Aggression in rats treated with dopa and 6-hydroxydopamine

Spontaneous fighting has been reported in rats treated with apomorphine (Senault, 1970), monoamine oxidase inhibitors and L-dopa (Scheel-Kruger & Randrup, 1967), and pargyline and diethyldithiocarbamate (Scheel-Kruger & Randrup, 1968).

We now report vigorous spontaneous fighting in rats first treated with intracisternal 6-hydroxydopamine and then given L-dopa and the peripheral decarboxylase inhibitor MK 486. These rats also showed a marked rise of the adrenal enzyme tyrosine hydroxylase. Three groups of male Sprague Dawley rats, 200–250 g, were observed: the first group (n = 16) received L-dopa (100 mg/kg, i.p.) 30 min after an injection of MK 486 (methyldopahydrazine, 10 mg/kg, i.p.). The second group (n = 10) had been injected four days before testing with 200 μg (i.c.) of 6-hydroxydopamine. On the days of testing, these rats received MK 486 and L-dopa in a similar way to group 1. The third group (n = 14) received 6-hydroxydopamine (i.c.) 10 days before testing. They then received the above doses of MK 486 and L-dopa, given 4 h after a dose of the disulfram analogue, FLA 63 (bis-4-methyl-1-homopiperazinylthiocarbonyldisulphide, 25 mg/kg, i.p. in suspension), which inhibits dopamine β-hydroxylase and consequently noradrenaline synthesis.

All rats previously treated with 6-hydroxydopamine when given MK 486 and L-dopa, regardless of whether FLA 63 was also given, showed vigorous and prolonged spontaneous fighting for at least 2 h after injection. When the rats were housed 8/cage, after 2 h all were blood-stained and appeared physically exhausted. However, no rats treated only with MK 486 and L-dopa engaged in spontaneous aggression.

In view of the severe exhaustion in the spontaneously fighting rats, adrenal concentrations of tyrosine hydroxylase were measured according to Nagatsu, Levitt & Udenfriend (1964). Rats treated with 6-hydroxydopamine, L-dopa, and MK 486 had elevated enzyme concentrations (Table 1). Further experiments made by Dr. Ng had shown no difference between adrenal tyrosine hydroxylase concentrations of control or hydroxydopamine injected rats.

Spontaneous fighting seen in 6-hydroxydopamine-treated rats given dopa and MK 486 with or without FLA 63, appears to involve a dopaminergic neural system. This view is entirely compatible with the effect of apomorphine which acts on dopamine receptor sites (Ernst, 1967; Andén, Rubenson & others, 1967) and also produces spontaneous aggression in rats (Senault, 1970). It is likely that the dopa given in our experiment was converted to dopamine, but not to noradrenaline since Everett & Borcherding (1970) have shown that intraperitoneal injections of dopa raise primarily cns dopamine. Further, our chosen dose of FLA 63 is adequate to block brain dopamine β-hydroxylase allowing for little conversion of exogenous dopa into noradrenaline (Corrodi, Fuxe & others, 1970). The necessity of pretreatment with 6-hydroxydopamine to elicit spontaneous fighting suggests that either a denervation hypersensitivity effect occurs with exogenous dopa or dopamine or that 6-hydroxy-

Table 1. *Adrenal tyrosine hydroxylase in spontaneously fighting rats*

Group	N	Tyrosine hydroxylase (nM dopa/h per pair of adrenals)
Naive rats	7	93·7 ± 4·5
Dopa + MK 486 (non-aggressive) ..	10	97·8 ± 2·8
6-hydroxydopamine, dopa + MK 486 (aggressive)	10	150·0 ± 9·3*

*$P < 0{\cdot}001$, *t*-test *vs* either naive or drug-injected control groups.

dopamine has functionally destroyed an inhibitory system which blocked the behavioral effect of dopa.

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Effect of phenylbutazone breakdown products on drug metabolism assay

Since the publication of a convenient method for the assay of phenylbutazone in biological material by Burns, Rose & others (1953, 1955) the drug has been used to measure the activity of liver microsomal drug metabolizing enzymes. The standardized enzyme preparation is then used to study the kinetics of hydroxylation reactions under various experimental conditions. Phenylbutazone has generally been regarded as a relatively stable compound and it has been normal practice to prepare and to store standard alkaline solutions of phenylbutazone. However, Pawelczyk & Wachowiak (1968, 1969) identified a number of breakdown products in aqueous solutions caused by hydrolysis and oxidation. The decomposition was independent of pH (between 7·0 and 10·6), buffer concentration or ionic strength, and caused a significant change in phenylbutazone concentration. The decomposition products were not the known metabolites of the drug, but appear likely to possess similar chemical characteristics. This raises doubts about the validity of enzyme experiments where freshly prepared solutions of phenylbutazone are not used. Some effects of the breakdown products on the apparent activity of the liver enzymes are now reported.

Solutions of phenylbutazone and its breakdown products, phenylbutazone carboxylic acid, α-hydroxyphenylbutazone carboxylic acid, 4-hydroxyphenylbutazone, n-caproylhydrazobenzene and re-crystallized azobenzene were prepared in acetone. These solutions were used as standards for g.l.c. and t.l.c. analyses. Phenylbutazone solutions in N and 0·1N sodium hydroxide were also prepared and stored at room temperature or −20°.

Both the acetone and aqueous phenylbutazone solutions rapidly developed a yellow-orange colour at room temperature. This also occurred at −20°, although more slowly. After various storage times the alkaline solutions were extracted for azobenzene with ether and for 4-hydroxyphenylbutazone and phenylbutazone with ether after pH adjustment to pH 7·2 and pH 2 respectively using hydrochloric acid. Gas chromatographic analyses of the ether extracts showed a decreasing phenylbutazone