Failure of dopamine to accumulate in central noradrenaline neurons after depletion with diethyldithiocarbamate

SIR,—Evidence has recently been obtained that treatment with the dopamine-β-oxidase inhibitor, disulfiram, results in an accumulation of dopamine in sympathetically innervated organs and that the dopamine accumulated in this way can be released on nerve stimulation (Thoenen, Haefely & others, 1966). Furthermore, in previous reports (see Carlsson, Fuxe & others, 1966) support has been obtained for the view that diethyldithiocarbamate inhibits dopamine-β-oxidase *in vivo*. Significant increases of dopamine, however, were observed only in the brain stem and in the adrenals. The low accumulation of dopamine in the central noradrenaline neurons may be due to breakdown by monoamine oxidase or to some other factor preventing the accumulation of dopamine in noradrenaline neurons. To further elucidate this problem a combined histochemical and biochemical study has been made to examine the effect of nialamide, a potent monoamine oxidase inhibitor, on central noradrenaline neurons depleted of this amine to a large extent, with the help of diethyldithiocarbamate.

Male, Sprague-Dawley rats (150–250 g) were treated three times with sodium diethyldithiocarbamate (500 mg/kg i.p. including water of crystallization) 10, 6 and 3 hr before death. Half the animals were also treated with nialamide (500 mg/kg, i.p.) $3\frac{1}{2}$ hr before death. Control rats received nialamide alone in the same way as described above. Some mice were also injected with diethyldithiocarbamate or diethyldithiocarbamate-nialamide as described, but only for behavioural studies. The animals were killed by decapitation under light chloroform anaesthesia. The brains of one and the same group were subjected to histochemical (see reviews by Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967) or biochemical analysis for dopamine, noradrenaline and 5-hydroxytryptamine (5-HT). Dopamine was measured by the method of Carlsson & Waldeck (1958) with the modification introduced by Carlsson & Lindqvist (1962) and noradrenaline by the method described by Bertler, Carlsson & Rosengren (1958). Usually dopamine and noradrenaline measurements were made on brain stem (di- and mesencephalon, pons and medulla oblongata) striatum and hemispheres.

After treatment with diethyldithiocarbamate alone there were marked to very marked decreases, mainly in intensity but also in number of noradrenaline nerve terminals in various parts of the brain. There always remained, however, weakly to strongly (hypothalamus) green-fluorescent noradrenaline nerve terminals in most of the areas examined. The fluorescent noradrenaline nerve cell bodies showed no certain decreases in intensity at the time-interval used. The dopamine nerve terminals and cell bodies of the brain were not affected by this treatment but remained strongly green-fluorescent. Biochemically, these effects were seen as marked decreases in the brain noradrenaline levels accompanied by an increase in the dopamine level of the brain stem (Table 1). The rats and mice were markedly sedated by this treatment. If nialamide was given $\frac{1}{2}$ hr before the last diethyldithiocarbamate injection there was only a small—if any—increase in the fluorescence intensity and number of the various noradrenaline nerve terminals of the brain, as compared to those of brains of rats treated with diethyldithiocarbamate alone. The dopamine neurons were hardly affected by the nialamide treatment. The 5-HT neurons showed distinct increases in fluorescence intensity and in number of fluorescent terminals. Biochemically, there was hardly any additional rise of dopamine in the brain stem by injection of nialamide to the diethyldithiocarbamate-treated mice.

TABLE 1. EFFECT OF DIETHYLDITHIOCARBAMATE ON CATECHOLAMINE LEVELS IN DIFFERENT AREAS OF THE BRAIN WITH OR WITHOUT MONOAMINE OXIDASE INHIBITION BEFORE THE LAST INJECTION OF THE DRUG.
Each experiment was performed on pooled tissue parts of 3 rats.

	Normal	Diethyldithiocarbamate 380 mg/kg × 3 i.p.		Diethyldithiocarbamate 380 mg/kg × 3 i.p. + nialamide 500 mg/kg	
	Ambient temperature				
	+22–23°	+22–23°	+29°	+22–23°	+29°
Brain area	Brain noradrenaline μg/g				
Stem	0·84 1·02	0·16	0·17	0·16	0·26
Hemispheres	0·36 0·38	0·03	0·03	0·03	0·05
Striatum	0·24 0·26	0·05	0·05	0·05	0·08
	Brain dopamine μg/g				
Stem	0·10 0·24	0·35	0·29	0·50	0·21
Hemispheres	0·08 0·18	0·11	0·04	0·14	0·11
Striatum	2·4 3·3	2·9	2·2	3·5	2·7

After dopamine-β-oxidase inhibition, only small amounts of dopamine were accumulated in the central noradrenaline neurons in spite of monoamine oxidase inhibition. This points to the importance of the β-hydroxyl group for a proper binding of the amine to the adenosine triphosphate-protein complex of the granules, as has been suggested previously (Musacchio, Kopin & Weise, 1965). Thus, since the dopamine formed probably cannot be sufficiently bound to the amine granules, it will lie in the axoplasm outside the granules. Since the uptake-concentration mechanism at the nerve cell membrane of the noradrenaline neurons seem to be even more efficient for dopamine than for noradrenaline (Burgen & Iversen, 1965), and since the monoamine oxidase is inhibited, there exist good possibilities for inhibition of the tyrosine-hydroxylase (Nagatsu, Levitt & Udenfriend, 1964), for example, which could cut off the synthesis and explain why dopamine is not accumulated in large amounts.

Treatment with diethyldithiocarbamate resulted in marked central nervous depression which could not be reversed by nialamide, but which was rather potentiated. The behavioural syndrome observed in mice after a large dose of nialamide was not complete in the diethyldithiocarbamate-treated mice. Thus, the general activation (e.g. continuous running) was not observed. The nialamide-syndrome is not prevented by a potent blocker of the first step in catecholamine biosynthesis whereas a blocker of both the tyrosine and the tryptophane hydroxylase causes a marked blockade of this syndrome (Corrodi, 1966). The present findings might suggest that the central noradrenaline neurons also are important for the nialamide syndrome, at least for its full development. However, the possibility must be considered that the central depressant action of diethyldithiocarbamate is largely due to some action other than inhibition of dopamine-β-oxidase.

Acknowledgements. This work has been supported by grants (B67-14X-155-03A, B67-12X-715-02) from the Swedish State Medical Research Council and by grants from "Stiftelsen M. Bergwalls Minne" and Knut and Alice Wallenberg's Foundation.

Department of Pharmacology,
University of Göteborg,
Göteborg, Sweden.

ARVID CARLSSON

Department of Histology,
Karolinska Institutet
Stockholm 60, Sweden.

KJELL FUXE
TOMAS HÖKFELT

May 3, 1967

References

Bertler, Å., Carlsson, A. & Rosengren, E. (1958). *Acta physiol. scand.*, **44**, 273–292.
Burgen, A. S. V. & Iversen, L. (1965). *Br. J. Pharmac. Chemother.*, **25**, 34–39.
Carlsson, A., Fuxe, K., Hökfelt, T. & Lindqvist, M. (1966). *J. Pharm. Pharmac.*, **18**, 60–62.
Carlsson, A. & Lindqvist, M. (1962). *Acta physiol. scand.*, **54**, 83–86.
Carlsson, A. & Waldeck, B. (1958). *Ibid.*, **44**, 293–298.
Corrodi, H. (1966). *J. Pharm. Pharmac.*, **18**, 197.
Corrodi, H. & Jonsson, G. (1967). *J. Histochem. Cytochem.*, in the press.
Hillarp, N.-Å., Fuxe, K. & Dahlström, A. (1966). In *International symposium on mechanisms of release of biogenic amines*, Stockholm: Pergamon Press.
Musacchio, J., Kopin, I. J. & Weise, V. K. (1965). *J. Pharmac. exp. Ther.*, **148**, 22–28.
Nagatsu, T., Levitt, M. & Udenfriend, S. (1964). *J. biol. Chem.*, **239**, 2910.
Thoenen, H., Haefely, W., Gey, K. F. & Hürlimann, A. (1966). *Helv. physiol. Acta*, **24**, C118.

Brain dopamine and the amphetamine-reserpine interaction

SIR,—It seems that in rats the amphetamine excitatory response including stereotyped activity (continuous sniffing, licking and biting) is effected by some interaction or synergism of amphetamine with the brain catecholamines, dopamine and noradrenaline. It can be prevented by inhibition of the synthesis of dopa, the physiological precursor of these amines (Weissman, Koe & Tenen, 1966; Randrup & Munkvad, 1966a) and then restored by the injection of dopa (Randrup & Munkvad, 1966a; Hanson, 1966). In very large doses dopa alone can produce stereotyped activity (Randrup & Munkvad, 1966b; Ernst, 1965).

Further experiments showed that specific inhibition of the synthesis of noradrenaline did not affect the stereotyped activity induced by amphetamine or dopa. This activity, therefore, seems to depend exclusively on dopamine, while noradrenaline seems to be involved in other forms of activity such as locomotion and aggressive behaviour (Randrup & Scheel-Krüger, 1966; Scheel-Krüger & Randrup, 1967).

With this background it becomes necessary to explain why reserpine, which completely depletes the brain both of dopamine and noradrenaline, does not prevent the amphetamine excitatory response.

To investigate this problem we made some experiments on the influence of reserpine and amphetamine upon brain catecholamines. Male Wistar rats weighing 210 to 280 g were injected with various combinations of reserpine (7·5 mg/kg s.c. 20 to 20½ hr before death), the monoamine oxidase inhibitor, nialamide (100 to 500 mg/kg s.c. 2½ hr before death), and (+)-amphetamine sulphate (10 mg/kg s.c. 2 hr before death). The rats were killed by a blow on the back of the neck and the catecholamines together with their *O*-methylated metabolites were measured in brain (Häggendal, 1962, 1963; Scheel-Krüger & Randrup, 1967; Carlsson & Waldeck, 1964).