3-Hydroxy-4-methoxyphenethylamine: the endogenous toxin of parkinsonism?

Parkinsonism is a disease characterized by a profound depletion of dopamine in the extrapyramidal areas of the brain together with degeneration of the nigro-striatal neuron system. The distressing symptoms of tremor, rigidity, and hypokinesia associated with parkinsonism have been attributed to hyperactivity of healthy cholinergic systems in the brain released from the inhibitory influence of these damaged dopaminergic pathways as well as to hypoactivity of the latter *per se* (Calne, 1970). Normal motor function in man may depend upon neurochemical equilibria involving dopamine, noradrenaline, 5-hydroxytryptamine, and other transmitter substances (Brimblecombe & Pinder, 1972), and parkinsonian symptomatology may well be associated with disturbances in such equilibria as a result, or as a cause, of pathological changes in the extrapyramidal brain areas. Nevertheless, the fundamental biochemical lesion involved in producing the imbalances and the neuronal degeneration is unknown. We propose that 3-hydroxy-4-methoxyphenethylamine (*p-O*-methyldopamine, HMPEA), produced endogenously by aberrant methylation of dopamine, is the entity responsible for the changes.

Previous hypotheses have included the closely related one of Barbeau (1968) who suggested that 3,4-dimethoxyphenethylamine (DMPEA) might be present in abnormal amounts in parkinsonian patients. DMPEA is present in urine from these subjects, although it may arise from causes other than abnormal methylation of dopamine, (Barbeau, 1970); it produces akinesia and tremor in experimental animals in addition to stimulating dopamine turnover and blocking central dopamine receptors (Brimblecombe & Pinder, 1972). Abnormal quantities of DMPEA have not been demonstrated in parkinsonian brains, and Barbeau's hypothesis remains as speculative as that implicating 6-hydroxydopamine. The latter compound produces a chemical sympathectomy in experimental animals by degradation of catecholaminergic, particularly noradrenergic, neurons, while direct injection into the substantia nigra also gives pathological changes similar to those observed in the human condition of parkinsonism (Malmfors & Thoenen, 1971). It has been suggested (Stein & Wise, 1971) that in parkinsonism the normal resistance of the dopaminergic neurons to the toxic action of endogenous 6-hydroxydopamine is weakened after viral infection or as a result of a pathological gene.

Aberrant *p-O*-methylation of catecholamines has been proposed in schizophrenia as a result of disordered metabolism and to lead to the formation of psychotoxic compounds (see Himwich, Kety & Smythies, 1967). Our hypothesis for parkinsonian aetiology is based upon the following evidence:

(i) HMPEA is a normal, though minor, metabolite of dopamine. 3-Hydroxy-4-methoxyphenylacetic acid has been isolated from cerebrospinal fluid (Mathieu, Revol & Trouillas, 1972), and HMPEA itself has been recovered in human urine after L-dopa treatment (O'Gorman, Borud & others, 1970). However, L-dopa is normally methylated to 3-*O*-methyldopa *in vivo* (Bartholini, Kuruma & Pletscher, 1971) and HMPEA may be derived in this case by *p-O*-methylation of the amino-acid followed by decarboxylation.

(ii) Inhibitors of catechol-*O*-methyl transferase (COMT) potentiate the therapeutic action of L-dopa (Carlsson, 1970; Bartholini & others, 1971) while administration of methyl receptor substances may alleviate the involuntary movements in parkinsonism (Wurtman, Rose & others, 1970; Cotzias, Tang & others, 1971). L-Dopa therapy reduces COMT-activity in man (Weiss, Cohn & Chase, 1971) and apomorphine, also an anti-Parkinson drug, inhibits COMT *in vitro* and in experimental animals (White & McKenzie, 1971).

(iii) HMPEA, but neither the isomeric 4-hydroxy-3-methoxyphenethylamine nor DMPEA, accelerates the caeruloplasmin-catalysed oxidations of dopamine and noradrenaline but inhibits that of 5-hydroxytryptamine (Barrass & Coult, 1972). Parkinsonism may be associated with decreased catecholamine and increased 5-hydroxytryptamine levels (Orzeck & Barbeau, 1970), while serum caeruloplasmin is elevated in the disease and has been suggested as a systemic basis for its aetiology (Barbeau, 1969). Caeruloplasmin-like activity has been detected in the brain (VanderWende, 1964; Marsden, 1965) and may be localized in those brain areas, such as the caudate nucleus, where dopamine levels are particularly high and which undergo degeneration in parkinsonism.

(iv) HMPEA produces hypokinesia and tremors upon intraperitoneal or intrastriatal injection in experimental animals and is much more potent in this respect than is either DMPEA or 4-hydroxy-3-methoxyphenethylamine (Spoerlein & VanderWende, 1967; Little & Dill, 1969; Brimblecombe & Pinder, 1972).

Our hypothesis is therefore that HMPEA is formed in greater amounts in parkinsonians than in normals as a result of aberrant methylation, and that it interacts with caeruloplasmin or a caeruloplasmin-like enzyme in a similar manner as *in vitro* to enhance the oxidation of dopamine and thereby reduce dopamine levels in the brain. HMPEA may act both to deplete dopamine and as a toxin *per se* by producing hypokinesia and tremor. It is interesting to speculate that aberrant methylation arises as a result of a change in the function and character of COMT, which is known to methylate catecholamines in the 4- as well as the 3-position (Creveling, Dalgard & others, 1970). The fundamental biochemical lesion in parkinsonism may therefore be a change in COMT function arising from the effects of viral infection or pathological genes. To evaluate our hypothesis it will be necessary to confirm that (a) levels of HMPEA or its metabolites are higher in parkinsonians than in normals, (b) COMT-inhibition *per se* alleviates or ameliorates parkinsonism, and (c) caeruloplasmin or caeruloplasmin-like activity exists in the extrapyramidal areas of the brain and may be elevated in parkinsonism.

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Effects of sympathomimetics on water movement across toad isolated bladder

That antidiuretic hormone (ADH) increases water movement out of toad isolated bladder is well known. Some catecholamines inhibit this action of ADH (Handler, Bensinger & Orloff, 1968; Strauch & Langdon, 1969). Handler & others (1968) have suggested that this action of catecholamines is probably mediated by α-adrenoceptors. The basis of this suggestion was inhibition by adrenaline and noradrenaline but not by isoprenaline, and antagonism of this inhibition by phentolamine (10^{-4}M) and phenoxybenzamine (10^{-4}M) but not by propranolol (10^{-4}M). We have sought to confirm this conclusion and extend the observations in three directions—to show that the inhibitory action of directly-acting sympathomimetics is concentration dependent, to use a wider range of agonists, and to employ concentrations of antagonists likely to be more specific in their actions.

Methods described by Bentley (1958) and Handler & others (1968) were used. Dividing the bladder of *Bufo bufo* (25 to 40 g) into two half-bladders provided a concurrent paired control to every experiment. Pairing was effective in that the spontaneous water loss and the stimulation of water loss induced by ADH, 250 μU/ml (the experimentally determined ED 50), did not differ ($P \simeq 0{\cdot}7$) between the right and left half-bladder of 6 toads. We also checked that the water loss during a second 40 min period of observation did not differ from that during the first period (correlation coefficient 0·95, $P < 0{\cdot}01$).

(−)-Adrenaline (10^{-7}M) did not affect spontaneous water movement ($P \simeq 0{\cdot}55$). However it completely inhibited the water loss induced by ADH (250 μU/ml). (−)-Noradrenaline (8×10^{-8}M), (−)-phenylephrine (2×10^{-6}M), dopamine ($2{\cdot}5 \times 10^{-6}$M) and (±)-isoprenaline (5×10^{-4}M) also had no effect on the spontaneous water loss. All five sympathomimetics produced a concentration-dependent inhibition of water movement (Fig. 1.)

Phentolamine was studied at a concentration, $2{\cdot}8 \times 10^{-7}$M, which caused approximately ten-fold antagonism of sympathomimetics acting directly at the α-adrenoceptors of mammalian vascular smooth muscle (Ambalavanar, Foster, Kelly & Schnieden: unpublished observations). It did not affect either the spontaneous water loss from the toad bladder or the loss induced by ADH (250 μU/ml). Its effect on the activity of each of the five sympathomimetic amines was assessed using a null method. The mean water loss of six half-bladders treated with ADH (250