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Possible Involvement of Brainstem Norepinephrine in Pentylenetetrazol Convulsions in Rats

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OISHI, R., N. SUENAGA AND T. FUKUDA. *Possible involvement of brainstem norepinephrine in pentylenetetrazol convulsion in rats.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 57–61, 1979.—The thresholds of twitch and clonic convulsion induced by intravenous infusion of pentylenetetrazol (PTZ) were measured in rats treated with 6-hydroxydopamine (6-OHDA). In adult rats, intraventricularly applied 6-OHDA increased susceptibility to PTZ convulsions and decreased norepinephrine (NE) contents of the cortex, hypothalamus and brainstem. When 6-OHDA was applied intraventricularly at 8 days after birth, PTZ convulsion susceptibility was slightly decreased and brainstem NE content was significantly increased. However, the effects of 6-OHDA given at 20 days after birth were similar to those observed in adults. Significant decrease of cortical and hypothalamic NE contents, but no change in PTZ convulsion susceptibility, occurred following 6-OHDA injections into the dorsal and ventral NE bundles. These results suggest that the brainstem NE neurons play an inhibitory role on the development of PTZ convulsions.

Catecholamines Convulsions 6-Hydroxydopamine Pentylenetetrazol

REDUCTION of brain monoamines by reserpine or tetrabenazine increases the susceptibility of mice and rats to pentylenetetrazol (PTZ) convulsions [6,18], and Kilian and Frey [15] and Doteuchi and Costa [11] proposed that norepinephrine (NE) plays an essential role in these phenomena. Furthermore, Corcoran *et al.* [8] found that 6-hydroxydopamine (6-OHDA) lowered the threshold of PTZ convulsions in rats. These findings suggest that reduction of brain catecholamines is involved in the enhancement of PTZ convulsions, although serotonin also contributes to the regulation of PTZ convulsions [9, 17, 25]. The present study was carried out to examine the correlation between the susceptibility to the PTZ convulsions and changes of NE contents of specific brain regions.

Injected into the dorsal and ventral NE bundle (DB and VB, respectively), 6-OHDA produces relatively selective reduction of forebrain NE content [2, 20, 24]; the susceptibility to PTZ convulsions was examined in rats with localized DB or VB injections of 6-OHDA.

EXPERIMENT 1

METHOD

Animals

The animals were male Wistar King A rats supplied by Kyushu University Institute of Laboratory Animals, weighing 200-300 g at the time of PTZ challenge. The rats were housed in groups of 4–6 and given food (Oriental rat chow) and water ad lib. The colony room was maintained on a 12 hr light/dark cycle, and the room temperature was controlled at 22 ± 1°C.

Surgery

The rats were anesthetized with ether and placed on a stereotaxic instrument. After a suitable hole was made in the skull and the dura cut, an injection cannula made of stainless steel tube with 0.4 mm outer diameter was inserted into DB (A: 1.3, L: 0.7, H: −0.7) or VB (A: 0.5, L: 1.6, H: −2.1) according to the stereotaxic atlas of König and Klippel [16] and the histochemical map of Ungerstedt [30]. 6-OHDA (1 μg in 1 μl of 0.9% saline–0.1% ascorbic acid/side) was infused bilaterally at a constant rate of 1 μl/min. In the case of sham operated animals, the rats were injected into bilateral DB with vehicle (1 μl of 0.9% saline–0.1% ascorbic acid/side).

PTZ Convulsions Test

The test was carried out 20 days after surgery. The test rat was briefly restrained while a 5% solution of PTZ was infused into a lateral tail vein at a constant rate of 0.004 ml/sec. Infusion was performed by the syringe pump model 975 (Harvard Apparatus). Duration of infusion required to elicit

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the initial twitch and clonic convulsions were recorded. Twitch and clonic convulsions thresholds were expressed as the mean ± SEM dose of PTZ per kg of body weight.

After the test, each rat was immediately injected with diazepam (5 mg/kg, IV) in order to protect against death from overdose of PTZ.

Biochemical Determination

The rats were sacrificed by decapitation 10 days after PTZ convulsions. The brain was immediately removed and divided on ice into 3 parts, that is the cortex, hypothalamus and brainstem, according to the procedure of Glowinski and Iversen [13]. The tissue contents of NE were determined fluorometrically as described by Shellenberger and Gordon [27].

Statistical Evaluations

Statistical evaluations of the convulsions thresholds and catecholamine contents were performed using two-tailed Student's *t*-test.

RESULTS

Effective intravenous infusion of PTZ induced the following sequence of responses: following initial few twitches, the generalized clonic seizures and loss of righting reflex ensued, leading then to the myoclonic jerk followed by a tonic extension of the forepaws; finally a clonic convulsion developed.

As shown in Fig. 1, 6-OHDA injected into DB and VB did not cause significant effect on twitch and clonic convulsion thresholds. Table 1 shows the NE contents of the brain in three groups. DB and VB injections of 6-OHDA caused marked decrease of cortical NE; the degree of reduction was greater with DB than with VB lesion. Reduction of NE content occurred also in the hypothalamus; in this case, the reduction was greater with VB than DB lesion. NE content of the brainstem was not significantly affected by either DB or VB lesion.

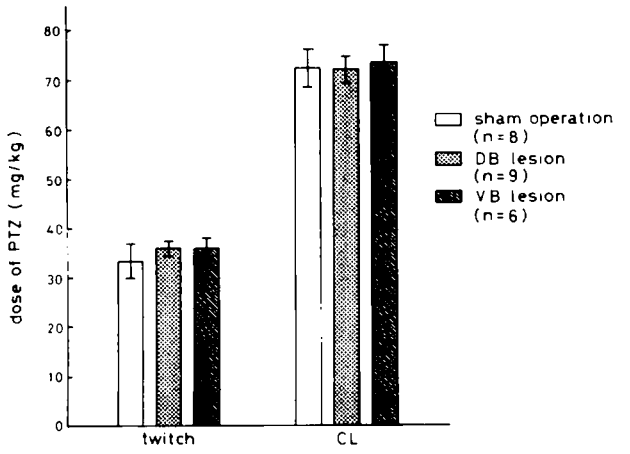

FIG. 1. Effects of bilateral chemical lesions of dorsal or ventral bundle on the thresholds of twitch and clonic convulsion induced by pentylenetetrazol infusion. 6-OHDA (1 μg/side) was injected 20 days before the test. Each threshold is expressed as the mean ± SEM dose of pentylenetetrazol per kg of body weight.

TABLE 1

BRAIN NOREPINEPHRINE CONTENTS IN THE DORSAL AND VENTRAL BUNDLE LESIONED RATS

Regions	Groups		
	Sham Operation	DB Lesion	VB Lesion
Cortex	0.25 ± 0.01‡	0.04 ± 0.01†	0.16 ± 0.01†
Hypothalamus	1.43 ± 0.05	0.90 ± 0.20*	0.44 ± 0.05†
Brainstem	0.47 ± 0.03	0.49 ± 0.02	0.48 ± 0.02

*$p<0.05$; †$p<0.001$, compared with sham operated group.
‡Each value is the mean content (μg/g) ± SEM of 6 or 7 animals.

DISCUSSION

Applied to DB and VB, 6-OHDA caused marked reduction of cortical and hypothalamic NE contents without affecting the threshold of PTZ convulsions. These results suggest that the enhancement by 6-OHDA of susceptibility to PTZ convulsions is not due to the reduction of NE contents of the regions innervated from DB or VB such as the cortex, hypothalamus, limbic area and other forebrain regions.

Several investigators have used 8 μg of 6-OHDA to produce DB lesion [3,21]. While 6-OHDA is a relatively selective neurotoxin with respect to catecholaminergic neurons, many investigators found that 6-OHDA produces significant changes in other systems [1, 5, 14, 23]. To minimize the nonspecific damage, very small doses of 6-OHDA (1 μg/side) were injected into DB and VB; yet, there was a marked decrease in cortical and hypothalamic NE content, indicating that 1 μg of 6-OHDA is enough to produce DB or VB lesions.

In this experiment, DB application of 6-OHDA caused a significant decrease in hypothalamic NE content. Electrical DB lesion did not cause significant decrease in hypothalamic NE content although it decreased markedly cortical NE content [22]. The reduction of the hypothalamic NE by DB application of 6-OHDA in the present experiment may be due to the diffusion of 6-OHDA to VB. This diffusion may also explain the reduction of cortical NE upon VB application of 6-OHDA, as cortical NE has DB as its major source [19, 26, 29, 30].

EXPERIMENT 2

The purpose of this experiment was to clarify whether intraventricular application of 6-OHDA produces an increase in the susceptibility of rat to PTZ convulsions in the present method.

METHOD

The animals were male Wistar King A strain supplied by Kyushu University Institute of Laboratory Animals, weighing 200–300 g at the time of PTZ challenge. 6-OHDA (150 μg in 10 μl of vehicle) was injected intraventricularly twice at 3 days interval; PTZ challenge was performed 20 days after the first treatment. Ten days after PTZ convulsions, the rats were sacrificed by decapitation and the brains were immediately removed and divided on ice into 4 parts, that is the cortex, hypothalamus, brainstem and striatum. The tissue contents of NE and dopamine (DA) were determined fluorometrically [27].

RESULTS AND DISCUSSION

As shown in Fig. 2, the threshold amounts of PTZ needed to elicit twitch in vehicle and 6-OHDA treated groups were 32.7 ± 1.9 and 27.6 ± 0.9 mg/kg, respectively; those needed to induce clonic convulsion were 73.9 ± 3.2 and 58.9 ± 2.0 mg/kg, respectively. 6-OHDA induced statistically significant effect on both twitch and clonic convulsion threshold ($p<0.05$ and $p<0.01$, respectively). Table 2 shows the effect of 6-OHDA treatment on catecholamine content of the brain. NE contents of the cortex, hypothalamus and brainstem, and DA content of the striatum were significantly decreased by the 6-OHDA treatment.

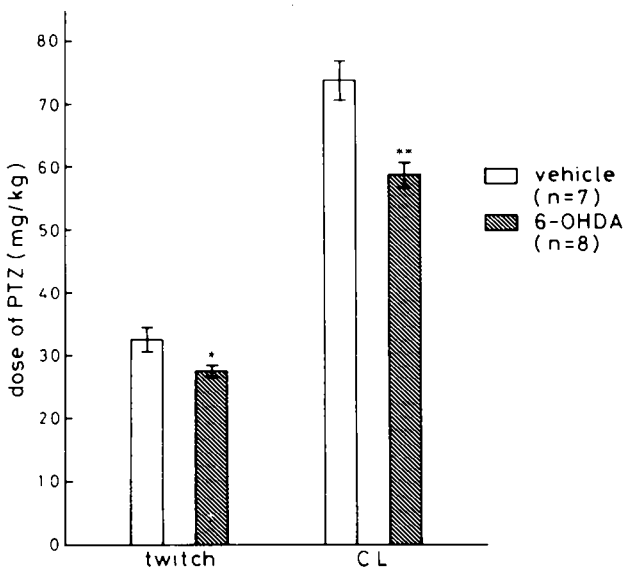

FIG. 2. Effects of intraventricularly applied 6-OHDA on the thresholds of twitch and clonic convulsion induced by pentylenetetrazol infusion. 6-OHDA (150 μg×2) was injected twice at 3 day intervals 20 days before the test. Each threshold is expressed as the mean ± SEM dose of pentylenetetrazol per kg of body weight. *$p<0.05$; **$p<0.01$, compared with the vehicle group.

TABLE 2

BRAIN CATECHOLAMINE CONTENTS IN THE RATS TREATED WITH 6-OHDA (150 μg×2)

Regions	Groups	
	Vehicle	6-OHDA
Norepinephrine		
Cortex	0.26 ± 0.02‡	0.09 ± 0.02†
Hypothalamus	1.44 ± 0.11	0.81 ± 0.04†
Brainstem	0.40 ± 0.03	0.17 ± 0.01†
Dopamine		
Striatum	12.45 ± 0.96	8.38 ± 0.70*

*$p<0.01$; †$p<0.001$, compared with the vehicle group.
‡Each value is the mean content (μg/g) ± SEM of 7 animals.

In adult rats, intraventricularly applied 6-OHDA caused an enhancement of susceptibility to PTZ convulsions accompanied by marked reduction of NE and DA contents of the brain, in agreement with the findings of Corcoran *et al.* [8].

EXPERIMENT 3

In adult rats, intraventricularly applied 6-OHDA reduced catecholamine contents of all brain regions. However, when 6-OHDA was administered intraventricularly during the early suckling age, the brainstem NE was usually increased, while the telencephalic NE was decreased [10, 12, 28, 31]; the increase of brainstem NE occurred with 6-OHDOPA treatment till the 9th day after birth [32]. As the treatment with 6-OHDA during neonatal period stunted growth [4,7], the susceptibility to PTZ convulsions was examined in the rats treated with 6-OHDA at 8 days after birth, in an attempt to increase brainstem NE content without producing a disturbance of growth; the results were compared with those carried out in rats treated intraventricularly with 6-OHDA at 20 days after birth.

METHOD

Male Wistar King A rats bred in our laboratory were used in this experiment. 6-OHDA (100 μg in 5 μl of vehicle) was injected intraventricularly twice at 3 day intervals, beginning at either 8 or 20 days after birth. A microsyringe was inserted 3.5 mm deep into the brain via the scalp at a position 4 mm rostral to the lambda, 1 mm lateral to the midline. The rats were weaned 30 days after birth. Then they were housed in groups of 4–6 and permitted free access to food and water. PTZ challenge was carried out 14–18 weeks after birth, when body weight of the rats was 200–250 g. Ten days after PTZ convulsions, the rats were sacrificed by decapitation and the removed brain was divided into 6 parts, that is the cortex, hypothalamus, midbrain, brainstem, cerebellum and striatum, according to the procedure of Glowinski and Iversen [13]. In addition the spinal cord from T1 to T12 was dissected out. The tissue contents of NE and DA were determined fluorometrically.

RESULTS

PTZ convulsions were tested in the rats treated with 6-OHDA at 8 days (6-OHDA(8) group) and at 20 days after birth (6-OHDA(20) group). As shown in Fig. 3, the threshold amount of PTZ needed to elicit twitch in 6-OHDA(8) group showed a statistically significant elevation as compared to the control group (37.1 ± 1.1 and 33.3 ± 1.1 mg/kg, respectively, $p<0.05$); however, the threshold of the 6-OHDA(20) group was not affected (34.2 ± 1.2 mg/kg). On the other hand, the clonic convulsion threshold was significantly reduced as compared to the control group in the 6-OHDA(20) group (73.9 ± 3.2 and 64.1 ± 2.1 mg/kg, respectively, $p<0.01$), but not in the 6-OHDA(8) group (75.9 ± 2.5 mg/kg).

Table 3 shows the effect of 6-OHDA administered at either 8 or 20 days after birth on catecholamine contents of various brain regions. The 6-OHDA(8) group showed marked decreases in NE contents of the cortex, hypothalamus, cerebellum and spinal cord, and DA content of the striatum. To the contrary, brainstem NE content was significantly increased, and midbrain NE content not af-

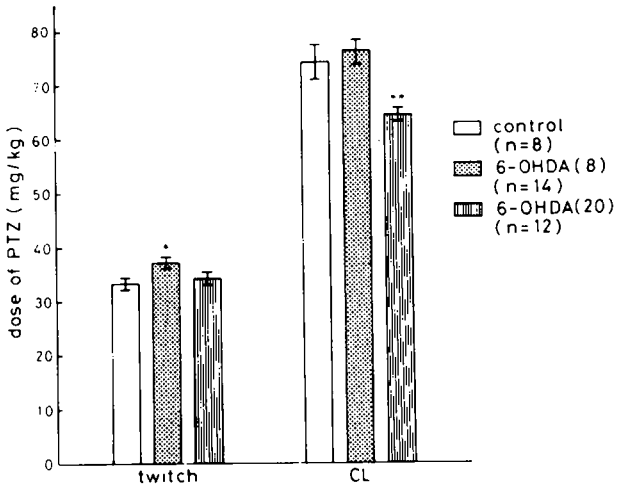

FIG. 3. Effects of intraventricularly applied 6-OHDA at suckling age on the thresholds of twitch and clonic convulsion induced by pentylenetetrazol infusion. 6-OHDA (100 μg × 2) was injected at either 8 or 20 days after birth twice at 3 day intervals. The test was performed 14–18 weeks after birth. Each threshold is expressed as the mean ± SEM dose of pentylenetetrazol per kg of body weight. $*p<0.05$; $**p<0.01$, compared with control.

fected significantly. On the other hand, the 6-OHDA(20) group showed marked decreases of NE contents of all regions, and the DA content of the striatum.

DISCUSSION

6-OHDA treatment at 20 days after birth produced both reduction of the threshold of PTZ clonic convulsions and decrease in NE contents of all regions; these results are similar to those obtained with 6-OHDA treatment in adult rats. In contrast, 6-OHDA treatment at 8 days after birth resulted in elevation of twitch threshold and increase in brainstem NE; NE contents of all other regions were decreased. These results suggest that decrease in brainstem NE is related to the enhancement of PTZ convulsion susceptibility. Not consistent with this explanation was that there was no change in twitch threshold of the 6-OHDA(20) group. However, this group exhibited lesser decrease in brainstem NE content than 6-OHDA treated adults (57.5% decrease in adults and 20.8% decrease in 6-OHDA(20) group).

TABLE 3

BRAIN CATECHOLAMINE CONTENTS IN THE RATS TREATED WITH 6-OHDA (100 μg×2) AT SUCKLING AGE

Regions	Groups		
	Control	6-OHDA (8)‡	6-OHDA (20)‡
Norepinephrine			
Cortex	0.20 ± 0.01§	0.00 ± 0.00†	0.07 ± 0.02†
Hypothalamus	1.54 ± 0.10	0.30 ± 0.02†	0.78 ± 0.06†
Midbrain	0.49 ± 0.01	0.40 ± 0.05	0.21 ±0.03†
Brainstem	0.48 ± 0.02	0.66 ± 0.03†	0.38 ± 0.04*
Cerebellum	0.16 ± 0.01	0.05 ± 0.01†	0.01 ± 0.01†
Spinal cord (T1-T12)	0.24 ± 0.02	0.02 ± 0.00†	0.02 ± 0.01†
Dopamine			
Striatum	10.03 ± 0.50	1.91 ± 0.73†	7.45 ± 0.67*

$*p<0.05$; $†p<0.001$, compared with control group.

‡6-OHDA was injected intraventricularly at 8 or 20 days after birth twice at 3 day intervals.

§Each value is the mean content (μg/g) ± SEM of 6 or 7 animals.

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