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Increase caused by desmethylimipramine in the production of [^{3}H]melatonin by isolated pineal glands

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We have recently found that the widely used antidepressant desmethylimipramine (DMI) causes an increase in pineal gland *N*-acetyltransferase activity *in vitro* and *in vivo* [1]. This enzyme is adrenergically regulated; DMI appears to act by blocking the neuronal uptake of catecholamines (uptake$_1$), thus causing an accumulation in pineal extracellular space of catecholamines which have been leaked or released from sympathetic nerve terminals within the gland [1]. Previous studies have indicated that *N*-acetyltransferase activity regulates large changes in melatonin production [2, 3]. It seemed probable, therefore, that DMI would also cause an increase in melatonin production.

We examined this possibility by measuring the amount of [^{3}H]melatonin in the culture medium of pineal glands incubated with [^{3}H]tryptophan and treated with DMI. Pineal glands were obtained from male Sprague–Dawley rats (100–120 g) which had been housed for 4 days in a room with an automatically regulated lighting schedule (L:D 14:10). Animals were killed by decapitation 6 hr after the lights were turned on; pineal glands were immediately removed and placed into organ culture. After 1 hr of incubation, glands were transferred to wells containing medium with 0.2 mM [^{3}H]tryptophan (sp. act. $\simeq$ 70 μCi/μmole) for a 6-hr treatment period.

A dose-dependent increase in both *N*-acetyltransferase activity and [^{3}H]melatonin production was observed after treatment of pineal glands with DMI (Table 1). At the higher concentration of DMI tested (100 μM), *N*-acetyltransferase activity and [^{3}H]melatonin production were statistically indistinguishable from the respective values measured after treatment with a maximally effective concentration [1] of epinephrine (EPI). Substantially more [^{3}H]*N*-acetylserotonin ([^{3}H]NAS), the immediate precursor of [^{3}H]melatonin, was also recovered from cultures containing glands treated with EPI or 100 μM DMI as compared with that from control cultures, in confirmation of the hypothesis that production of [^{3}H]melatonin increased because *N*-acetylation was increased. The amount of [^{3}H]serotonin ([^{3}H]5-HT) recovered from the medium of the cultures containing the DMI-treated glands, but not from that of EPI-treated glands, was about 3-fold greater than that recovered from the medium of control glands. Pineal 5-HT is partially stored out of solution in granules in sympathetic nerve terminals; it seems likely that the large increase in medium [^{3}H]5-HT in DMI-treated cultures is due to a blockade of neuronal uptake of 5-HT caused by DMI treatment [7, 8]. Likewise, this increased availability of free 5-HT may explain why DMI treatment did not result in a decrease in [^{3}H]5-hydroxyindoleacetic acid ([^{3}H]HIAA) and [^{3}H]hydroxytryptophol ([^{3}H]HTOH) levels substantially below those found in the media of control glands, as has been found after β-adrenergic stimulation [9], and as we observed here after treatment with EPI. It would appear that a portion of the released [^{3}H]5-HT may have been subsequently oxidized to [^{3}H]HTOH and [^{3}H]HIAA by monoamine oxidase in pinealocytes.

The effects of DMI reported above may be only of pharmacological interest because of the high concentration of DMI used. To determine whether a lower concentration of DMI might alter pineal indole metabolism, we treated pineal glands with 1 μM DMI or 1 μM norepinephrine (NE) or with a combination of these drugs. The combination was substantially more effective as a stimulant of *N*-acetyltransferase activity, [^{3}H]melatonin production, and [^{3}H]NAS production than either drug alone (Table 2). This apparent sensitizing effect of DMI derives from the pre-synaptic action of DMI as an inhibitor of uptake$_1$, as outlined in the model proposed by Trendelenburg [10]. Production of [^{3}H]5-HT relative to control values was increased by DMI treatment, depressed by NE treatment, and unaffected by treatment with the combination of drugs. Production of [^{3}H]HIAA and [^{3}H]HTOH, however, was unaffected by treatment with NE or DMI but was depressed by treatment with the combination of DMI + NE. The divergent effects of DMI + NE treatment

Table 1. Activity of *N*-acetyltransferase in tissue and the amount of derivatives of [^{3}H]tryptophan in the medium from pineal organ cultures treated with EPI or DMI*

	Drug treatment (1–7 hr)			
	None	EPI (100 μM)	DMI (100 μM)	DMI (10 μM)
N-acetyltransferase activity (nmoles product/gland/hr)	0.08 ± 0.003	6.0 ± 0.86†	8.5 ± 0.82†	4.8 ± 1.07†
[^{3}H]tryptophan derivatives‡ (nCi/gland/6 hr)				
Melatonin	0.3 ± 0.04	4.6 ± 0.38†	4.2 ± 0.29†	2.3 ± 0.52†
NAS	0.3 ± 0.04	2.6 ± 0.37†	0.9 ± 0.08†	0.3 ± 0.05
HIAA	7.1 ± 0.27	3.3 ± 0.28†	5.4 ± 0.43	5.4 ± 0.96
HTOH	0.8 ± 0.06	0.6 ± 0.06	0.7 ± 0.09	0.7 ± 0.06
5-HT	2.3 ± 0.17	3.7 ± 0.66	7.0 ± 1.06†	7.3 ± 1.16†

* Details of the culture method, *N*-acetyltransferase assay, and thin-layer chromatographic isolation of [^{3}H]tryptophan derivatives and the sources of the drugs, chemicals and animals used have been published [1, 4–6]. Data are based on four glands and are given as the mean (±S.E.).

† Significantly different from the control value, $P < 0.01$.

‡ Assuming that the specific activity of the [^{3}H]tryptophan derivatives and [^{3}H]tryptophan was the same (71.6 μCi/μmoles), 1 nCi of a derivative would be equivalent to 14.0 pmoles of compound.

Table 2. Activity of *N*-acetyltransferase in tissue and the amount of derivatives of [^{3}H]tryptophan in the medium from pineal organ cultures treated with NE and/or DMI*

	Drug treatment (1–7 hr)			
	None	NE (1.0 μM)	DMI (1.0 μM)	NE (1.0 μM) + DMI (1.0 μM)
N-acetyltransferase activity (nmoles product/gland/hr)	0.1 ± 0.01	0.1 ± 0.03	2.1 ± 0.07†	6.4 ± 0.95†
[^{3}H]tryptophan derivatives (nCi/gland/6 hr)				
Melatonin	0.3 ± 0.3	1.4 ± 0.65	1.0 ± 0.36	8.2 ± 0.76†
NAS	0.1 ± 0.06	0.7 ± 0.64	0.4 ± 0.30	4.5 ± 0.97†
HIAA	11.8 ± 1.33	10.9 ± 2.52	7.1 ± 1.72	5.2 ± 1.26†
HTOH	2.5 ± 0.26	2.2 ± 0.51	1.2 ± 0.51	0.8 ± 0.22†
5-HT	5.4 ± 0.77	3.1 ± 0.33‡	8.4 ± 0.62‡	4.8 ± 1.69

* The specific activity of [^{3}H]tryptophan in this experiment was 66.2 μCi/μmole; 1 nCi of derivative would be equivalent to 15 pmoles, if the specific activity of tryptophan was equal to that of the tryptophan derivatives.
† Significantly different from the control value, $P < 0.01$.
‡ Significantly different from the control value, $P < 0.05$.

on medium [^{3}H]melatonin and [^{3}H]NAS as compared with that on medium [^{3}H]5-HT, [^{3}H]HIAA and [^{3}H]HTOH support the conclusion that *N*-acetylation of 5-HT, and not uptake or hydroxylation of tryptophan, is altered by this treatment.

The activity of *N*-acetyltransferase and the concentration of melatonin in the rat pineal gland increase at night [2, 3]. Recent studies have indicated that serum and urine melatonin in humans also increase at night [11–13]. If melatonin production in humans is regulated through an adrenergic mechanism similar to that in the rat, DMI treatment might increase melatonin production in humans; some of the observed effects of DMI treatment might be mediated by the pineal gland.

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REFERENCES

1. A. Parfitt and D. C. Klein, *Endocrinology* **99**, 540 (1976).
2. J. Axelrod, *Science, N.Y.* **184**, 1341 (1974).
3. D. C. Klein, in *The Neurosciences, Third Study Program* (Eds. F. O. Schmitt and F. G. Worden), p. 509. MIT Press, Cambridge, Mass. (1974).
4. D. C. Klein and J. L. Weller, *J. Pharmac. exp. Ther.* **186**, 516 (1973).
5. A. Parfitt, J. L. Weller and D. C. Klein, *Neuropharmacology* **15**, 353 (1976).
6. A. Parfitt, J. L. Weller, D. C. Klein, B. Marks and K. Sakii, *Molec. Pharmac.* **11**, 241 (1975).
7. Jaim-Etcheverry and L. M. Zieher, in *The Neurosciences, Third Study Program* (Eds. F. O. Schmitt and F. G. Worden), p. 917. MIT Press, Cambridge, Mass. (1974).
8. N. H. Neff, R. E. Barret and E. Costa, *Eur. J. Pharmac.* **5**, 348 (1969).
9. D. C. Klein and G. R. Berg, *Adv. Biochem. Psychopharmac.* **3**, 241 (1970).
10. U. Trendelenburg, *Pharmac. Rev.* **18**, 629 (1966).
11. G. W. Vaughan, R. W. Pelham, K. L. Sandock and M. K. Vaughan, *J. clin. Endocr. Metab.* **37**, 341 (1973).
12. J. Arendt, L. Paunier and P. C. Sizonenko, *J. clin. Endocr. Metab.* **40**, 347 (1975).
13. H. J. Lynch, R. J. Wurtman, M. A. Moskowitz, M. C. Archer and M. H. Ho, *Science, N.Y.* **187**, 169 (1975).