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ADRENALINE, NORADRENALINE AND DOPAMINE LEVELS IN BRAIN AND HEART AFTER ADMINISTRATION OF 6-HYDROXYDOPAMINE AND GUANETHEDINE TO NEWBORN MICE

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Abstract— The adrenaline, noradrenaline and dopamine levels of the whole brain and heart of mice were measured spectrofluorimetrically after subcutaneous injection at birth with 6-hydroxydopamine or guanethedine. 6-Hydroxydopamine decreased noradrenaline and dopamine levels in brain tissue within 15 days after administration. Dopamine recovered within 45 days to an almost normal level. Both noradrenaline and dopamine decreased initially after guanethedine treatment, but differences from control values were insignificant 7 months after treatment. Dopamine and noradrenaline levels in heart decreased after both 6-hydroxydopamine and guanethedine treatment. The effect on adrenaline levels in brain and heart was statistically insignificant. It is suggested that 6-hydroxydopamine and guanethedine may cause long-lasting lesions in some catecholamine containing neurons in the central nervous system.

ELECTRON microscopic, fluorescence microscope, biochemical and pharmological studies[1,2] show that 6-hydroxydopamine can provoke selective destruction of peripheral adrenergic nerve terminals when administered parenterally. Brain catecholamines are not altered by 6-hydroxydopamine unless the blood brain barrier is bypassed by direct intraventricular or intracisternal injection.[3,4] Selective and permanent destruction of the peripheral sympathetic system is obtained when 6-hydroxydopamine is given to newborn animals.[5] Under these experimental conditions all the pre- and paravertebral ganglia are reduced to small sclerotic nodules almost completely deprived of nerve-cells. In these animals the noradrenaline content of peripheral organs is drastically reduced while in the brain only moderate reduction is observed.[6]

Recently it has been shown that selective lesions of the sympathetic cells can be induced also by guanethedine, [2-(hexahydro-1(2H)-azocinyl)ethyl]guanidine, both in newborn and in adult animals.[7,8]

The purpose of this study was to investigate the time course of depletion and eventual recovery of adrenaline, noradrenaline and dopamine in brain and heart after subcutaneous administration of 6-hydroxydopamine and guanethedine to newborn mice.

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MATERIALS AND METHODS

Swiss Webster mice were injected subcutaneously 5 days after birth with 50 mg 6-hydroxydopamine/kg body wt or with 50 mg guanethedine/kg. The injections were repeated on the following 5 days. The compounds were dissolved in saline solution containing 0·1% (w/v) of ascorbic acid. Control animals received only the diluent.

The animals were sacrificed by cervical dislocation at various time intervals. Brain and heart were excised, weighed, and then homogenized in 6 ml 0·4 M perchloric acid. After standing overnight at 4° the homogenate was centrifuged twice at 5000 *g*. The supernatant was adjusted to pH 6 by dropwise addition of 4 N NaOH. The whole supernatant was applied on an Amberlite type GC120 column (2·0 cm long × 0·4 cm dia.).[9] Adrenaline and noradrenaline were eluted with 10 ml of 1 N HCl, and dopamine with 9 ml of 2 N HCl.[3]

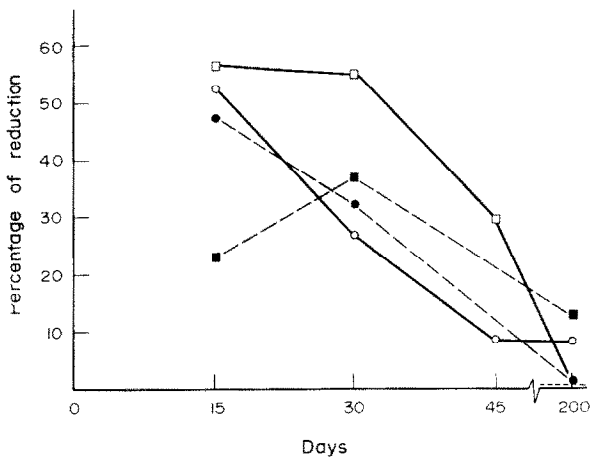

FIG. 1. Time course of per cent recovery of catecholamines in mouse brain after treatment at birth with 6-hydroxydopamine or guanethedine. (□) Noradrenaline after 6-hydroxydopamine treatment; (■) noradrenaline after guanethedine treatment; (○) dopamine after 6-hydroxydopamine treatment; (●) dopamine after guanethedine treatment.

Adrenaline and noradrenaline were measured spectrofluorimetrically by the THI-method[10] and dopamine by the iodine oxidation method.[11] Reproducibility of the methods was assessed carrying out the whole procedure with known amounts of adrenaline, noradrenaline and dopamine. Within a range of 15 per cent all starting material was recovered. No correction for loss of material has been carried out.

6-Hydroxydopamine.HCl was obtained from Aldrich Chemical Co. Inc., Milwaukee, and guanethedine.sulphate from Ciba SpA, Milan.

Statistical analyses were performed by the Student's *t*-test.

RESULTS

There was a significant reduction of whole brain noradrenaline content 15 days after treatment at birth with 6-hydroxydopamine (Table 1). The reduction persisted for a further 15 days. A 50 per cent reduction of whole brain dopamine content was observed 15 days after 6-hydroxydopamine treatment, but it recovered to no more than 70 per cent of the normal value after 30 days of treatment. After 45 days the

TABLE 1. NORADRENALINE AND DOPAMINE CONTENT OF MOUSE BRAIN AFTER TREATMENT AT BIRTH WITH 6-HYDROXYDOPAMINE OR GUANETHEDINE

Days		Dopamine (μg/g)	Noradrenaline (μg/g)	No. of experiments
15	Control	0·64 ± 0·06	0·39 ± 0·06	8
	6-OHDA	0·30 ± 0·04*	0·17 ± 0·05§	6
	Guan.	0·35 ± 0·05*	0·30 ± 0·02*	7
30	Control	0·55 ± 0·04	0·32 ± 0·05	8
	6-OHDA	0·38 ± 0·05*	0·15 ± 0·02§	6
	Guan.	0·39 ± 0·05†	0·19 ± 0·02*	10
45	Control	0·64 ± 0·05	0·33 ± 0·06	6
	6-OHDA	0·58 ± 0·06‡	0·23 ± 0·03†	8
200	Control	0·68 ± 0·07	0·30 ± 0·03	8
	6-OHDA	0·60 ± 0·03‡	0·33 ± 0·05‡	6
	Guan.	0·68 ± 0·04‡	0·25 ± 0·05‡	6

Figures represent the mean value (± standard error) of micrograms of catecholamine per gram of fresh tissue. Student's *t*-test. P-values significance. * ⩽ 0·05, † ⩽ 0·01, ‡ insignificant, § ⩽ 0·001. 6-OHDA, 6-hydroxy-dopamine; guan., guanethedine.

reduction of noradrenaline was much less pronounced and the reduction of dopamine, although still present was not statistically significant. Guanethedine had less effect on whole brain amine content during the 30-day period than 6-hydroxydopamine. The reduction of noradrenaline increased from 25 to 35 per cent between the 15th and 30th day, whereas dopamine reduction diminished from 45 to 30 per cent under the same experimental conditions. The effects of the two drugs on dopamine and noradrenaline levels were insignificant 200 days after treatment.

Noradrenaline levels in heart were reduced by 40 per cent (Table 2) both at 15 and 30 days after 6-hydroxydopamine treatment, and this reduction persisted until

TABLE 2. NORADRENALINE AND DOPAMINE CONTENT OF MOUSE HEART AFTER TREATMENT AT BIRTH WITH 6-HYDROXYDOPAMINE OR GUANETHEDINE

Days		Dopamine (μg/g)	Noradrenaline (μg/g)	No. of experiments
15	Control	1·00 ± 0·12	0·69 ± 0·09	6
	6-OHDA	0·75 ± 0·10*	0·40 ± 0·05*	6
	Guan.	0·53 ± 0·07§	0·27 ± 0·04§	8
30	Control	1·01 ± 0·09	0·66 ± 0·07	6
	6-OHDA	0·57 ± 0·04*	0·41 ± 0·07*	6
	Guan.	0·58 ± 0·05*	0·45 ± 0·04*	8
45	Control	1·01 ± 0·08	0·65 ± 0·05	4
	6-OHDA	0·59 ± 0·05§	0·50 ± 0·06†	8
200	Control	1·04 ± 0·09	0·58 ± 0·04	8
	6-OHDA	0·76 ± 0·09†	0·28 ± 0·03†	6
	Guan.	0·92 ± 0·08‡	0·35 ± 0·06§	6

Figures represent the mean value (± standard error) of micrograms of catecholamine per gram of fresh tissue. Student's *t*-test. P-values, significance. * ⩽ 0·05, † ⩽ 0·01, ‡ insignificant, § ⩽ 0·001. 6-OHDA, 6-hydroxydopamine; guan., guanethedine.

200 days after treatment. Dopamine reduction continued between the 15th and 30th day, but a moderate recovery was observed after 200 days. Guanethedine injections at birth caused a 60 per cent reduction of noradrenaline after 15 days, which was reduced to 30 per cent 30 days after treatment. Dopamine was reduced initially by 40 per cent, but after 200 days the difference was insignificant.

Adrenaline levels in whole brain of control animals were 0·04 μg/g wet tissue and in heart 0·05 μg/g. No significant changes between experimental and control animals were observed.

In all cases the drug effect on catecholamine levels was measured on separate groups of male and female mice. There were no significant differences between the sexes.

DISCUSSION

The results reported above indicate that some impairment of catecholamine neurons in the central nervous system is induced when large doses of 6-hydroxydopamine and guanethedine are given to newborn mice. In adult animals neither of these drugs, given parenterally, is able to cross the blood–brain barrier. In newborn animals, however, the blood–brain barrier may not yet be completely developed and may allow at least a partial access of the drugs to the brain compartment. Under these experimental conditions treatment with 6-hydroxydopamine caused a significant and long-lasting reduction of brain biologenic amines; it is of interest to note that while the noradrenaline content only partially recovered with time, dopamine levels gradually recovered and returned to almost normal values after a 7-week period. This might be explained, by a differential action of 6-hydroxydopamine on catecholamine neurons in the central nervous system, the dopaminergic cells being less vulnerable to the effect of the drug. A similar trend was observed when large doses of guanethedine were administered to newborn mice. Brain catecholamines were significantly reduced after treatment; dopamine levels, however, recovered faster than noradrenaline levels. However, these data do not allow us to assess to what extent any specific catecholamine centre has been damaged by the two drugs.

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