The irritant properties of dopamine-β-hydroxylase inhibitors in relation to their effects on L-dopa-induced locomotor activity

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Inhibitors of dopamine-β-hydroxylase (DBH) (EC : 1.14.2.1) are extensively employed as research tools in neuropharmacology for their ability to specifically block the synthesis of noradrenaline thereby depleting the cerebral stores of this transmitter (Johnson, Boukma & Kim, 1969; Svensson & Waldeck, 1969; Florvall & Corrodi, 1970). These compounds have provided a valuable technique for assessing the role of noradrenaline in various types of animal behaviour and using such an approach it has been suggested that noradrenergic stimulation is involved in motor activity induced by L-dopa (Ahlenius & Engel, 1971; Maj, Grabowska & Mogilnicka, 1971; Stromberg & Svensson, 1971; Dolphin, Jenner & Marsden, 1976).

The interpretation of such behavioural experiments, however, depends on the specificity which may be attributed to the dopamine-β-hydroxylase inhibitors (DBHI) used. Indeed, it has been suggested that the effects of the DBHI disulfiram and U14,624 (1-phenyl-3-(2-thiazolyl)-2-thiourea) in reducing spontaneous locomotor activity are due to non-specific peritoneal irritation caused by injection of the drugs as unstable solutions or suspensions (Thornburg & Moore, 1971; von Voigtlander & Moore, 1970). We have therefore tested the hypothesis that the inhibitory effect of intraperitoneally administered DBHI's on L-dopa-induced motor activity is mediated by non-specific irritation rather than a specific reduction in cerebral noradrenaline concentrations. This has been done by comparing the activity of the DBHI, FLA63 (bis 4-methyl-l-homopiperazinyl-thiocarbonyl disulphide) and U10,157 (1,1-dimethyl-3-phenyl-2-thiourea) with two known irritants, the sulphated polysaccharide carrageenan and the particulate irritant kaolin, in two tests of irritant potency. We have also compared the ability of the standard irritants and the DBHI, in concentrations which have similar irritant properties, to reduce L-dopa-induced motor activity and that induced by the dopaminergic agonist apomorphine in both normal and reserpinized mice.

Male 'Swiss S' mice (20–25 g; Animal Suppliers Ltd) were used. Reserpine (10 mg kg^{-1}, i.p.; Halewood Chemicals Ltd) was administered 18–24 h before testing. FLA-63 (0·5–4·0 mg ml^{-1}; Labkemi AB) was dissolved in 0·5 N HCl and then neutralized with 0·5 N NaOH. These concentrations correspond to doses of 5–40 mg kg^{-1} when 0·20–0·25 ml was administered (i.p.). U10,157 (Upjohn Inc.) and kaolin (Macarthy's Ltd) were adminstered in a concentration of 10 mg ml^{-1} as a fine suspension in 2 % methylcellulose corresponding to doses of 100 mg kg^{-1} when 0·20–0·25 ml was administered (i.p.). Carrageenan (Viscarin Marine Colloids, Maine) was administered as a 2 % colloidal solution in 0·9 % saline.

Irritancy in the mouse paw was tested by subcutaneous administration of the above compounds or their vehicles in a volume of 0·025 ml in the subplantar region of the right hind paw. Three h after administration the animals were killed by cervical dislocation, both hind paws were amputated at the median malleolus and weighed. The difference in weight between the left and

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Table 1. *A comparison of the irritant ability and effect on locomotor activity (LMA) of two known irritants, carrageenan and kaolin, and two known DBHI, FLA-63 and U10,157, in normal mice.*

Test	Vehicles		Irritants		DBHI	
	Saline 0·9 %	Methylcellulose 2 %	Carrageenan 2 %	kaolin 10 mg ml^{-1}	FLA-63 1·0 mg ml^{-1}	U10,157 10 mg ml^{-1}
Paw oedema (mg)	8 ± 1	56 ± 3*	94 ± 19*	59 ± 6	60 ± 3*	32 ± 3
Peritoneal exudation (ΔOD 605 nm)	0·08 ± 0·01	0·23 ± 0·03*	0·41 ± 0·01*	0·42 ± 0·03*	0·21 ± 0·03*	0·35 ± 0·01*
L-Dopa LMA (counts/4 h)	23412 ± 1822	25829 ± 1542	20136 ± 1283	20941 ± 641	15897 ± 1605*	14681 ± 1676*
Apomorphine LMA (counts/2 h)	4538 ± 373	3998 ± 263	3735 ± 216	3840 ± 501	4498 ± 314	4417 ± 351

All results are expressed as the mean of at least 6 determinations ± 1 s.e.m. An asterisk indicates a significant difference ($P < 0·01$) (Student's *t*-test) when the results using U10,157 or kaolin are compared to those obtained with their vehicle, methylcellulose, and when the results using FLA-63 and carrageenan and methylcellulose are compared to those obtained with saline.

right paw was taken as a measure of the inflammatory response. Peritoneal irritant activity was measured following intraperitoneal administration of the substances under test or their vehicles in a volume of 0·20–0·25 ml to animals pretreated intravenously with Evan's (azovan) blue dye (0·2 ml of a 4 mg ml^{-1} solution). Three h later the animals were killed by ether inhalation, a small incision was made in the abdominal wall through which 1 ml heparinized 0·9 % saline was injected. The mouse was gently rocked to mix the peritoneal contents and the incision was then enlarged to facilitate aspiration of the fluid from the peritoneum. The fluid was centrifuged at 500 *g*, the supernatant diluted 5-fold in water and the optical density recorded at 605 nm on a Cecil spectrophotometer model C.E.303.

Motor activity was recorded using batches of 3 mice in Animex activity meters as previously described (Dolphin & others, 1976). The substances under test were administered (0·20–0·25 ml, i.p.) 1 h before L-dopa (except for the long-acting DBHI U10, 157 which was administered 4 h before L-dopa) or 3 h before apomorphine. Animals were placed in the recording cages immediately following administration of L-dopa (200 mg kg^{-1}, i.p.; Roche Products Ltd) plus the peripheral decarboxylase inhibitor MK 486 (α-methyldopahydrazine; 25 mg kg^{-1}; MSD Ltd) as a fine suspension in 2 % methylcellulose; or following s.c. administration of apomorphine HCl (0·5 mg kg^{-1}; Evans Medical Ltd). Activity was recorded for 4 h after L-dopa administration and for 2 h after apomorphine administration, and was measured as total Animex counts registered during the duration of the experiment. Results are expressed as the mean total counts recorded by 6 batches of mice ± 1 s.e.m.

FLA-63 and carrageenan were both highly irritant compared to their vehicle saline (Tables 1 and 2). In normal mice sub-plantar administration of 0·025 ml FLA-63 (1·0 mg ml^{-1}) produced a 7·5-fold increase ($P < 0·01$) and 0·025 ml carrageenan (2 %) an 11·5-fold increase ($P < 0·01$) in paw oedema. Similarly, administration of 0·25 ml FLA-63 (1·0 mg ml^{-1} i.p.) produced a 2·5-fold increase in peritoneal exudation ($P < 0·01$) and 0·25 ml carrageenan (2 %) a 6-fold increase ($P < 0·01$). In reserpinized mice FLA-63 (1·0 mg ml^{-1}) produced a 2-fold increase in paw oedema ($P < 0·01$) and a 2-fold increase in peritoneal exudation. Similarly, carrageenan (2 %) produced a 2-fold increase in both paw odema and peritoneal exudation ($P < 0·01$).

A comparison of irritant properties of kaolin (10 mg ml^{-1}) and U10,157 (10 mg ml^{-1}) showed no clear increase in irritant potency compared to their vehicle methylcellulose either in the induction of paw oedema in normal or reserpinized mice or in the stimulation of the peritoneal exudation in reserpinized animals (Tables 1 and 2). Both compounds, however, were active in stimulating peritoneal exudation in normal mice and methylcellulose itself was irritant when compared to saline in both tests of irritancy.

To examine the inhibiting effects on locomotor activity the drugs and irritants were administered in the same doses as those used in the peritoneal exudation test. Administration of 0·25 ml FLA-63 (1·0 mg ml^{-1}) corresponding to a dose of 10 mg kg^{-1}, inhibited L-dopa-induced motor activity by more than 30 % in both normal and reserpinized mice ($P < 0·01$). Similarly, 0·25 ml U10, 157 (10 mg ml^{-1}) corresponding to a dose of 100 mg kg^{-1} inhibited L-dopa-induced motor activity by 43 % in normal mice ($P < 0·01$) and by 35 % in reserpinized mice ($P < 0·01$) compared to its vehicle, methylcellulose. Neither kaolin nor carrageenan nor the vehicle methylcellulose produced any inhibition of L-dopa-induced motor activity, in reserpinized or normal mice.

By contrast, neither the irritants carrageenan, kaolin or methylcellulose nor the DBHI FLA-63 or U10,157 had any effect on apomorphine-induced motor activity in either normal or reserpinized mice (Tables 1 and 2).

A dose response curve of the irritant activity of FLA-63 compared with its effects on locomotor activity is shown in Fig. 1. In normal mice L-dopa-induced motor

Table 2. *A comparison of the irritant ability and effect on locomotor activity (LMA) of two known irritants, carrageenan and kaolin, and two known DBHI, FLA-63 and U10,157 in reserpinized mice.*

	Vehicles		Irritants		DBHI	
	Saline 0·9 %	Methylcellulose 2 %	Carrageenan 2 %	Kaolin 10 mg ml^{-1}	FLA-63 1·0 mg ml^{-1}	U10,157 10 mg ml^{-1}
Paw oedema (mg)	20 ± 6	26 ± 5	39 ± 5*	43 ± 7	43 ± 5*	26 ± 5
Peritoneal exudation (Δ OD 605 nm)	0·09 ± 0·01	0·13 ± 0·02	0·18 ± 0·02*	0·15 ± 0·03	016 ± 0·03*	0·16 ± 0·01
L-Dopa LMA (counts/4 h)	18396 ± 1450	21499 ± 2340	20751 ± 955	19719 ± 2595	9547 ± 2240*	14032 ± 1706*
Apomorphine LMA (counts/2 h)	5284 ± 302	5218 ± 290	4774 ± 462	4501 ± 697	4753 ± 706	4530 ± 462

The data are expressed and the significances calculated as described in the legend to Table 1.
* $P < 0·01$.

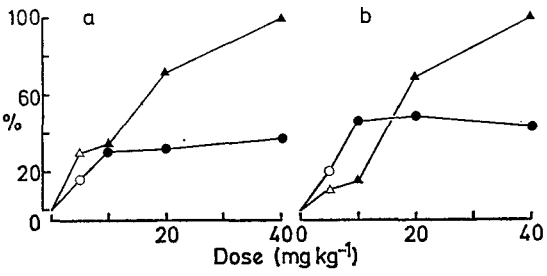

FIG. 1. Comparison of FLA-63 as a peritoneal irritant (-▲-; -△-) and as an inhibitor of L-dopa-induced motor activity (-●-;-○-) in a) normal and b) reserpinized mice (10 mg kg^{-1}; 18–24 h previously). Locomotor activity is expressed in terms of percent inhibition compared to groups of animals receiving L-dopa plus MK 486 alone. Peritoneal irritation is expressed as a percentage of the irritation caused by the highest dose of FLA-63 (mg kg^{-1}) administered. All results are the mean of at least 6 determinations. The open symbols indicate results which do not differ significantly from animals receiving vehicle alone and the closed symbols indicate a significant difference ($P < 0.01$) compared to control groups.

activity was reduced by 32 % ($P < 0.01$) using 10 mg kg^{-1} FLA-63 (Fig. 1a) and in reserpinized mice a 48 % reduction ($P < 0.01$) was found with this dose (Fig. 1b). In neither case was a greater reduction in motor activity produced by increasing the dose of FLA-63 to 20 or 40 mg kg^{-1}. By contrast it is apparent that although FLA-63 in a dose of 10 mg kg^{-1} was irritant as judged by the degree of intraperitoneal inflammation ($P < 0.01$) the extent of this irritation was increased 2-fold in normal mice and 3·5-fold in reserpinized animals by increasing the dose of FLA-63 administered to 40 mg kg^{-1} ($P < 0.01$, compared to 10 mg kg^{-1} FLA-63 for both normal and reserpinized mice).

In the two tests of irritancy which we have employed in the present work, the two DBHI examined FLA-63 and U10,157 have been found to be inflammatory agents of similar potency to the two irritants carrageenan and kaolin in the concentrations used (Tables 1 and 2). Interestingly, the effect of all compounds was less in the reserpinized animal than in the normal mouse due perhaps to the depletion of biogenic amines, including 5-HT, caused by reserpine. However, only FLA-63 and U10,157 were effective in attenuating L-dopa-induced locomotor activity in both normal and reserpinized mice, at doses which we have previously shown to inhibit noradrenaline synthesis from L-dopa (Dolphin & others, 1976).

The dose-response relationship of FLA-63 in both irritancy tests (Fig. 1) shows that its irritant properties are dose-dependent, whereas the effect of FLA-63 on L-dopa-induced locomotor activity in both normal and reserpinized mice reaches a plateau at doses between 10 and 40 mg kg^{-1} FLA-63 in agreement with its effect on cerebral noradrenaline concentrations (Svensson, 1973), and on timing behaviour (Ahlenius & Engel, 1972).

Apomorphine-induced locomotor activity is thought to be caused purely by stimulation of the dopaminergic pathways involved in the control of motor activity and does not involve a noradrenergic component or require the presence of endogenous noradrenaline (Ernst, 1967; Andén, Rubenson & others, 1967; Andén, Strombom & Svensson, 1973). Thus the inhibition of noradrenaline synthesis by DBHI would not be expected to affect apomorphine-induced locomotor activity, and if any effect had been found it might be attributable to the irritant properties of the DBHI. However, neither FLA-63 or U10,157, nor the two standard irritants caused any reduction in the motor activity resulting from apomorphine administration to normal or reserpinized mice.

That only FLA-63 and U10,157 and not the irritant agents carrageenan and kaolin produce any inhibition of L-dopa-induced locomotor activity, suggests that the observed attenuation of L-dopa-induced activity does not result from the irritant properties of the DBHI, and lends further evidence to the possibility that there is a causal relation between the inhibition of DBH by these drugs and the observed effects in this and other behavioural systems. However, it has been found previously that administration of DBHI in the diet of mice does not inhibit motor activity, although producing a similar reduction in noradrenaline to that gained by their intraperitoneal administration (von Voigtlander & Moore, 1970). It has been suggested that this is because dietary administration is not a stressful procedure. However, very little is known of the effects of possible variations in absorption, distribution and metabolism of DBHI, which probably occur following differing routes of administration, on the inhibition of DBH (Dolphin & others, 1976). It is possible that slower absorption of the drug from the alimentary tract allows supersensitivity of central noradrenaline receptors to develop, which compensates for the reduction in stored noradrenaline produced by DBH inhibition.

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REFERENCES

AHLENIUS, S. & ENGEL, J. (1971). *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **27**, 349–360.
AHLENIUS, S. & ENGEL, J. (1972). *Psychopharmac. (Berl.)*, **24**, 243–246.
ANDÉN, N.-E., RUBENSON, A., FUXE, K. & HOKFELT, T. (1967). *J. Pharm. Pharmac.*, **19**, 627–632.

ANDÉN, N.-E., STROMBOM, U. & SVENSSON, T. H. (1973) *Psychopharmac. Berl.*, **29**, 289–298.
DOLPHIN, A., JENNER, P. & MARSDEN, C. D. (1976). *Eur. J. Pharmac.*, **35**, 135–144.
ERNST, A. M. (1967). *Psychopharmac. (Berl.)*, **10**, 316–323.
FLORVALL, L. & CORRODI, H. (1970). *Acta pharm. Suecica*, **7**, 7–22.
JOHNSON, G. A., BOUKMA, S. J. & KIM, E. C. (1969). *J. Pharmac. exp. Ther.*, **168**, 229–234.
MAJ, J., GRABOWSKA, M. & MOGILNICKA, E. (1971). *Psychopharmac. (Berl.)*, **22**, 162–171.
STROMBERG, U. & SVENSSON, T. H. (1971). *Ibid.*, **19**, 53–60.
SVENSSON, T. H. (1973). *J. Pharm. Pharmac.*, **25**, 73–75.
SVENSSON, T. H. & WALDECK, B. (1969). *Eur. J. Pharmac.*, **7**, 278–282.
THORNBURG, J. E. & MOORE, K. E. (1971). *Archs Int. Pharmacodyn. Thér.*, **194**, 158–167.
von VOIGTLANDER, P. F. & MOORE, K. E. (1970). *Proc. Soc. exp. Biol. Med.*, **133**, 817–820.

Chronic guanethidine and adrenal medullary function in the rat

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The adrenal medulla has long been likened to a modified sympathetic ganglion. Recently it has become well documented that high doses of guanethidine chronically administered are markedly toxic to sympathetic ganglia (Angeletti & Levi-Montalcini, 1970; Heath, Hill & Burnstock, 1974) in both adult (Burnstock, Evans & others, 1971; Juul & McIsaac, 1973; Jensen-Holm & Juul, 1971) and newborn (Eranko & Eranko, 1971a, b; Angeletti, Levi-Montalcini & Caramia, 1972; Johnson, Cantor & Douglas, 1975) rats. Ganglionic cellular lysis and a decreased cholinesterase activity have been reported by Jensen-Holm & Juul (1968, 1970) and Juul & McIsaac (1973). Burnstock & others (1971), using histochemical fluorescence, found that less than 2% of the nerve cell bodies remained in the superior cervical ganglion after six weeks of guanethidine treatment (25 to 30 mg kg^{-1} day^{-1}, i.p.). Thus, to ascertain whether guanethidine-induced neurotoxicity extends to the adrenal medulla, we have examined the effect of chronic guanethidine treatment upon the release of medullary catecholamines.

Male Sprague-Dawley rats (175–200 g), fed standard rat pellets and with free access to water, were randomly divided into four groups (2 control and 2 test). One test group was injected with guanethidine monosulphate 20 mg kg^{-1} day^{-1} (i.p.) for 14 days, and the other with 100 mg kg^{-1} day^{-1} (i.p.) for 14 days. Both control groups received an equal volume of 0·9% w/v sodium chloride day^{-1} (i.p.) for 14 days. To more clearly control any chronically induced changes, one of the control groups received an acute dose of guanethidine (20 mg kg^{-1}, i.p.) 2 h before use.

On the day of study, animals were pretreated with atropine (1 mg kg^{-1}, i.p.), anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and pithed to enable stimulation of the entire sympathetic outflow of the thoraco-lumbar region of the spinal cord according to the method of Gillespie & Muir (1967). Rats were surgically prepared for the recording of blood pressure and for the intravenous injection of drugs. Selective field stimulation of the whole left adrenal gland was made as described previously (Romanyshyn, Asaad & Clarke, 1974; Clarke & Romanyshyn, 1976). The method assesses adrenal medullary release by comparing the blood pressure rise following electrical stimulation with dose-effect curves to intravenous adrenaline.

Fig. 1 shows the frequency-response curves to adrenal field stimulation and the corresponding dose-effect curves to adrenaline. Adrenal release appears largely unimpaired regardless of whether guanethidine was administered acutely or chronically. For instance, compared with acute guanethidine, the frequency-response

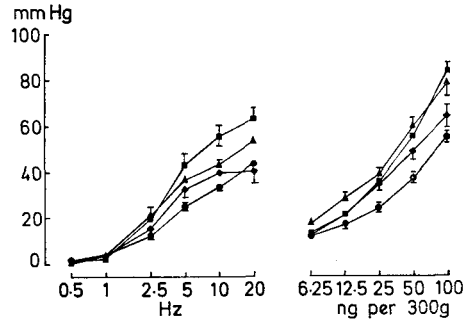

FIG. 1. Relation between blood pressure increase (mm Hg) and log frequency (Hz) of adrenal field stimulation (1 ms, 20V for 20 s) or dose of intravenously injected adrenaline (ng per 300 g body weight) in rats. ● control (n = 6). ■ guanethidine (20 mg kg^{-1}, i.p. 2 h previously, n = 7). ▲ guanethidine (20 mg kg^{-1} day^{-1}, i.p. for 14 days, n = 7). ◆ guanethidine (100 mg kg^{-1} day^{-1}, i.p for 14 days, n = 7).

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