Mechanism of *p*-Chlorophenylalanine-Mediated Increase in Seizure Susceptibility: Inhibition by Cerebellar Ablation (36206)

LENORE M. KOPELOFF AND GEORGE J. ALEXANDER

Division of Neuroscience, New York State Psychiatric Institute, New York, New York 10032; and Neurotoxicology Research Unit, Bronx State Hospital, Bronx, New York 10461

Recent interest in the effect of *p*-chlorophenylalanine (*p*-CPA) on serotonin metabolism in structures located near and functionally connected with the cerebellum, such as the raphe neurons and the pineal, has prompted us to investigate whether prior removal of cerebellum would influence the enhancing effect of *p*-CPA on convulsive seizures.

We have recently demonstrated that *p*-chlorophenylalanine, a selective depletor of brain serotonin (5-HT), increased susceptibility to seizure in normal rats treated with the convulsant pentamethylenetetrazol (Metrazol) (1). Similar effects have been obtained by others (2).

There is ample evidence of a relationship between cerebellar activity and resistance to convulsive seizures. Inactivation of selected areas by temporary freezing or permanent removal of cerebellum produced a marked incease in epileptiform manifestations following audiostimulation, photic stimulation, or administration of β-methyl-β-ethylglutarimide (Megimide) (3). An increase in spontaneous convulsions was observed in cerebellectomized rats (3), which had been previously rendered epileptic by cobalt powder application to frontal cortex (4). Conversely, convulsive seizures were inhibited in rats by electrical stimulation of certain areas of cerebellum or by stimulating effects of additional irritative cobalt lesion applied to the ipsilateral side of the cerebellum (5). An anticonvulsant, diphenylhydantoin, has been shown to stimulate the rate of discharge of cerebellar Purkinje cells in the cat (6).

To the best of our knowledge there is no study of the relationship between seizure susceptibility and cerebellar 5-HT metabolism. Cerebellum contains little 5-HT, while pineal gland is rich in 5-HT, and raphe neurons may be serotonin-dependent (7). *p*-CPA has a profound but puzzling effect on the 5-HT-like fluorescence of these structures. Ninhydrin fluorescence decreases in all areas of the brain but increases in the cerebellum following *p*-CPA administration (8). Raphe nuclei of animals treated with *p*-CPA and observed histochemically after exposure to paraldehyde increase in fluorescence which, however, may prove not to be due to serotonin (7).

Materials and Methods. Male Wistar rats, 250–300 g, purchased from Manor Research, Sloatsburg, NY, were maintained in individual cages on standard Teklad diet and water *ad libitum*. DL-*p*-Chlorophenylalanine (*p*-CPA) and serotonin creatinine sulfate were obtained from Mann Research (Schwarz Bio-Research, Orangeburg, NY). Pentamethylenetetrazol was purchased from Knoll Pharmaceutical Co., Orange, NJ.

Cerebellectomy was performed on 25 rats by aspiration under amobarbital sodium anesthesia (75 mg/kg, ip) as descrbed by Dow *et al.* (3) and Dow and Moruzzi (9). The skull was removed over the occipital region by electric drill and forceps, dura was split and cerebellum was ablated by gentle suction through a Pasteur pipette. Sham-operation included removal of skull over occipital region leaving dura intact. The extent of cerebellar removal was noted at autopsy.

Postoperatively, both groups of rats were injected subcutaneously with a nutrient solution (Ambex Eli Lilly, 3 ml/rat) and 5% dextrose in saline (10 ml, b.i.d.) for 10 days, in addition to standard diet. The sham-operated series was restricted in food and water intake for 1 month following operation to parallel the amounts consumed by the cerebellectomized rats. After a recovery period of 6–8 weeks, *p*-CPA, suspended in 5% Tween-20

TABLE I. Metrazol Threshold in Rats after Cerebellectomy and Treatment with *p*-Chlorophenylalanine (*p*-CPA).

Expt. no.	(weeks) After operation	Sham-operated				Cerebellectomized			
		No. of rats	*p*-CPA (mg/kg)	Metrazol (mg/kg)	Threshold change[a] (%)	No. of rats	*p*-CPA (mg/kg)	Metrazol (mg/kg)	Threshold change[a] (%)
1	Prior	10	—	58.8 ± 3.1		9	—	64.2 ± 4.2	
	4	10	—	53.4 ± 1.8	— 9.2	9	—	57.6 ± 3.7	—10.3
	6	10	300	44.4 ± 1.9	—16.9	9	300	50.0 ± 2.8	—13.2
	11	10	—	45.6 ± 3.6		9	—	48.9 ± 3.6	
	14	10	300	39.6 ± 1.7	—13.2	9	300	42.0 ± 2.4	—14.1
2	6	4	—	49.5 ± 3.3		6	—	45.0 ± 4.7	
	8	4	600	36.0 ± 2.8	—27.3	4	600	45.0 ± 4.5	0
	10	4	—	54.0 ± 4.9		4	—	48.0 ± 4.0	
	12	4	600	39.0 ± 2.0	—27.8	6	600	51.3 ± 6.8	+ 6.9

[a] Av net change based on immediately preceding value.

(120 mg/ml) as recommended by Weitzman *et al.* (8) was injected intraperitoneally into 18 cerebellectomized rats and 14 sham-operated controls. Brain serotonin assays were performed 8 weeks after cerebellectomy or sham operation and 48 hr following injection of *p*-CPA or vehicle. Brains were removed within 2–3 min after decapitation and serotonin was determined in a spectrophotofluorimeter (as a ninhydrin complex) (10) with excitation wavelength of 385 mμ (uncor.).

In the series in which seizure thresholds were measured, Metrazol was injected 48 hr after *p*-CPA or vehicle treatment by a procedure consisting of a series of intramuscular injections at 15-min intervals, beginning with 18 mg/kg of Metrazol (18 mg/ml) and followed by 6 mg/kg (6 mg/ml), as described previously (1). Seizure intensity was graded from mild (1+) to very strong (4+). Rats exhibiting very severe seizures were treated with amobarbital sodium (50 mg/kg, ip) to avoid fatalities. Mild seizures were usually clonic only and of very brief duration. Tonic–clonic seizures, not requiring medication, fell into intermediate categories. In Expt. 1, thresholds were determined prior to operation and again 4 weeks later to assess the effect of cerebellectomy on seizure threshold, then, *p*-CPA (300 mg/kg) was administered twice. In Expt. 2, 600 mg/kg of *p*-CPA were administered 8 weeks after operation and again 4 weeks later.

Results. Effects of cerebellectomy. After an initial weight loss of 30–50 g which occurred during the first week, there was satisfactory recovery and continued weight gain. Among the symptoms observed, typical of those reported elsewhere for cerebellectomized rats (9), were varying degrees of loss of balance, weakness of limbs and tail with occasional stiffness of hind legs and tail, and extreme difficulty in locomotion. These symptoms persisted for the duration of the studies, although a definite degree of adaptation was noted with time in all animals. They appeared otherwise healthy and continued to gain weight at the same rate as the sham-operated controls. At autopsy, the extent of cerebellar removal was found to have exceeded 75% in all animals, except for 2, in which 50% removal was noted. Gross examination of the pineal body failed to reveal any noticeable damage. No significant difference in Metrazol seizure threshold was observed as a result of cerebellectomy (Table I). A slight decrease in Metrazol threshold upon repeated testing was observed, which was consistent with decreases reported previously (1, 11). Removal of cerebellum led, however, to a sharp increase in the *intensity* of seizures: control rats exhibited only mild seizures at convulsive threshold level, while cerebellectomized rats under the same conditions suffered many strong or very strong seizure attacks (Fig. 1). Serotonin levels in the cerebral hemispheres were found to be the same before, and 8 weeks after, the operation

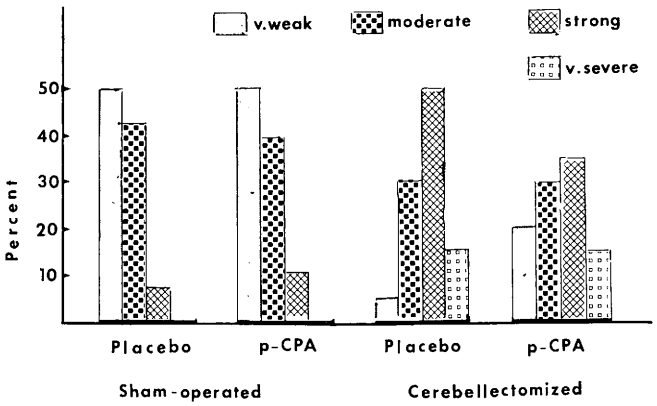

FIG. 1. Distribution of rats graded according to seizure intensity following Metrazol: Effect of cerebellectomy and *p*-CPA (300 mg/kg, 48 hr prior to Metrazol).

(Table II).

Effect of p-CPA (300 mg/kg). Treatment of sham-operated rats with 300 mg/kg of *p*-CPA led to a 16.9% decrease in Metrazol threshold, approximately 8% below the level expected as a result of repeated Metrazol testing (Table I, Expt. 2). A second treatment with the same dose led to a somewhat smaller decrease which was still greater, however, than expected from Metrazol alone.

Cerebellectomized rats tested after first treatment with 300 mg/kg of *p*-CPA showed a 13.2% drop in threshold value (3% below level without *p*-CPA), and after second treatment, a similar decrease of 14.1%. While none of the differences in response between the sham-operated and cerebellectomized rats after treatment with 300 mg/kg of *p*-CPA were statistically significant (*t* test), a slight trend was apparent towards a lessened efficacy of *p*-CPA treatment after cerebellectomy. Treatment with *p*-CPA did not affect seizure intensity in control rats but appeared to have a slight moderating effect in cerebellectomized animals. None of the sham-operated group given either placebo or 300 mg/kg of *p*-CPA responded with very strong seizures: 50% of each group showed weak activity (Fig. 1). Treatment of cerebellectomized rats with 300 mg/kg of *p*-CPA increased the percentage of weak convulsions.

Effect of p-CPA (600 mg/kg). In control rats, a marked decrease in seizure threshold to Metrazol (27.3%) was observed after initial treatment with 600 mg/kg of *p*-CPA (Table I, Expt. 2). In a subsequent trial, treatment

TABLE II. Brain Serotonin Levels of Rats after Cerebellectomy and *p*-CPA Treatment.[a]

Operation	Previous treatment	*p*-CPA (mg/kg)	No. of rats	Serotonin (μg/g)		
				Cerebrum	Cerebellum	Pineal
None	None	0	4	0.46 ± 0.02	0.15 ± 0.01	22.0 ± 2.2
	None	600	4	0.26 ± 0.03[b]	0.40 ± 0.02[b]	0
Cerebellectomy	None	0	3	0.45 ± 0.02	—	35.3 ± 9.0
	None	600	4	0.20 ± 0.03[b]	—	0
Sham	Expt. 2	0	5	—	—	20.8 ± 2.0
	Expt. 2	600	4	—	—	0
Cerebellectomy	Expt.2	0	4	—	—	15.5 ± 4.4

[a] Serotonin measured 48 hr after *p*-CPA treatment.
[b] $p < .05$.

with 600 mg/kg caused a similar decrease of 27.8%. In the cerebellectomized group, no net change in seizure threshold was produced by 600 mg/kg of *p*-CPA on initial trial, and a small rise following second treatment. The difference between sham-operated and cerebellectomized rats derived from the two tests with 600 mg/kg of *p*-CPA proved significant at the $p < .05$ level (t test.).

The serotonin fluorescence in cerebral hemispheres of control rats 48 hr following treatment with 600 mg/kg was decreased by 40%; with a complete disappearance of serotonin from the pineal gland (Table II). Results were similar in both sham-operated and cerebellectomized rats.

Discussion. Treatment of rats with *p*-chlorophenylalanine, a tyrosine analog, which blocks hydroxylation of phenylalanine and tryptophan, leads to a selective decrease in serotonin content of the brain (2, 12–14). Animals so treated become more susceptible to convulsive seizures induced by the chemical convulsant, Metrazol, averaging 25% decrease in threshold (1, 2). Other agents known to reduce brain serotonin also lower Metrazol thresholds (15). On the other hand, agents found to cause an increase in brain serotonin (5-hydroxytryptophan, alone or in combination with iproniazide), decrease susceptibility to seizures. These facts have led to a hypothesis ascribing to serotonin a protective function against seizures (15) and excitation in the brain (16). Our data show that *p*-CPA lowered convulsive threshold: less Metrazol was required to produce a seizure response. However, *p*-CPA did not appear to affect the intensity of seizures.

Although it is well known that the cerebellum has both facilitatory and inhibitory functions, complete or near-complete removal has indicated a predominance of inhibitory effects (3, 9). In animals rendered chronically epileptic by implantation of cobalt powder (4, 17, 18), convulsive seizures induced by Megimide, photic, or audiostimulation became more intense following either temporary inactivation of cerebellum by local freezing with dry ice or total cerebellectomy (3).

Levels of acetylcholine and serotonin are significantly lower in cerebellum than in other areas of the brain (10, 14, 15, 19). Cerebellar 5-HT content was found to decrease slightly in rats following *p*-CPA treatment when measured as a phthalate complex (14), but not with the ninhydrin procedure (10). *p*-CPA interfered with the latter assay, particularly when serotonin levels were low (20), inasmuch as ninhydrin adducts of *p*-CPA and 5-HT have similar fluorescence excitation and emission maxima. We found the intensity of the 5-HT complex to be approximately 400 times greater than that of the *p*-CPA complex. While presumably only a small portion of the *p*-CPA injected in our study penetrated the brain, most of which was removed during processing, sufficient amounts appear to have persisted to interfere with 5-HT assay. Serotonin-like fluorescence, which decreased in the pineal and in the cerebrum, rose in cerebellum from an average of 0.15 μg/g in our control rats to nearly 3 times that much in rats pretreated with 600 mg/kg of *p*-CPA 48 hr earlier. A similar increase was found by Weitzman *et al.* (8) in cerebellum of monkeys treated with *p*-CPA. Whether this increase was due to *p*-CPA, serotonin, or some other unidentified material remains to be established (7).

Our data on the serotonin content in the brain of cerebellectomized animals indicate that changes following *p*-CPA treatment are parallel both cerebellectomized and control groups. Although it is possible to relate the observed decrease in seizure threshold with the general decrease in brain serotonin, as observed in controls treated with *p*-CPA, the mechanism is more complex after cerebellectomy where the overall brain serotonin content decreased but seizure thresholds did not. One might postulate from these data that *p*-CPA exerted its effect on seizure activity primarily through the cerebellum or such structures as the raphe neurons, which are functionally related to the cerebellum. Other data indicate the possibility of *p*-CPA inhibition of cerebellar function. Prolonged treatment of dogs with *p*-CPA (100 mg/kg/day, orally) produced "an unusual oblique gait, tremors, and muscle tenseness reminiscent of certain syndromes in decerebellated dogs" (13). Cerebellum has neuronal connections with cerebral cortex as well as with sensory (visual, auditory) areas and controls input to the CNS through a form of feedback "loop"

system. It appears from our data that a similar cerebellar control mechanism regulates the intensity and duration of seizures, but has no influence on convulsive threshold levels. It is possible that some cerebellar inhibitory pathways are serotonin-dependent; *p*-CPA may inactivate these and thereby partially decrease cerebellar protective function. The fact that cerebellum is low in 5-HT need not, in itself, negate the above hypothesis. Effect on raphe neurons could also account for the results observed after cerebellectomy.

Summary. Cerebellectomy in rats produced no change in the convulsive threshold to pentamethylenetetrazol but a marked increase in the intensity and duration of seizures.

The effect of *p*-chlorophenylalanine, which lowered seizure thresholds to pentamethylenetetrazol in sham-operated animals, was abolished by cerebellectomy.

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