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References

- Beahrs, O. H., and Sakulsky, S. B. (1968). *Archives of Surgery*, **96**, 512.
- Beck, J. S. (1971). In *Immunofluorescence Techniques in Immunopathology and Histopathology*, Broadsheet 69, Association of Clinical Pathologists. London, British Medical Association.
- Billewicz, W. Z., et al. (1969). *Quarterly Journal of Medicine*, **38**, 255.
- Cattell, R. B. (1949). *Journal of Clinical Endocrinology and Metabolism*, **9**, 999.
- Crile, G., and McCullagh, E. P. (1951). *Annals of Surgery*, **134**, 18.
- Crooks, J., Murray, I. P. C., and Wayne, E. J. (1959). *Quarterly Journal of Medicine*, **28**, 211.
- Fulthorpe, A. J., Roitt, I. M., Doniach, D., and Couchman, K. (1961). *Journal of Clinical Pathology*, **14**, 654.
- Greig, W. R. (1965). *Journal of Clinical Endocrinology and Metabolism*, **25**, 1411.
- Hedley, A. J., Flemming, C. J., Chesters, M. I., Michie, W., and Crooks, J. (1970a). *British Medical Journal*, **1**, 519.
- Hedley, A. J., Scott, A. M., Weir, R. D., and Crooks, J. (1970b). *British Medical Journal*, **1**, 556.
- Hedley, A. J., Michie, W., Duncan, T., Hems, G., and Crooks, J. (1971). In press.
- Hobbs, J. R., Bayliss, R. I. S., and MacLagan, N. F. (1963). *Lancet*, **1**, 8.
- Mourant, A. E., Kopeć, A. C., and Domaniewska-Sobczak, K. (1958). *The ABO Blood Groups*, Oxford, Blackwell Scientific.
- Nofal, M. M., Beierwaltes, W. H., and Patno, M. E. (1966). *Journal of the American Medical Association*, **197**, 605.
- Olsen, W. R., Nishiyaric, R. H., and Graber, L. W. (1970). *Archives of Surgery*, **101**, 175.
- Painter, N. S. (1960). *British Journal of Surgery*, **48**, 291.
- Riddell, V. (1962). *British Journal of Surgery*, **49**, 465.
- Werner, S. C., and Spooner, M. (1955). *Bulletin of the New York Academy of Medicine*, **31**, 137.
- Wilson, G. M. (1967). In *Symposium on Thyroid Disease and Calcium Metabolism*, p. 51. Edinburgh, Royal College of Physicians.

Brain Amine Concentrations after Monoamine Oxidase Inhibitor Administration

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Summary

The concentrations of 5-hydroxytryptamine (5HT), noradrenaline, and dopamine were estimated post mortem in brain stem, hypothalamus, and caudate nucleus in 33 patients who had been treated with isocarboxazid, clorgyline, or tranylcypromine and 11 controls. Similar and highly significant increases in 5HT and noradrenaline concentration occurred with all three drugs. The distribution was unimodal, but about a quarter of the patients showed only a small increase in brain amines. Tranylcypromine seemed to have a significantly greater effect on dopamine in caudate nucleus and hypothalamus compared with isocarboxazid and clorgyline. In the doses used chlorpromazine did not reduce the amine concentrations. Four patients with Parkinson's syndrome had low concentrations of dopamine in caudate nucleus in spite of monoamine oxidase inhibitor administration.

Introduction

Monoamine oxidase inhibitors (M.A.O.I.s) are valuable antidepressants. However, some patients, clinically similar to those who have responded well, respond badly, and the reason for this is still obscure. Variability in the causation of the depressive symptoms or variability in drug absorption, distribution, or metabolism may all play a part but a full explanation may not be possible until there is a clear understanding of their mode of action.

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They are known to inhibit a number of enzyme systems. Often it is assumed that inhibition of monoamine oxidase is the most likely way in which they affect mental depression, but this is by no means certain. Recently their action as M.A.O.I.s has been complicated by the discovery of multiple forms of this enzyme (Youdim *et al.*, 1969; Collins *et al.*, 1970) in human brain mitochondria, each with a characteristic anatomical distribution and each with its own substrate specificity and sensitivity to inhibitors (Johnson, 1968; Fuller *et al.*, 1970).

In 1965 we showed that it is possible to assess the effect of M.A.O.I.s on the concentration of 5-hydroxytryptamine (5HT) in human brain (Maclean *et al.*, 1965). We have now extended this work to investigate the effect of three M.A.O.I.s on the concentrations in human brain of 5HT, noradrenaline, and dopamine. The inhibitors used were: isocarboxazid, a typical hydrazide inhibitor; tranylcypromine, a non-hydrazide with amphetamine-like actions; and clorgyline, a non-hydrazide which is reported to have a pronounced difference in its potency against two of the monoamine oxidase isoenzymes (Johnson, 1968; Hall *et al.*, 1969).

Subjects and Methods

One of us (W.J.N.) often treats terminally ill patients with M.A.O.I.s. Patients with illnesses regarded as "terminal," such as inoperable neoplasms or extensive cerebrovascular disease, may live for a considerable time. In such patients a depressed mood is extremely common and treatment can make the terminal period more comfortable for the patient, the relatives, and the nursing staff.

For the purposes of this investigation 33 such patients were treated with M.A.O.I.s and, unless their management contra-indicated it, were continued on these drugs until death. Patients who died within 10 days of starting M.A.O.I. or who had received the drug for over 100 days before death were excluded from the series. Eleven patients received isocarboxazid, 13 clorgyline, and 9 tranylcypromine, the average age being 80.6, 85.1, and 85.3 years respectively. Eleven patients with an average age of 79.9 years served as controls.

Drugs.—In addition to the standard medication necessary for their medical condition the 33 patients received clorgyline (20 mg), isocarboxazid (30 mg), or tranylcypromine (30 mg)

TABLE I—Concentrations ($\mu\text{g/g}$ Tissue $\pm$ S.E. of Mean) of 5HT, Noradrenaline, and Dopamine in Brain Stem, Hypothalamus, and Caudate Nucleus in Controls and in Patients Receiving Isocarboxazid, Clorgyline, or Tranylcypromine in Doses of 30, 20, and 30 mg Daily Respectively

	No. of Cases	Days on Drug	5HT in Brain Stem	Noradrenaline in Hypothalamus	Dopamine in	
					Hypothalamus	Caudate Nucleus
Controls	11	—	0.39 ($\pm$ 0.025)	0.49 ($\pm$ 0.10)	0.25 ($\pm$ 0.024)	1.55 ($\pm$ 0.21)
Isocarboxazid	11	26.1	0.80 ($\pm$ 0.079)	0.93 ($\pm$ 0.127)	0.36 ($\pm$ 0.028)	1.99 ($\pm$ 0.24)
Clorgyline	13	33.2	0.84 ($\pm$ 0.059)	1.53 ($\pm$ 0.34)	0.36 ($\pm$ 0.036)	2.50 ($\pm$ 0.50)
Tranylcypromine	9	37.4	1.00 ($\pm$ 0.13)	1.46 ($\pm$ 0.29)	0.56 ($\pm$ 0.103)	2.91 ($\pm$ 0.33)

daily in divided doses. Nine treated patients and two controls were receiving chlorpromazine 50-300 mg daily (mean 115 mg/day) for at least one week before death. They were compared with 19 and six respectively who were not given chlorpromazine or other major tranquillizers.

Dissection and Estimations of 5HT, Noradrenaline, and Dopamine in Brain Stem, Hypothalamus, and Caudate Nucleus.—The general technique, precautions, and laboratory estimations were as described by Pare *et al.* (1969) with the following exceptions. Hypothalamus as previously defined (Pare *et al.*, 1969) is difficult to isolate by gross dissection. In this series dissection was facilitated, after removal of the brain stem, by a midline sagittal section to divide the cerebral hemispheres. This enabled the dissection of the dorsal border of hypothalamus, defined by the hypothalamic sulcus, to be made under direct vision. This modification produced a consistently smaller mass of hypothalamic tissue than in the previous series; it also enabled a larger portion of the caudate nucleus to be taken.

The mean time between death and necropsy was 49.5 hours for the 11 controls and 43.1 hours for the 33 patients receiving M.A.O.I.s.

Results and Discussion

Controls.—The concentrations of 5HT in brain stem were very similar to those found in our previous investigations (Maclean *et al.*, 1965; Pare *et al.*, 1969). The concentrations of noradrenaline in hypothalamus and dopamine in caudate nucleus were not comparable because of the differing dissection techniques. None of the amines showed any significant fall in concentration when patients coming to necropsy more than 36 hours after death were compared with those examined earlier (Maclean *et al.*, 1965).

Effect of M.A.O.I.s on Concentrations of Brain Amines.—The mean period of administration of the three M.A.O.I.s differed but when patients who had received the drugs for similar periods were compared, the changes seen in Table I persisted. The increase in concentrations of 5HT and noradrenaline after the three M.A.O.I.s were qualitatively similar, the difference compared with controls being highly significant, *P* varying from <0.02 to <0.001 . 5HT was estimated only in brain stem, but similar increases in concentrations of noradrenaline were found in the brain stem and caudate nucleus as in the hypothalamus. The increase in dopamine concentration in caudate nucleus was less pronounced and when compared with controls reached a level of significance only with tranylcypromine ($P < 0.01$). The concentration of dopamine in the hypothalamus was increased with all three compounds but the increase with tranylcypromine was almost double that of the other two ($P < 0.05$). Small quantities of dopamine were found in the brain stem and these were not raised in those patients receiving M.A.O.I.s. This difference in the effect of tranylcypromine on dopamine concentration is of interest in relation to the finding of Youdim *et al.* (1971), who reported that in the brains of patients taking M.A.O.I.s tranylcypromine inhibited the isoenzyme of monoamine oxidase which oxidized dopamine whereas isocarboxazid and clorgyline did not do so.

Speed of Action of M.A.O.I.s.—Five patients were excluded from the series because of the brief duration of M.A.O.I. therapy (from two to eight days; mean four days). These cases

are of interest as they suggest that the concentrations of brain amines may increase relatively early, similar to the improvement noted occasionally in clinical practice. The mean concentrations of 5HT, noradrenaline, and dopamine in the brain stem, hypothalamus, and caudate nucleus respectively were 0.71, 0.84, and 1.62 $\mu\text{g/g}$.

Effect of Chlorpromazine (Table II).—When the 11 patients who had received chlorpromazine were compared with the 25 who had not, no sign of reduction was found in the concentrations of 5HT, noradrenaline, or dopamine either in controls or in those receiving M.A.O.I.s. This suggests that chlorpromazine in a dose of about 100 mg daily does not antagonize the effect of M.A.O.I.s in increasing brain amine concentrations and in this dosage is not contraindicated as an adjunct therapy.

Parkinson's Syndrome (Table III).—Four patients had Parkinson's syndrome; one was a control and the other three had M.A.O.I.s, each being treated with a different type of

TABLE II—Effect of Chlorpromazine (average = 115 mg/day) on Concentrations of 5-Hydroxytryptamine, Noradrenaline (NA), and Dopamine (DA) ($\mu\text{g/g}$ Tissue) in Brain Stem, Hypothalamus, and Caudate Nucleus in Patients Receiving M.A.O.I.s and Controls

	Chlorpromazine	No.	Days on M.A.O.I.	5HT in Brain Stem	NA in Hypoth.	DA in Caud. N.
Controls	No	6	—	0.37	0.40	1.59*
	Yes	2	—	0.41	0.33	1.81
M.A.O.I.s	No	19	32.0	0.87	1.02	2.29†
	Yes	9	31.0	0.82	1.08	2.66

*Excluding one patient with Parkinson's disease.

†Excluding three patients with Parkinson's disease.

TABLE III—Concentrations ($\mu\text{g/g}$ Tissue) of 5-Hydroxytryptamine, Noradrenaline, and Dopamine in Brain Stem, Hypothalamus, and Caudate Nucleus of Parkinsonian and Non-Parkinsonian Patients with or without Treatment with M.A.O.I.s

		No.	Days on Drug	5HT Brain Stem	NA Hypoth.	DA Caud. N.
Controls	All patients	11	—	0.39	0.49	1.55
	Parkinson's syndrome	1	—	0.34	0.41	0.44
M.A.O.I.s	All patients	33	31.7	0.87	1.31	2.44
	Parkinson's syndrome	3	51.0	0.82	0.75	0.48

drug. These four patients had concentrations of 5HT and noradrenaline within the normal range. The concentration of dopamine in the caudate nucleus, however, was markedly low in the control patient and little different in the three patients receiving M.A.O.I.s. Dopamine in the brain stem and hypothalamus did not differ significantly from that in non-Parkinsonian patients. These findings are in conformity with the clinical experience that M.A.O.I.s are relatively ineffective in this condition, and in contrast to levodopa, which has been reported to increase the concentration of dopamine in the caudate nucleus when patients were examined post mortem (Rinne *et al.*, 1971).

REASONS FOR INEFFECTIVENESS OF M.A.O.I.S

Since all three M.A.O.I.s produced similar changes in the brain stem 5HT the data were grouped together for evidence of a

bimodal distribution which might account for the clinical finding that some depressed patients respond and some do not respond to these drugs. In fact there was no evidence of a bimodal distribution. On the other hand, there was a wide scatter of values, some patients showing very little increase in amine concentration compared with controls. (Alexanderson *et al.* (1969) showed a pronounced variability in the steady-state plasma levels of patients on tricyclic antidepressants and this may be correlated with the therapeutic response (Åsberg *et al.*, 1971). We found that tissue response with M.A.O.I.s may show similar variability. Some patients evidently need a larger dose of M.A.O.I.s and Akindele *et al.* (1970) showed a correlation between clinical improvement and disappearance of rapid eye movement (R.E.M.) sleep which in certain patients occurs only with large doses of an M.A.O.I.

By doubling the dose of isocarboxazid or giving an equivalent (oral) dose of nialamide intramuscularly previously unresponsive patients may improve (Pare and Jenner, 1966). This is borne out biochemically when the findings of the present investigation are combined with those of Maclean *et al.* (1965). Ten out of 44 patients receiving a "standard" M.A.O.I. (isocarboxazid 30 mg/day, nialamide 300 mg/day, clorgyline 20 mg/day) for a minimum period of 10 days had brain 5HT concentrations of less than 0.6 µg/g. Of those patients receiving clinically more effective therapy (tranylcypromine 30 mg/day, isocarboxazid 60 mg/day, or nialamide 500 mg intramuscularly four times a week) only one out of 24 had a brain stem concentration of less than 0.6 µg/g. Nevertheless some patients do appear clinically quite unresponsive to M.A.O.I.s yet respond dramatically to tricyclic antidepressants, and, of course, the reverse is also true. The familial association of these cases has led to the suggestion that

they may represent genetically different types of depression (Angst, 1961, 1964; Pare *et al.*, 1962; Pare and Mack, 1971).

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References

- Akindele, M. A., Evans, J. I., and Oswald, I. (1970). *Electroencephalography and Clinical Neurophysiology*, 29, 47.
 Alexanderson, B., Evans, D. A. P., and Sjöqvist, F. (1969). *British Medical Journal*, 4, 764.
 Angst, J. (1961). *Psychopharmacologia*, 2, 381.
 Angst, J. (1964). *Arzneimittel-Forschung*, 14, 496.
 Åsberg, M., Crönholm, B., Sjöqvist, F., and Tuck, D. (1971). *British Medical Journal*, 3, 331.
 Collins, G. G. S., Sandler, M., Williams, E. D., and Youdim, M. B. H. (1970). *Nature*, 225, 817.
 Fuller, R. W., Warren, B. J., and Molloy, B. B. (1970). *Biochemical Pharmacology*, 19, 2934.
 Hall, D. W. R., Logan, B. W., and Parsons, G. H. (1969). *Biochemical Pharmacology*, 18, 1447.
 Johnson, J. P. (1968). *Biochemical Pharmacology*, 17, 1285.
 Maclean, R., Nicholson, W. J., Pare, C. M. B., and Stacey, R. S. (1965). *Lancet*, 2, 205.
 Pare, C. M. B., and Jenner, G. C. (1966). *Diseases of the Nervous System*, 27, 193.
 Pare, C. M. B., and Mack, J. (1971). *Journal of Medical Genetics*, 8, 306.
 Pare, C. M. B., Rees, L., and Sainsbury, M. J. (1962). *Lancet*, 2, 1340.
 Pare, C. M. B., Yeung, D. P. H., Price, K., and Stacey, R. S. (1969). *Lancet*, 2, 133.
 Rinne, U. K., Sonninen, V., and Hyyppä, M. (1971). *Life Sciences*, 10, 549.
 Youdim, M. B. H., Collins, G. G. S., and Sandler, M. (1969). *Nature*, 223, 626.
 Youdim, M. B. H., *et al.* (1972). *Nature*. In press.

Changes in Total Body Calcium and Sodium in Osteomalacia

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Summary

Serial measurements were made of total body calcium and sodium, using whole body neutron activation analysis, in six patients being treated for osteomalacia. All showed a rise in total body calcium ranging from 3 to 30%; this continued for up to 24 months after the start of treatment, though complete healing had apparently occurred after 6 months on the basis of histological examination of biopsy specimens of bone. As the total body calcium rose there was a fall in the non-exchangeable sodium pool in bone.

Introduction

Quantitation of the changes in bone mineralization which occur in osteomalacia is difficult. Assessment by conventional

radiography is relatively insensitive (Simon, 1965). Measurement of cortical thickness or area (Barnett and Nordin, 1960) was a useful advance but is more suited to investigation of osteoporosis than osteomalacia. In this latter condition the cortex may be ill-defined and difficult to measure precisely. Cortical width may often be relatively well preserved and less affected than cortical bone density. Techniques using γ-ray absorption (Cameron and Sorenson, 1963) or x-ray densitometry (Anderson *et al.*, 1966) offer a considerable improvement in sensitivity, detecting changes in bone mineralization with an error of around 5% in practice. These measurements made on the peripheral skeleton suffer from the disadvantage that in some metabolic bone diseases cortical and trabecular bone may not be affected to the same degree. This may be a feature of osteoporosis or uraemic osteodystrophy, but the use of spinal densitometry (Nordin *et al.*, 1968) in combination with measurements of the peripheral skeleton might be expected to avoid this difficulty.

Measurement of total body calcium avoids problems of asymmetrical skeletal involvement in metabolic bone disease and provides information about the natural history or response to treatment.

Previous studies (Anderson *et al.*, 1964; Chamberlain *et al.*, 1968a, 1968b) showed that in-vivo neutron activation analysis in man was technically feasible and able to provide an assessment of total body calcium and sodium while administering a radiation dose (Bush, 1971) which was ethically acceptable.

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