REGIONAL CHANGES IN BRAIN CATECHOLAMINE CONTENT FOLLOWING ADMINISTRATION OF GUANETHIDINE TO NEONATAL RATS

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Abstract—Norepinephrine(NE), dopamine(DA) and serotonin(5-hydroxytryptamine, 5-HT) contents were estimated in the different regions of the developing rat brain following guanethidine injection at the neonatal period for the purpose of determining the influence of guanethidine on development of the monoamine neuron in the brain. Despite a nonsignificant change in the weights of all regions of the brain, guanethidine caused a significant reduction of NE content in the limbic-striatum at day 7 and 30 and an increase in mesencephalon-ponsmedulla at day 30. DA concentration in the limbic-striatum at day 7, 14 and 30 and in the neocortex at day 7 showed a decrease with guanethidine treatment. The change in 5-HT content was not induced with guanethidine in all regions and all days examined. These results suggest that guanethidine, crossing the blood-brain barrier at the neonatal stage, induced the degeneration of the nerve terminals and 'collateral accumulation' of catecholamines in the central NE and DA neurons.

It has been introduced and proposed that 6-hydroxydopamine (6-OHDA) (1) and guanethidine (2) are valuable tools for selective sympathectomy. There are several reports concerning guanethidine-induced lesion on the sympathetic nerve (3-5) and the cytotoxic effect of guanethidine on the cultural sympathetic chain ganglia (6). Recently, Liuzzi et al. (7) found that guanethidine injected into neonatal mice induced the reduction of norepinephrine(NE) and dopamine (DA) contents in the whole brain as well as in the heart. Although many workers have reported in detail of the degenerative action of 6-OHDA or 6-hydroxydopa on the monoamine neurons in the rat brain following the injection to the neonatal animals (8-12), there are few reports on the regional influence of guanethidine on monoamine content in the brain. The present study was designed to examine the regional changes of NE, DA and serotonin (5-hydroxytryptamine, 5-HT) contents and of the monoaminergic innervation in the developing brain following injection of guanethidine into neonatal rats.

MATERIALS AND METHODS

Treatment of animals with guanethidine

Wistar strain rats of both sexes were used. The neonatal rats from the same litter were separated into treated and control groups and were given guanethidine sulphate s.c. within 10 hr after birth. A dose of 50 mg/Kg (0.1 ml/10 g of body weight) of the drug, being dissolved in the saline containing 0.1% ascorbic acid, was injected. The administration

was repeated at days 2, 4 and 6 after birth. Control animals were injected only with the vehicle according to the same schedule. Treated and control animals were maintained in a room temp. of 23 C and kept under normal daylight conditions. The weaning of the pups was carried out at 21 days after birth. At the age of 7, 14 and 30 days, animals were sacrificed by decapitation and the wet weights and the monoamine contents of the brain samples were estimated.

Preparation of each region of the brain

Regional dissection was carried out by the method of Nomura et al. (13). The regions analyzed were neocortex, limbic system-striatum (limbic-striatum), diencephalon, mesencephalon-pons-medulla (lower brain stem) and cerebellum. Pineal gland and meninges were discarded. The neocortex consisted of the cerebral cortex except for hippocampus, amygdala and septum. The limbic-striatum contained olfactory bulb, hippocampus, amygdala, striatum and septum. The diencephalon was separated from remaining brain stem at the anterior border of superior colliculus and the posterior border of optic chiasm. The cerebellum was isolated by cutting its peduncular connection with the brain stem. The lower brain stem was separated from the spinal cord by cutting under the obex area.

Control and treated rats at the various days after birth were sacrificed by decapitation between 10:00 a.m. and 3:00 p.m.. Dissections were performed over ice. The brain samples were washed with the cold saline, weighed and homogenized in acidified n-butanol for amine determination.

Estimation of NE, DA and 5-HT

NE and DA were extracted by the techniques of Maickel et al. (14). Assay of both amines was carried out spectrophotofluorometrically by the trihydroxyindole method of Chang (15). 5-HT was extracted and determined spectrophotofluorometrically by the method of Curzon and Green (16).

Materials used were guanethidine sulphate (Ismelin, Ciba), L-noradrenaline, dopamine hydrochloride and serotonin creatinine sulphate (Nakarai Chemicals Ltd.).

RESULTS

Lethal effect and the influence of guanethidine on the body weight and the regional brain weight

When guanethidine (50 mg/Kg, s.c.) was injected into the neonatal rats at 10 hr after birth and followed by 4 consecutive doses every 24 hr, 57% of the rats died in the first week. Injection of guanethidine (50 mg/Kg, s.c.) at day 0, 2, 4 and 6, used in the present study, was however, lethal to less than 12% of the animals.

At the age of 7, 14 and 30 days after birth, the body weights were lower in the treated group than in control group. Animals which died following this treatment revealed such syndromes as the decrease of motor activity and an abnormal posture before death. The weight of each region of the brain was compared between the control and treated groups. A trend of reduction, which was not significant, was observed in all regions of the treated animals (Table I).

TABLE 1. Influence of guanethidine on regional brain weight at 7, 14 and 30 days after birth

Treatment	Days after birth	Whole brain	Regional Neocortex	brain weight Limbic-striatum	(g) Diencephalon	Lower brain stem	Cerebellum
Control	7	0.642 ± 0.033 (6)	0.203 ± 0.011 (6)	0.218 ± 0.019 (6)	0.076 ± 0.005 (6)	0.107 ± 0.006 (6)	0.039 ± 0.006 (6)
	14	1.168 ± 0.096 (15)	0.367 ± 0.036 (15)	0.384 ± 0.034 (15)	0.120 ± 0.008 (15)	0.173 ± 0.012 (15)	0.118 ± 0.018 (15)
	30	1.480 ± 0.067 (12)	0.450 ± 0.024 (12)	0.474 ± 0.010 (12)	0.133 ± 0.010 (12)	0.235 ± 0.010 (12)	0.188 ± 0.009 (12)
Guanethidine	7	0.604 ± 0.038 (3)	0.197 ± 0.027 (3)	0.201 ± 0.002 (3)	0.069 ± 0.002 (3)	0.103 ± 0.003 (3)	0.034 ± 0.004 (3)
	14	1.064 ± 0.118 (9)	0.328 ± 0.032 (9)	0.360 ± 0.049 (9)	0.117 ± 0.013 (9)	0.158 ± 0.017 (9)	0.102 ± 0.018 (9)
	30	1.476 ± 0.062 (10)	0.433 ± 0.029 (10)	0.491 ± 0.032 (10)	0.135 ± 0.007 (10)	0.233 ± 0.009 (10)	0.184 ± 0.014 (10)

The results are expressed as the mean value $\pm$ S.E.M. with the number of experiments in parentheses.

Influence of guanethidine on regional NE content

At day 30, guanethidine induced a significant reduction of NE content in the limbic-striatum and its increase in the lower brain stem (Fig. 1). NE concentration in limbic-striatum of treated animals also decreased on days 7 and 14. On the contrary, guanethidine did not cause a significant alteration in NE content in such regions as the neocortex, the

FIG. 1. Influence of guanethidine on regional norepinephrine concentration in the brain at 7, 14 and 30 days after birth.

Rats were injected with guanethidine sulphate (50 mg/kg, s.c.) within 10 hr after birth and the injection was repeated on days 2, 4 and 6. Control animals were injected with the vehicle according to the same schedule as guanethidine treated group. The ordinate represents the endogenous norepinephrine content ($\mu\text{g/g}$ tissue) and the abscissa represents the age (days after birth) and the brain region (neocortex, limbic-striatum, diencephalon, lower brain stem and cerebellum). Each column (white; control, black; guanethidine treatment) is the mean value $\pm$ S.E.M. of 3–12 determinations. * Significantly different from control group, $P < 0.01$.

FIG. 2. Influence of guanethidine on regional dopamine concentration in the brain at 7, 14 and 30 days after birth.

Each column is the mean value $\pm$ S.E.M. of 4–10 determinations.

* Significantly different from control group, $P < 0.01$; ** $P < 0.05$.

Details as in Fig. 1.

diencephalon and the cerebellum.

Influence of guanethidine on regional DA content

In the limbic-striatum, DA content in the treated group was significantly lower than the level of the control group at all days examined (Fig. 2). DA content in the neocortex showed a tendency toward reduction with guanethidine treatment and the reduction at day 7 was significant. This same reduction occurred in the cerebellum. DA concentration in the diencephalon and the lower brain stem of the treated group tended to increase both on days 7 and 14. In all the regions, DA levels at day 30 were lower in the treated brain than in the control.

Influence of guanethidine on regional 5-HT content

With administration of guanethidine there was a tendency toward reduction of 5-HT content in both the limbic-striatum, the neocortex and the diencephalon but there was an increase in 5-HT level in the lower brain stem (Fig. 3). These changes were, however, not statistically significant.

FIG. 3. Influence of guanethidine on regional 5-hydroxytryptamine concentration in the brain at 7, 14 and 30 days after birth.

Each column is the mean value $\pm$ S.E.M. of 3–8 determinations.

Details as in Fig. 1.

DISCUSSION

Systemic injection of guanethidine into neonatal rats had a different influence on NE and DA contents according to different regions of the brain. The reduction of the endogenous NE and DA content in the limbic-striatum may be due to destruction of the nerve terminal of the catecholamine neuron. In fact, Hill et al. (6) observed that guanethidine induced a greater degeneration in the terminal than in the cell body in the preparation of the sympathetic ganglion culture. Liuzzi et al. (7) found that the decrease of NE and DA content in the whole brain as well as in the heart of the mice was induced with the injection of guanethidine at the neonatal stage. The present results, however, demonstrated not only the decrease of NE and DA contents in the limbic-striatum but also a significant increase of NE content in the lower brain stem on days 30 and a tendency toward increase of DA content in the diencephalon and the lower brain stem on days 7 and 14. Such evidence

indicates that guanethidine induces a greater degeneration of the nerve terminal than the cell body of the NE and DA neurons and that thereafter NE and DA are accumulated in the collateral from the intact axon which is proximal to the cell body. The reduction of NE and DA contents in the limbic-striatum and the increase of NE content on days 30 and DA content on days 7, 14 in the lower brain stem may be the result of this mechanism. Jonsson et al. (11) demonstrated histochemically 'collateral accumulation', proposed by Anden et al. (17), of NE in pons-medulla areas following the treatment of 6-OHDA at the neonatal period. The phenomenon of the amine accumulation in the collaterals has been established and maximal accumulation is attained within a few days after lesion and continues for several weeks (18). Although the change in regional catecholamine content induced with guanethidine had a low statistical significance, the present results suggest that guanethidine induces 'collateral accumulation' which probably occurs in the neighbourhood of locus coeruleus (NE) and of substantia nigra (DA). Why there was a significant reduction of DA in the lower brain stem at day 30 when guanethidine was administered, is the subject of future papers.

5-HT content was not significantly influenced with guanethidine. It has been reported that the terminal was more susceptible to guanethidine than the cell body and that this might be due to a greater uptake of guanethidine in the terminal than in the cell body (6). Guanethidine, either not at all or only slightly taken up into the terminal of the 5-HT neuron, could not be expected to induce a degeneration of the 5-HT neuron but rather the selective degeneration of the NE and DA neurons in the developing brain.

The present results show that guanethidine injected into the neonatal rat causes a significant change in regional NE and DA contents in the brain. This degeneration and 'collateral accumulation' in the central catecholamine neuron caused with guanethidine are weaker than that with 6-hydroxydopa and guanethidine has a more lethal effect than 6-hydroxydopa (12).

Regarding the mechanism of the toxic action of guanethidine, it has been reported that this compound inhibits oxidative phosphorylation in isolated mitochondria (19) and induces structural damage to the mitochondria in the adrenergic neuron (2, 20, 21). That such a toxic action to the adrenergic neuron of guanethidine may induce degeneration and 'collateral accumulation' in the central catecholamine neurons should also be taken into consideration.

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