Effect of catechol-*O*-methyltransferase inhibitors on brain apomorphine concentrations and stereotyped behaviour in the rat

The catechol-*o*-methyltransferase (COMT) inhibitors pyrogallol (Missala, Lal & Sourkes, 1973; McKenzie & White, 1973), tropolone and 8-hydroxyquinoline (McKenzie & White, 1973) prolong apomorphine-induced stereotyped behaviour (ASB) in the rat. We now report the effect of these three inhibitors on brain apomorphine concentrations.

Male Sprague-Dawley rats, ~150 g, were injected intraperitoneally with pyrogallol (J. T. Baker, New Jersey) (250 mg kg^{-1}), tropolone (Aldrich Chemical Co., Inc., Wisconsin) (100 mg kg^{-1}), 8-hydroxyquinoline (Fisher Scientific, Montreal) (100 mg kg^{-1}), physiological saline or propylene glycol (Fisher) 60 and 30 min before apomorphine (Merck Frosst, Kirkland, PQ) (10 mg kg^{-1}, i.p.). Drugs were dissolved in saline, except 8-hydroxyquinoline which was dissolved in propylene glycol. Injection volume was 1·0 ml except for 8-hydroxyquinoline and propylene glycol (0·25 ml). Doses are expressed as the base. Groups of rats were killed at 5, 10, 20, 50, 90 or 120 min, and in some cases at 75 min, after injection of apomorphine.

The brains were assayed for apomorphine by a modification of the procedure of Burkman, Notari & Van Tyle (1974). Each brain was homogenized in an ice-cold mixture of 0·1 M HCl (5 ml), Na_2SO_3 (0·06 ml, 12·5%, anhydrous), EDTA Na_2 (0·06 ml, 10%) and ethyl acetate (2·0 ml) Sequanal grade (Pierce Chemical Co., Rockford, Illinois) in a Teflon-glass homogenizer. The homogenate was diluted to 10 ml with 0·1 M HCl. Four ml was transferred to a 50 ml glass centrifuge tube, saturated with solid NaCl and thoroughly shaken for 2 min with ethyl acetate (5 ml). The mixture was centrifuged for 5 min at 1300 *g* to separate the phases. After the ethyl acetate layer was removed by aspiration and discarded, the pH of the aqueous phase was adjusted to 6·5–7·5 with $NaHCO_3$ solution (1·5 ml, 0·5 M), 10 ml ethyl acetate was added and the extraction repeated. Fluorescence of the ethyl acetate extract was measured in an Aminco–Bowman spectrophotofluorimeter set at excitation and emission wavelengths of 270 and 370 nm (uncorrected), respectively. Calibration curves relating fluorescence intensity and apomorphine concentration were constructed by assaying duplicates of standards; these were prepared by diluting known amounts of apomorphine in 0·1 M HCl to 10 ml with pooled homogenate of brains from untreated rats. Appropriate fluorescence (blank) corrections were made by subtracting the mean fluorescence of samples containing no apomorphine from fluorescence readings of standard samples and sample extracts for each control and COMT-inhibitor-treated rat. Treatment with the individual COMT inhibitors alone did not affect the fluorescence blanks. A linear relation was demonstrated between fluorescence intensity and amount of apomorphine in the standard solution prepared in brain homogenate up to 2·80 $\mu g\ ml^{-1}$ or more ($r = 0{\cdot}99$). Recovery experiments were conducted by adding known amounts of apomorphine to brain before homogenization and assaying the homogenate in duplicate as described above. The mean recovery was 99·4 ± 2·0% (s.e.m.). Concentrations of apomorphine as little as 4 ng ml^{-1} gave fluorescence readings of 1·5 × blank.

Following injection of apomorphine the presence of ASB was recorded by direct observation (Lal & Sourkes, 1973). Behavioural observations were also made on control rats receiving individual COMT inhibitors or drug diluent alone.

In control rats receiving apomorphine, the peak concentration of the alkaloid in the brain occurred at 5 min ($\bar{x} = 5{\cdot}35\ \mu g\ g^{-1}$) and then quickly declined so that at 90 min apomorphine was either undetectable or present only in trace amounts

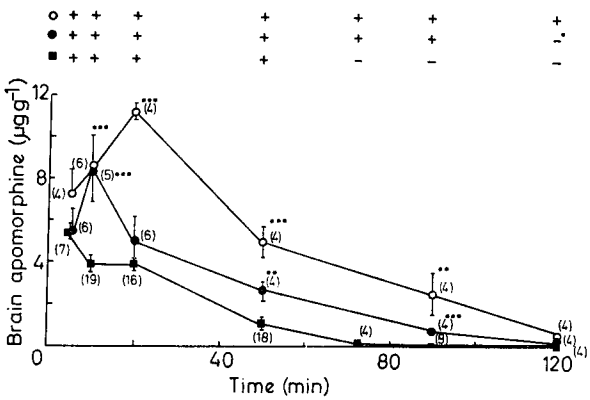

FIG. 1. Rats were given pyrogallol, 250 mg kg^{-1}, (○——○), tropolone, 100 mg kg^{-1}, (●——●) or saline intraperitoneally (■——■) 60 and 30 min before apomorphine (10 mg kg^{-1}). The animals were killed at 5, 10, 20, 50, 90 or 120 min and brains assayed for apomorphine. Saline-pretreated rats (controls) were also killed at 75 min. Each point is expressed as the mean ± standard error of the mean; the number of animals for each point is given in parentheses. Significance of difference between experimental and control rats is given by Student's t test; *** = $P < 0{\cdot}001$; ** = $P < 0{\cdot}005$. Stereotyped behaviour (ASB) is recorded as either present (+) or absent (−) at each time interval; ○ = pyrogallol, ● = tropolone, ■ = saline pretreated rats; * one of the rats still manifested ASB.

($\leqslant 0{\cdot}03$ μg g^{-1}) (Fig. 1). In animals pretreated with tropolone and pyrogallol peak apomorphine values occurred at 10 and 20 min ($\bar{X} = 8{\cdot}32$ and $11{\cdot}19$ μg g^{-1}, respectively). At all time intervals studied, the concentration of brain apomorphine was higher than in controls if the rats were pretreated with either of these two COMT inhibitors. The differences between the two groups of animals were significant at each time, except at 5 min in the case of pyrogallol treatment and 5 and 20 min in the case of tropolone. At 120 min the apomorphine concentration was still elevated, with values ranging from 0·09–0·22 μg g^{-1} (after tropolone) and 0·14–0·73 μg g^{-1} (after pyrogallol).

In control rats receiving propylene glycol before apomorphine, peak brain concentrations of apomorphine occurred at 5 min and then rapidly declined as in the case of saline-pretreated rats (Table 1). Prior treatment with 8-hydroxyquinoline (dissolved in propylene glycol) resulted in a significantly higher peak of apomorphine concentration at 5 min ($P < 0{\cdot}05$) and higher mean values at other times but these were not significantly greater than control values. In two of the 8-hydroxyquinoline-pretreated animals killed at 120 min, the concentration of apomorphine in the brain was still elevated (0·07 and 0·11 μg g^{-1}).

In all rats, ASB commenced within a minute or two of apomorphine injection. Control rats receiving apomorphine alone exhibited ASB at 50 min but not at 75 min or beyond this time. In contrast, all animals pretreated with tropolone exhibited ASB at 90 min, although not at 120 min except in one of the four animals.

After pyrogallol the rats became prostrated and this had some masking effect on ASB. Stereotyped behaviour was nevertheless clearly identifiable, though initially diminished in intensity, and continued for 120 min, when the last group of animals was killed. In the 8-hydroxyquinoline experiments, only one experimental and one control rat exhibited ASB beyond 70 min and none beyond 85 min. None of the rats receiving COMT inhibitor alone or diluent alone manifested ASB.

This assessment of the time of elimination of apomorphine from whole brain of rats shows that the duration of ASB is related to the concentration of apomorphine

Table 1. *Effect of 8-hydroxyquinoline on brain apomorphine concentrations.*[1]

Treatment	Time (min) 5	10	20	50	90	120
Propylene glycol	4·96 ± 0·79 (6)	4·76 ± 1·13 (6)	2·53 ± 0·13 (4)	0·58 ± 0·17 (6)	0·05 ± 0·02 (4)	0·00 (4)
8-Hydroxy-quinoline	7·74 ± 0·20[2] (4)	4·45 ± 0·97 (6)	3·90 ± 1·47 (6)	1·05 ± 0·15 (4)	0·08 ± 0·07 (4)	0·04 ± 0·02 (4)

[1] Rats were injected with 8-hydroxyquinoline (100 mg kg^{-1}) dissolved in propylene glycol or propylene glycol alone i.p. at 60 and 30 min before apomorphine (10 mg kg^{-1}). Values are expressed as $\mu g\ g^{-1}$, mean ± standard error of the mean; number of rats in parentheses. None of the rats killed at 90 or 120 min exhibited stereotyped behaviour.
[2] $P < 0.05$.

in brain. This is in keeping with the results of Butterworth & Barbeau (1975). In the present work, pyrogallol and tropolone prolonged ASB by elevating the concentration of apomorphine in the brain and extending its stay in that organ. In contrast to the finding of McKenzie & White (1973), 8-hydroxyquinoline did not prolong ASB. In keeping with this observation 8-hydroxyquinoline did not consistently extend the stay of apomorphine in brain. Our results suggest that ASB is observed if the concentration of apomorphine in whole brain is at least 0·14 $\mu g\ g^{-1}$.

There is evidence that apomorphine is *O*-methylated *in vitro* (Cannon, Smith & others, 1972; Missala & others, 1973; McKenzie & White, 1973). The present observations with pyrogallol and tropolone are compatible with an inhibition of *O*-methylation of apomorphine *in vivo*. The failure of 8-hydroxyquinoline to prolong the stay of apomorphine in brain consistently may indicate that the duration of COMT inhibition with this compound is too short to affect the metabolism of apomorphine beyond the first 5 min when the latter is injected 30 min after the inhibitor. It is possible, of course, that pyrogallol and tropolone inhibit apomorphine degradation by inhibition of glucuronidation, the major metabolic pathway in the rat (Kaul, Brochmann-Hanssen & Way, 1961), and in this way elevate brain apomorphine concentration.

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