Phenylethyldithiocarbamate: a new dopamine-β-hydroxylase inhibitor

SIR,—It has been shown that disulfiram (tetraethylthiuram disulphide) and its metabolite diethyldithiocarbamate are potent dopamine-β-hydroxylase inhibitors *in vivo* (Goldstein, Anagnoste, Lauber & McKereghan, 1964; Collins, 1965; Carlsson, Fuxe, Hökfelt & Lindqvist, 1966). Dithiocarbamates have been used widely as fungicides and some new derivatives have been investigated in this respect (Thorn & Ludwig, 1962; Rieche, Hilgetag, Martini, Nejedly & Schlegel, 1960). Among the most potent of these was phenylethyldithiocarbamate. The potency of phenylethyldithiocarbamate as a dopamine-β-hydroxylase inhibitor has now been investigated.

Male Sprague-Dawley rats, 200–250 g were used. The weight was within a range of ±10 g in controls and treated animals before an experiment. Phenylethyldithiocarbamate was synthesized according to Rieche & others (1960) and was given subcutaneously as the sodium salt. Noradrenaline in brain and heart was measured (Euler & Lishajko, 1961), and, from the same eluate, brain dopamine was oxidized and measured (Carlsson & Waldeck, 1958). The adrenals from one rat were assayed for adrenaline (Gunne, 1963), the small amount of noradrenaline present being read as adrenaline against the adrenaline standard.

Phenylethyldithiocarbamate was compared with disulfiram for inhibition of the noradrenaline synthesis in whole brain and heart (Table 1). When given

TABLE 1. EFFECT OF REPEATED ADMINISTRATION OF PHENYLETHYLDITHIOCARBAMATE AND DISULFIRAM ON NORADRENALINE AND DOPAMINE CONTENT IN THE WHOLE BRAIN AND HEART.

Disulfiram: 2 × 1 g/kg, orally; doses were given 18, and 3 hr before death. Phenylethyldithiocarbamate: 3 × 200 mg/kg, s.c.; doses were given 51, 27, and 3 hr before death. The results are mean values ± s.e.m., and given as μg/g. Figures in brackets refer to number of experiments.

		Controls (3)	Disulfiram (3)	Controls	Phenylethyldithiocarbamate
Heart	Noradrenaline	0·62 ± 0·02	0·41 ± 0·05*	0·80 ± 0·03 (6)	0·52 ± 0·09*(7)
Brain	Noradrenaline	0·40 ± 0·02	0·22 ± 0·01†	0·40 ± 0·02 (9)	0·21 ± 0·02‡(9)
	Dopamine	0·83 ± 0·06	0·81 ± 0·06	0·67 ± 0·08 (5)	0·74 ± 0·09 (5)

* = $P < 0.05$. † = $P < 0.01$. ‡ = $P < 0.001$.

repeatedly, disulfiram (2 × 1 g/kg, orally) and phenylethyldithiocarbamate (3 × 200 mg/kg, s.c.) caused a decrease of noradrenaline of the same magnitude both in brain and heart, being 45 and 34% respectively. Note that the route of administration and the amount of drug differed in the two experiments. There was no increase of dopamine in the whole brain with either compound. This can be explained by the finding that the dopamine content in the dopamine nerve terminals is unchanged when dopamine-β-hydroxylase is inhibited (Carlsson & others, 1966).

A twofold increase of the dopamine content in the brain stem was found after a single injection (300 mg/kg, s.c.) of phenylethyldithiocarbamate (Table 2). At the same time there was a concomitant decrease of noradrenaline to about 33% of the control value. In this acute experiment no effect was observed on the noradrenaline level in the heart. Phenylethyldithiocarbamate however, produced a marked effect on the catecholamine content in the adrenals, both in

TABLE 2. EFFECT OF PHENYLETHYLDITHIOCARBAMATE ON NORADRENALINE AND DOPAMINE CONTENT IN BRAIN STEM AND HEART

300 mg/kg, s.c. of phenylethyldithiocarbamate was given 3 hr before death. The results are mean values ± s.e.m., and given as μg/g. Figures in brackets refer to number of experiments.

		Controls (5)	Phenylethyldi-thiocarbamate (5)
Heart	Noradrenaline	0·60 ± 0·05	0·60 ± 0·03
Brain stem	Noradrenaline	0·55 ± 0·02	0·18 ± 0·02‡
	Dopamine	0·34 ± 0·02	0·66 ± 0·08†

† = $P < 0.01$. ‡ = $P < 0.001$.

TABLE 3. ADRENALINE AND DOPAMINE IN THE ADRENALS AFTER SINGLE AND REPEATED ADMINISTRATION OF PHENYLETHYLDITHIOCARBAMATE

Single injection: 300 mg/kg was administered s.c. 3 hr before death. Repeated injections: 200 mg/kg was administrated s.c. 51, 27, and 3 hr before death. The results are mean values ± s.e.m.; noradrenaline is calculated as adrenaline, and the total amount given as μg/2 adrenals. Figures in brackets refer to number of experiments.

	Single injection		Repeated injections	
	Controls (5)	Treated (5)	Controls (6)	Treated (6)
Adrenaline	31·8 ± 0·81	9·5 ± 0·60*	22·7 ± 0·21	8·5 ± 1·80*
Dopamine	0·28 ± 0·03	1·78 ± 0·11*	0·21 ± 0·02	1·11 ± 0·12*

* = $P < 0.001$.

acute (300 mg/kg, s.c.) and chronic (3 × 200 mg/kg, s.c.) experiments (Table 3). The adrenaline content was decreased to about 30 and 40% respectively. Dopamine on the other hand increased five- to sixfold. A similar elevation of dopamine in rat adrenals has been found with diethyldithiocarbamate (Carlsson & others, 1966).

Treatment with disulfiram or with phenylethyldithiocarbamate induced sedation in the rats, but the latter drug seemed to be more potent as judged by the difficulty encountered in arousing the animals. From the present results it appears that phenylethyldithiocarbamate is a dopamine-β-hydroxylase inhibitor. This interpretation is supported by the decrease of noradrenaline and a concomitant increase of dopamine, both in brain stem and adrenals.

The possibility of an *in vivo* decomposition of phenylethyldithiocarbamate should also be pointed out. Such a decomposition is known to occur *in vitro* with sodium diethyldithiocarbamate (Hallaway, 1959). Phenylethylamine may be formed and release noradrenaline from the stores (Jonsson, Grobecker & Holtz, 1966). The phenylethylamine component of this substance may enhance depletion of noradrenaline when dopamine-β-hydroxylase is inhibited.

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Adamantanamines and their derivatives as sensitizing agents for 5-hydroxytryptamine-induced contraction of smooth muscle

SIR,—It has been shown *in vitro* that smooth muscle contractions induced by 5-hydroxytryptamine (5-HT) or noradrenaline can be sensitized with different drugs. Sigg, Soffer & Gyermek (1963) reported the sensitizing effect of imipramine on the 5-HT- and noradrenaline-induced contraction of the nictitating membrane of the cat. Rossum (unpublished) found that the log concentration-response curve of noradrenaline is shifted to lower concentrations if imipramine or cocaine is used as sensitizer. Offermeier (1965) has described an increase in the response of the rat fundus strip to 5-HT and of the rat vas deferens to noradrenaline after preincubation with imipramine or cocaine. Using cocaine, with some preparations shifts have been obtained with a factor of almost 100. Continuing our study of receptors of neurotransmitters (Wesemann & Zilliken, 1966) we now describe the influence of adamantanamines and their derivatives on 5-HT-induced contractions of the rat isolated fundus strip.

Male rats, strain Wistar II, 160–180 g, starved for 48 hr but given water *ad libitum*, were used for the fundus strip preparation according to Vane (1957). The mucosa was carefully removed to facilitate the washing out of drugs. The muscle strip was incubated in oxygenated Tyrode (10 ml) at 37°. The strip was fixed to one end of a lightly loaded isotonic lever giving about 20 times magnification. Cumulative dose-response curves were obtained by gradually increasing the dose without washing out (Ariëns & de Groot, 1954).

The antiviral compound amantadine (adamantan-1-amine) inhibits the penetration of influenza A_2 virus into the cell (Davies, Grunert, Haff & others, 1964). We were unable to demonstrate a significant inhibition of influenza virus neuraminidase (A_2-Japan virus 1957 E.C.3.2.1.18). However amantadine, in concentrations higher than 10^{-5} M, sensitizes the rat fundus strip to 5-HT (tested with J. Offermeier). With a concentration of 10^{-4} M of the compound the dose-response curve for 5-HT is shifted to the left by a factor of about 10. Maximal sensitization (usually a factor of about 100) is achieved with 10^{-3} M