Effect of 6-Hydroxydopamine on Behaviour and Cerebral Amine Content in Rats

6-Hydroxydopamine (6-OH-DA) selectively destroys peripheral adrenergic nerve endings in various species[1,2]. It decreases the norepinephrine (NE) content of various peripheral tissues for several weeks[2-5] as well as in the brain after intraventricular application[6]. It was suggested that 6-OH-DA has a selective effect on central NE neurons since the uptake of NE into the striatum, containing predominantly dopamine neurons, was unimpaired[6]. However, using histochemical[7] and biochemical[8] methods, both NE and dopamine neurons were found to be depleted of their amine content. A different effect of 6-OH-DA on dopaminergic and noradrenergic central neurons would be surprising in view of the structural similarity of dopamine and 6-OH-DA and the vicinity of the striatal dopaminergic nerve endings to the lateral ventricle. It therefore seemed of interest to determine NE, dopamine and 5-hydroxytryptamine in the brain of rats injected intraventricularly with 6-OH-DA and to correlate changes of the amine content with behaviour.

Male rats from a closed randomized colony (Wistar descent, 180–200 g) were anaesthetized (i.v.) with 75 mg/kg propanidid (EPONTOL, Bayer). Immediately afterwards 250 γ/rat of 6-OH-DA dissolved in 20 µl physiological saline gased with nitrogen was injected into the right lateral ventricle[9,10]. Controls received the same treatment with saline only. The rats were kept under normal conditions for 10 days. Thereafter, groups of 6 rats were studied singly in an open field test[11] and the exploratory activity was expressed by measuring the number of rearings and the number of sections crossed (ambulation) during 3 min. In parallel series the locomotor activity of 4 groups of 3 rats was measured in activity cages (Lehigh-Valley, Electronics Inc. Mod. A 1497) simultaneously with 2 control groups. The number of interruptions of light beams during 1 h was recorded over 14 consecutive hours. The animals were then decapitated and the total brain immediately frozen in petrol ether, cooled with solid CO_2. The organ was stored at -80 °C for not longer than 10 days in plastic vials. Thereafter, the brains were homogenized, extracted (according to SHORE and OLIN[12]) and the content of NE (modification of SHORE and OLIN[12]), dopamine (modification of BERTLER et al.[13]) and 5-hydroxytryptamine[14] measured.

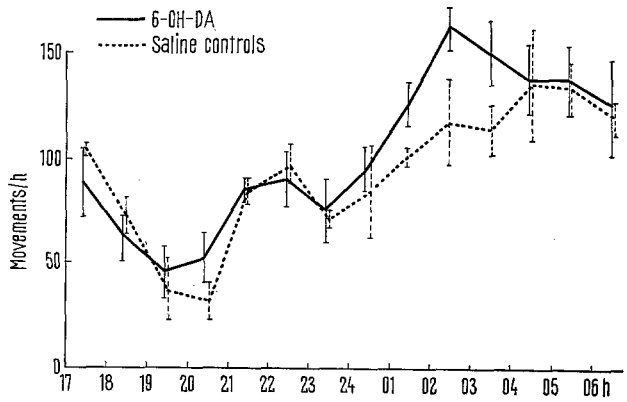
The present results show that 10 days after intraventricularly applied 6-OH-DA, NE decreases to 47%, dopamine to 57%, whereas 5-hydroxytryptamine remains practically unchanged (Table). These findings indicate that 6-OH-DA markedly affects both noradrenergic and dopaminergic neurons but has only negligible effect on 5-hydroxytryptamine neurons. The exploratory behaviour of the animals as measured in the open field test was virtually unaltered 10 days after 6-OH-DA. The number of sections crossed was equal in treated and control animals whereas the number of rearings was only slightly but significantly decreased (Table). The locomotor activity studied in activity cages over a period of 14 h was not statistically significant from the animals injected with saline (Figure).

Although depletion of catecholamines was of remarkably long duration, evidence for a degeneration of central dopaminergic and noradrenergic nerve endings is still lacking. Experiments are underway designed to find out whether central catecholamine neurons show the same different sensitivity of their axons and cell body towards the action of 6-OH-DA as peripheral adrenergic neurons. The unaltered motor behaviour of rats treated with 6-OH-DA is interesting in view of the role suggested for

Influence of 6-OH-DA on amine content of the rat brain and exploratory activity 10 days after intraventricular application

Test	Parameter	Controls µg/g	6-OH-DA treated in % of controls (=100)	p
Brain amine	5-hydroxytryptamine	0.38±0.01 (17)	84± 4 (14)	<0.05
	dopamine	0.98±0.07 (22)	57± 7 (24)	<0.001
	norepinephrine	0.36±0.03 (20)	47± 8 (22)	<0.001
Exploratory activity	ambulation	–	90±12 (24)	>0.1
	rearing	–	64±15 (24)	<0.05

The numbers are mean ± standard error (number of determinations).



Locomotor activity 10 days after intraventricular injection of 6-OH-DA. The vertical bars represent standard error of the mean.

[1] J. P. TRANZER and H. THOENEN, Experientia *24*, 155 (1968).
[2] H. THOENEN and J. P. TRANZER, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. *261*, 271 (1968).
[3] G. HAEUSLER, W. HAEFELY and H. THOENEN, J. Pharmac. exp. Ther., in press (1969).
[4] R. A. MUELLER, H. THOENEN and J. AXELROD, Science *163*, 468 (1969).
[5] P. MOLINOFF and J. AXELROD, Science *164*, 428 (1969).
[6] N. J. URETSKY and L. L. IVERSEN, Nature *221*, 557 (1969).
[7] U. UNGERSTEDT, Europ. J. Pharmac. *5*, 107 (1968).
[8] F. BLOOM, A. GROPPETTI, A. REVUELTA and E. COSTA, 4th Int. Congress on Parmacology, Basel 1969, Abstracts p. 218.
[9] E. P. NOBLE, R. J. WURTMAN and J. AXELROD, Life Sci. *6*, 281 (1967).
[10] J. GLOWINSKI, J. J. KOPIN and J. AXELROD, J. Neurochem. *12*, 25 (1965).
[11] P. A. J. JANSSEN, A. H. M. JAGENEAU and K. H. L. SCHELLEKENS, Psychopharmacologia *1*, 389 (1960).
[12] P. A. SHORE and J. S. OLIN, J. Pharmac. exp. Ther. *122*, 295 (1958).
[13] A. BERTLER, A. CARLSSON and E. ROSENGREN, Acta physiol. scand. *44*, 273 (1958).
[14] D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, J. Pharmac. exp. Ther. *117*, 82 (1956).
[15] J. J. SCHILDKRAUT and S. S. KETY, Science *156*, 21 (1967).

[16] The technical assistance of Mr. E. A. LIENGME is gratefully acknowledged.

adrenergic neurons (for review see e.g. Schildkraut and Kety[15,16]).

Zusammenfassung. 250 γ 6-Hydroxydopamin intraventrikulär pro Ratte vermindern nach 10 Tagen den Gehalt von Noradrenalin und Dopamin etwa um 50%. Hingegen wird 5-Hydroxytryptamin nur geringfügig beeinflusst. Das gleichzeitig gemessene exploratorische Verhalten und die lokomotorische Aktivität sind praktisch unverändert.

W. P. Burkard, M. Jalfre and J. Blum

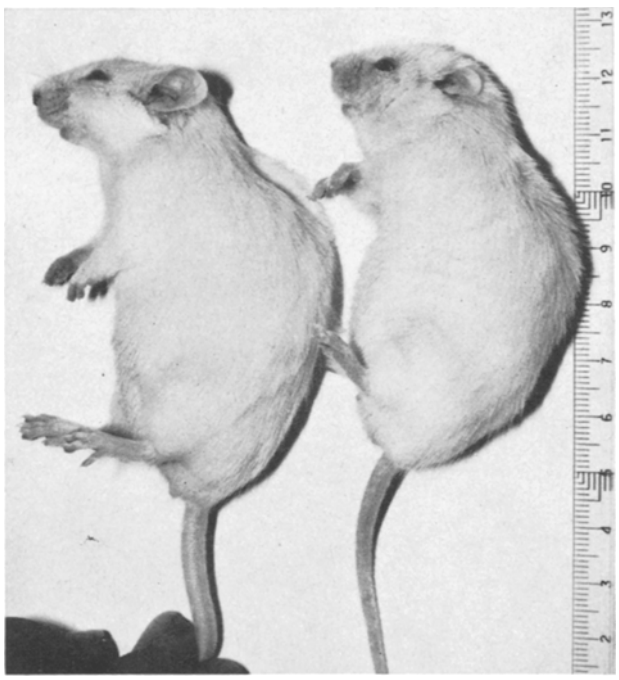
Abteilung für experimentelle Medizin der F. Hoffmann-La Roche and Co. AG., Basel (Switzerland), 13 August 1969.

Malformations after Treatment of New-Born Mice with a Single Dose of Cyclophosphamide

The lethal and teratogenic effects of cytostatics and other drugs administered to fetus are the subject of much experimental study, while much less attention is paid to their effects when given to new-born animals. In investigations on the late effects of a single injection of cyclophosphamide in rats[1–3] and mice, we found that a single dose given to new-born mice resulted in malformations. As the drug is much used in human medicine a brief report may be indicated.

Swiss albino mice, less than 24 h of age and weighing 1–1.5 g, were given a s.c. injection of cyclophosphamide (Sendoxan, supplied by AB Pharmacia, Sweden), dissolved in distilled water, in doses ranging from 0.04–0.2 mg/mouse. In the experiments here described, all littermates were given the same treatment, as preliminary experiments had shown that water-treated controls grew much better than their cyclophosphamide-treated littermates and therefore might oust the latter from suckling.

At a dose of 0.2 mg/mouse or more there was a lethality of 100% (40 mice out of 40 died) within 7 days. At a dose of 0.08–0.1 mg/mouse, 18 out of 48 mice are alive after 6 months. All these mice are malformed, externally seen as a small stunted nose and short forelegs (Figure), but they show a normal behaviour. During the period of growth they are, however, much delayed, and grow more slowly and get hair much later than the controls. No detailed analysis has as yet been performed on the internal organs.



6-month-old mice. Right, treated with 0.08 mg cyclophosphamide at less than 24 h of age. Left, water-treated control. Note the stunted nose, short forelegs and ears of the treated mouse. Measurements shown in cm.

At a dose of 0.05 mg/mouse, 9 mice out of 10 are alive 6 months later, and at a dose of 0.04 mg/mouse none of 10 treated mice has died. In these 2 groups there are only a few showing malformations like the above-described.

A few of the treated females became pregnant. Those treated with a dose of 0.05 mg/mouse or less, apparently passed through their pregnancy and delivery without complications. Of females treated with a dose of 0.08–0.1 mg when new-born, however, 3 died during delivery, apparently because of a narrow pelvis. It should also be mentioned that of the surviving mice, treated with a dose of 0.08–1.0 mg, only 2 out of 18 are females. Apart from the 3 females mentioned, it has not been examined if those which died were pregnant.

As mentioned, treatment of new-born animals with cyclophosphamide does not seem to have been reported previously. The drug has, however, been much used in children in the treatment of malignancies, even down to an age of 9 days[4]. Cyclophosphamide is also used in children in non-malignant diseases as nephrosis[5]. To our knowledge no permanently adverse effects have been noted. Our observations may be a warning that during the period of growth such may appear, other than those intended on the hematopoetic and immunological systems[6].

Zusammenfassung. Cyclophosphamid in Einzeldosis an neugeborene Mäuse verabreicht, bewirkt Missbildungen von Kopf und Vorderextremitäten sowie Geburtshindernis.

H. Nordlinder

University of Uppsala, Institute of Pathology, Box 553, S-751 22 Uppsala (Sweden), 24 July 1969.

[1] U. Stenram and H. Nordlinder, Nature *219*, 1154 (1968).
[2] U. Stenram, R. Berg and E. Nilsson, Z. Krebsforsch. *72*, 119 (1969).
[3] U. Stenram, Experientia, in press (1969).
[4] H.-J. Holweg, Monatschr. Kinderheilk. *111*, 307 (1963).
[5] M. W. Moncrieff, R. H. H. White, C. S. Ogg and J. S. Cameron, Br. med. J. *1*, 666 (1969).
[6] This work was supported by grants from the Swedish Medical Research Council (Project No. K69-12X-623-05), the Swedish Cancer Society (Project No. 95-K69-03X) and AB Pharmacia, Sweden.