a general toxic effect since we did not observe comparable changes in the concentrations of dopamine in corpus striatum or serotonin in the remainder of the brain under these conditions.

Other investigators have recently reported that the content of norepinephrine in brain was not reduced after chronic administration of imipramine (20 mg/kg/day) by intraperitoneal injection (for 10 days) or by oral administration (for 19 days).[11] Since in our laboratory we have consistently observed a decrease in brain norepinephrine after 3 weeks of treatment with imipramine as well as desmethylimipramine or protriptyline in comparable doses,[3,4] we cannot readily account for this apparent discrepancy in findings.

The decrease of norepinephrine after chronic administration of DMI may be related to other effects of the drug such as the block of uptake and decrease in deamination of norepinephrine which we have found to persist after chronic administration.[10] In addition to the well known effect of DMI on the uptake of norepinephrine at the neuronal membrane, effects of this drug on granules or the granular membrane are indicated in a number of experiments.[12–16] High concentrations of DMI have been shown to release norepinephrine from rat heart slices,[17] and if adrenergic nerves in brain were to have a specific affinity for DMI, the drug could conceivably accumulate in sufficient localized concentrations to release norepinephrine from storage granules. It is also possible that after chronic administration of DMI the uptake of dopamine into noradrenergic storage granules may be impaired with a consequent decrease in the conversion of dopamine to norepinephrine by dopamine-beta-hydroxylase which is situated in the storage granules. The further possibility that the decrease in norepinephrine content during chronic administration of tricyclic antidepressants may represent an adaptive response to the more efficient utilization of norepinephrine has been discussed elsewhere.[4]

A decrease in tyrosine hydroxylase activity has recently been reported to occur in various regions of rat brain, including corpus striatum, during chronic administration of tricyclic antidepressants.[18] While this could conceivably account for the decrease in norepinephrine in various regions of rat brain during chronic administration of DMI, it would leave unexplained the failure to find a comparable decrease in dopamine in the corpus striatum.

The lowered content of norepinephrine in brain after chronic but not acute DMI is but one of several differences between the effects of acutely and chronically administered DMI on noradrenergic neurons in brain.[10] Some or all of these may be related to the clinical antidepressant effects of the tricyclic antidepressants which are usually achieved only after several weeks of treatment.

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Interaction of metronidazole with human and bovine plasma albumin

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METRONIDAZOLE [1-(β-hydroxyl ethyl)-2-methyl-5-nitromidazole] has been shown to have tri. chromonacidal activity.[1] Clinical trials[2] have confirmed that this drug is of great value in humans-Although metronidazole is usually well absorbed after oral administration, some patients fail to respond to treatment and this poor response may be due to a low systemic concentration of the drug. Whether this is due to relatively poor absorption from the gastrointestinal tract[3] or to rapid metabolism of the drug[4] is open to question. In view of the fact that protein-binding affects pharmacokinetic properties of drugs;[5] we examined the thermodynamics of the binding of this compound to serum albumins.

Metronidazole was the generous gift of May & Baker (Dagenham, Essex). Human serum albumin (HSA), Sigma Chemical Co., U.S.A., and bovine serum albumin (BSA), British Drug Houses, Ltd, Pool, U.K. were used as supplied by the manufacturers. We considered it unnecessary to purify further the crystalline albumins. All solutions were prepared in 0·067 M sodium phosphate buffer, pH 7·4 (I = 0·170). The albumin solutions were made at a concentration of 0·4 mg/ml. A 200 μM solution of the drug was made and diluted as required.

The uv spectrum of metronidazole was recorded with a Perkin–Elmer (Model 137 UV) spectrophotometer. We recorded only one maximum value. This measurement is the longest wavelength, in the 260–400 nm region, at which a peak absorption occurs for the compound. A knowledge of this value was necessary and useful in our binding experiments.

The method of equilibrium dialysis which was employed to observe albumin binding has been described by Bassir and Bababunmi.[6] This method permits easy determination of protein interaction with drugs.[7] In the present studies, 10 cm lengths (3 cm dia.) or 15 cm lengths (1·5 cm, dia.) of Visking cellophane tubing (Scientific Instrument Centre Ltd, London, U.K.) were used. They were cleaned by rinsing in a shaking bath (Gallenkamp, U.K.) of de-ionized water for 72 hr and were then stored in the 0·067 M sodium phosphate buffer at 4°. The tubing was washed thoroughly with the buffer before use. Optical measurements of the drug were made at 318 nm with a Carl Zeiss Model PMQ II spectrophotometer, using rectangular quartz spectrophotometer cells having a 1 cm light path.

The standard method for the measurement of free and bound drug was as follows: 8·0 ml of albumin solution was placed inside the bag and dialysed against 15·0 ml of medium containing metronidazole in a 50 ml borosilicate AG glass tube and covered with cotton wool. Four different concentrations (25, 50, 100 and 150 μM) of the drug were used while the concentration of the protein was kept constant. For each concentration of the drug four tubes were placed and rocked at 150 cycles/min for 18 hr in the water bath which was regulated either at 15 ± 1° or 25 ± 1°. At 18 hr equilibrium