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SHORT COMMUNICATIONS

Effects of Org 6582, chlorimipramine and desmethylimipramine on the depletion of biogenic amines from the rat brain *in vivo*

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The biogenic amine hypothesis of depression postulates that the clinical symptoms of the disease arise from a deficiency of one or more of these amines at receptor sites within the brain. This hypothesis is substantiated by studies on the turnover and uptake of noradrenaline and serotonin following the administration of clinically effective antidepressants which block the uptake of these amines [1–5]. The credibility of this amine hypothesis has been the subject of a detailed review recently [6].

We have been studying the mode of action of antidepressant drugs of diverse chemical structure with a view to determining whether the clinical efficacy is related (a) to their ability specifically to reduce the re-uptake of noradrenaline and/or serotonin into central neurones *in vivo*, (b) to an action on amine metabolism which is independent of an effect on the uptake system. Our approach has been encouraged by the finding that a new tetracyclic antidepressant drug, Org GB94, is devoid of any action on the amine uptake systems *in vivo* [7,8] and furthermore, unlike the dibenzazepine antidepressants of the imipramine type, it increases rather than decreases noradrenaline turnover *in vivo* [9]. Iprindol is another example of a clinically effective antidepressant which does not affect amine re-uptake and in addition does not affect amine turnover *in vivo*; its main action appears to be related to a direct effect on post synaptic noradrenergic receptors [9,10].

Org 6582 is a bicyclononene derivative which was to some extent modelled on *p*-chloramphetamine, a drug which has less central stimulant activity than *d*-amphetamine and which appears to act primarily by reducing the cerebral synthesis of serotonin [11,12]. *p*-Chloroamphetamine has been shown to have antidepressant properties in man [13]. Sugrue and colleagues [14] have recently shown that Org 6582 is quite specific in its ability to reduce the activity of serotoninergic neurones *in vivo*. Furthermore, these authors found that the reduction in the concentration of noradrenaline in the rat heart caused by metaraminol or 6-hydroxydopamine was unaffected by pretreatment with Org 6582 [14]. We were therefore interested in determining the effects of Org 6582 on the depletion of noradrenaline, dopamine and serotonin *in vivo* in the rat brain and to compare its action with desmethylimipramine (DMI), a tricyclic antidepressant which is a potent inhibitor of catecholamine uptake [15] and with chlorimipramine, an inhibitor of serotonin uptake [16]. It has been shown that some substituted tyramine derivatives deplete brain catecholamines and serotonin after they have been transported into the neurone by means of specific amine carrier mechanisms [17]. A drug which inhibits this uptake mechanism will therefore reduce the depletion of brain amines by the tyramine derivatives. This provides a method whereby an assessment can be made of the effect of a drug on amine uptake *in vivo*.

In these experiments, male Wistar rats (95–105 g) were used. They were housed under normal animal house conditions until the commencement of the experiment and then randomly assigned to cages in groups of five. Groups of rats were then injected at time 0 and 2 hr with either 4-α-dimethyl-*m*-tyramine (H77/77) or with 4-methyl-α-ethyl-*m*-tyramine (H75/12). These substituted tyramine derivatives have been shown by Carlsson [17] to have a high specificity for the uptake and binding mechanism of the intraneuronal storage granules for the catecholamines and serotonin respectively. At time 0 some groups were also injected with Org 6582 (40 mg/kg i.p.), with DMI (20 mg/kg i.p.) or with chlorimipramine (20 mg/kg i.p.) either alone or in combination with one of the substituted tyramine derivatives. After 2 hr these animals were again treated either with Org 6582 (20 mg/kg i.p.) or DMI (20 mg/kg i.p.) or chlorimipramine. Those groups treated with the tyramine derivatives were injected at times 0 and 2 hr with either 12.5 mg/kg i.p. of H77/77 or with 25 mg/kg i.p. of H75/12. The control group was injected with physiological saline. During the period of drug treatment each cage consisted of one control rat and one rat from each treatment group. By using such a block design it has been shown

Table 1. Effect of Org 6582 and desmethylimipramine on the depletion of brain catecholamines caused by 4-α-dimethyl-*m*-tyramine (H77/77)

	Control	H77/77 alone	H77/77 + Org 6582	Org 6582 alone	H77/77 + DMI	DMI alone
Noradrenaline	0.147	0.084* (−50, −35) −43%	0.077* (−54, −40) −48%	0.128 (−24, 0) −13%	0.102*† (−39, −21) −31%	0.129 (−23, 0) −12%
Dopamine	0.794	0.639* (−31, −6) −20%	0.622* (−33, −9) −22%	0.721 (−22, +6) −9%	0.646* (−30, −5) −19%	0.738 (−21, +8) −7%

Each figure represents the mean value (μg/g wet wt of whole brain) of 5 rats. Ninety-five % confidence limits are shown in parenthesis.

* Difference between the treated and control group significant at $P < 0.05$.

† Difference between the group treated with H77/77 alone and that treated with H77/77 + DMI significant at $P < 0.05$.

Table 2. Effect of Org 6582 and chlorimipramine on the depletion of brain serotonin caused by 4-methyl-α-ethyl-*m*-tyramine (H75/12)

	Control	H75/12 alone	H75/12 + Org 6582	Org 6582 alone	H75/12 + chlorimipramine	Chlorimipramine alone
Serotonin	0.52	0.44* (−30, −2) −17%	0.57† (−9, +27) +8%	0.55 (−12, +23) +4%	0.52† (−16, +17) −1%	0.55 (−11, +25) +6%

Each figure represents the mean value (μg/g wet wt of whole brain) of 5 rats. Ninety-five per cent confidence limits are shown in parenthesis.

* Difference between the treated and control group significant at $P < 0.05$.

† Difference between the group treated with H75/12 alone and that treated with H75/12 + Org 6582 significant at $P < 0.05$.

that differences between the various treatment groups due to environmental changes are minimized [24].

All animals were decapitated 4 hr after the initial injection, the whole brain minus cerebellum removed, rapidly weighed and homogenized in a dilute HCl: sodium edate medium (0.01 N HCl containiing 10% (w/v) ethylene diamine tetraacetic acid as the disodium salt). After centrifugation (800 *g* for 20 min), aliquots of the clear supernatant were taken for spectrophotoflourimetric assay of noradrenaline and dopamine, following alumina purification [18] or serotonin following *n*-butanol extraction [20]. The values for the amine concentrations were not corrected for 100 per cent recovery. The recoveries of serotonin and dopamine were approximately 80 per cent, and that for noradrenaline 60 per cent.

The results, expressed as the mean and 95% confidence limits, were analysed statistically as described by van Riezen and Delver [24]. This method of expressing the results enables the block differences between the various treatment groups to be eliminated.

The results of this study are shown in Tables 1 and 2. It can be seen that H77/77 has a more marked effect in depleting noradrenaline than it does on dopamine (Table 1). Furthermore, whereas DMI was effective in partly reversing the depletion of noradrenaline by H77/77 it had no effect on the depletion of dopamine, Org 6582 was ineffective in reversing the tyramine induced depletion of both noradrenaline and dopamine. The results of this experiment suggest that different mechanisms possibly regulate the uptake of these catecholamines, a view which was suggested by Halaris, Belendiuk and Freedman [21] from their study of the effects of a number of tricyclic antidepressants on the uptake of tritiated dopamine into synaptosomal suspensions and further substantiated by the kinetic studies of labelled noradrenaline and dopamine uptake by Snyder and Coyle [22].

Unlike its effect on catecholamine uptake, Org 6582 significantly reversed the depletion of brain serotonin by H75/12, an effect which it shared with chlorimipramine (Table 2). The depletion of brain serotonin by H75/12 is relatively small compared with the effects of this agent on other rat strains [17]. Other studies have shown that Org 6582 differs from chlorimipramine in terms of its specificity of action on amine uptake systems.

Thus Halaris and co-workers [21] have shown in their *in vitro* studies that of the nine tricyclic antidepressants studied, chlorimipramine was the most potent in blocking tritiated dopamine uptake, whereas from the present study it would seem unlikely that Org 6582 has this effect. The results of the present study therefore extend and confirm the views of other investigators [14] that Org 6582 could be a specific inhibitor of serotonin uptake into central neurones *in vivo* with a neurochemical profile more closely resembling 3-(*p*-trifluoro-methyl-phenoxy)-*N*-methyl-3-phenylpropylamine (Lilly 110140) than the tricyclic antidepressant chlorimipramine [23].

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REFERENCES

1. J. J. Schildkraut, *Am. J. Psychiat.* **122,** 509 (1965).
2. W. E. Bunney and J. M. Davis, *Archs. gen. Psychiat.* **13,** 483 (1965).
3. J. J. Schildkraut and S. S. Kety, *Science, N.Y.* **156,** 21 (1967).
4. A. Coppen, *Br. J. Psychiat.* **113,** 1237 (1967).
5. A. Glassman, *Psychosom. Med.* **31,** 107 (1969).
6. B. E. Leonard, *Int. Rev. Neurobiol.* **18,** 357 (1975).
7. B. E. Leonard, *Psychopharmacologia, Berlin* **36,** 221 (1974).
8. W. F. Kafoe and B. E. Leonard, *Archs int. Pharmacodyn.* **206,** 389 (1973).
9. B. E. Leonard and W. F. Kafoe, *Biochem. Pharmac.* **25,** 1939 (1976).
10. E. Szabadi, P. Bevan and C. M. Bradshow, *Exp. Brain Res.* **23,** suppl., 195 (1975).
11. A. Pletscher, G. Bartholini, H. Bruderer, W. P. Burkard and K. F. Gey, *J. Pharmac. exp. Ther.* **145,** 334 (1964).
12. B. E. Leonard, *Psychopharmacologia, Berlin* **46,** 11 (1976).
13. H. M. van Praag and J. Korf, *J. clin. Pharmac.* **13,** 3 (1973).
14. M. F. Sugrue, I. Goodlet and S. E. Mireylees, *Eur. J. Pharmac.* **40,** 121 (1976).
15. A. H. Horn, J. J. Coyle and S. H. Snyder, *Molec. Pharmac.* **7,** 66 (1971).
16. A. Carlsson, H. Corrodi, K. Fuxe and T. Hokfelt, *Eur. J. Pharmac.* **5,** 357 (1969).
17. A. Carlsson, *Bayer-Symposium II*, p. 223. "Effects of drugs on uptake and release of catecholamines" (Eds. H. J. Schümann and G. Kroneberg). Springer-Verlag, Berlin (1970).
18. A. H. Anton and D. F. Sayre, *J. Pharmac. exp. Ther.* **138,** 360 (1962).
19. A. H. Anton and D. F. Sayre, *J. Pharmac. exp. Ther.* **145,** 326 (1964).
20. S. H. Snyder, J. Axelrod and H. Zweig, *Biochem. Pharmac.* **14,** 831 (1965).
21. A. E. Halaris, K. T. Belenduik and D. X. Freedman, *Biochem. Pharmac.* **24,** 1896 (1975).
22. S. H. Snyder and J. T. Coyle, *J. Pharmac. exp. Ther.* **165,** 78 (1969).
23. R. W. Fuller, K. W. Perry, H. D. Snoddy and B. Molloy, *Eur. J. Pharmac.* **28,** 233 (1974).
24. H. van Riezen and A. Delver, *Arzneim. Forsch.* **21,** 1562 (1971).

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