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Susceptibility to Seizures Produced by Chemical Convulsants and Maximal Electric Shock in Rats After Electrolytic Lesions Into the Red Nucleus

K. KOLASA,* Z. KLEINROK,* S. CONSOLO,† P. FERRARIO‡ AND L. ZECCA‡

**Department of Pharmacology, Medical School, Jaczewskiego 8, 20090 Lublin, Poland*
†Institute of Pharmacology "Mario Negri," Via Eritrea 62, 20157 Milan, Italy
‡CNR-ITBA, Via Ampere 56, 20131 Milan, Italy

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KOLASA, K., Z. KLEINROK, S. CONSOLO, P. FERRARIO AND L. ZECCA. *Susceptibility to seizures produced by chemical convulsants and maximal electric shock in rats after electrolytic lesions into the red nucleus.* PHARMACOL BIOCHEM BEHAV **41**(1) 99–103, 1992.—Bilateral electrolytic lesions into the red nucleus (RN) of rat elicit an increase in susceptibility to seizures induced by pilocarpine, kainic acid, isoniazid, pentylenetetrazole, bicuculline and maximal electric shock (MES). It was also observed that carbachol-induced wet-dog shakes were increased in the RN-lesioned rats. The brain acetylcholine (ACh) and gamma-aminobutyric acid (GABA) concentrations were significantly decreased in the striatum and substantia nigra, respectively. There were no changes in electroencephalogram (EEG) recordings in the RN-lesioned group compared with sham-operated rats. Based on the results it is proposed that the RN is involved in the generalization and acceleration of seizure activity through the cholinergic and GABA-ergic system.

Red nucleus lesions Chemical and electric seizures Wet-dog shakes ACh and GABA level Rats

THE red nucleus (RN) is an upper brainstem nucleus from which descending motor pathways originate. It receives input from the ipsilateral motor cortex and the controllateral nucleus interpositus of cerebellum. On the other hand, the RN together with the retrorubral field (RRF), is a part of the ventral midbrain tegmentum (VMT) which includes substantia nigra pars compacta (SNc) substantia nigra lateralis (SNl) substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA) (Fig. 1). In this way it has access either directly or indirectly to many structures of the brain (2, 3, 6, 13, 18).

Histochemical studies have shown the presence of acetylcholinesterase in the neuropil of the cat RN, suggesting a cholinergic input to this nucleus (1,15). In addition, Kimura et al. have reported the existence of choline acetyltransferase in the RN cholinergic perikarya of cat and rat (9,10). Furthermore, the red nucleus is known to contain measurable amounts of GABA and its synthesizing enzyme, glutaminic acid decarboxylase (GAD) as shown in humans and cats (11, 14, 28).

Recently it has been reported that the RN is related to epileptic manifestations (17). This could be due to the connections between RN and SN. Thus the SNr has been identified as one of principal region of seizures (24,25). The inhibition of the SN efferents, mostly GABA-ergic in nature, would be able to modify the progression of convulsive discharges (5,20). Selective lesions in the efferents from the SN precipitated the appearance of kindling (17).

In the light of these observations, we set out to determine whether the RN plays a role in the susceptibility to seizures produced by maximal electric shock and several chemical convulsants, i.e., pilocarpine, kainic acid, isoniazid, pentylenetetrazole, bicuculline. We determine also the effect of RN lesion on ACh and GABA level of rat brain areas which innervate the nucleus or are known to receive projections from it and from RRF.

METHOD

Animals

Male Wistar rats were used for behavioral experiments and Charles River rats for biochemical experiments, all weighing 260–300 g. The rats were housed in a controlled room temperature (22 ± 1°C) on a 12-h light cycle with free access to food and water.

Surgery for the RN Lesions

Rats were anesthetized with methohexital sodium (Brietal) (Lilly, France) 12.5 mg/kg IP and mounted in a stereotaxic frame, positioned 5 mm above the horizontal. A small burr-hole was made in the skull and the dura was slit to expose the surface of the cortex. Each rat received an electrolytic lesion (current 2 mA for 15 s) in the right and left RN using a tungsten wire electrode, insulated except 0.5 mm at the tip. In the sham-control group the RN lesion was simulated. Coordinates of the site of the lesion were 5.8 mm posterior to bregma, 0.7 mm lateral from the midline and 7.5 mm below the dura (16).

Lesions were assessed by light microscopy 7 days later. The

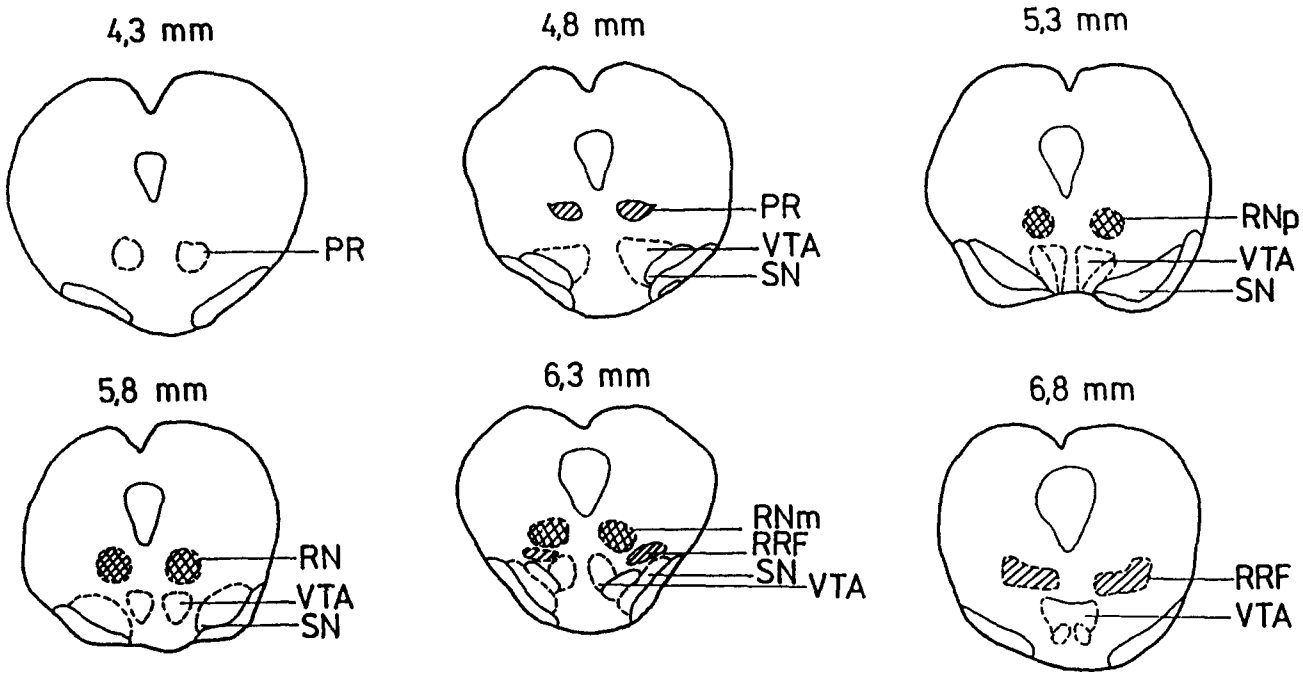

FIG. 1. Schema of red nucleus situation in the rat brain according to stereotaxic atlas of Paxinos and Watson. The area shaded in crossed and slashed lines indicates the position and extention of the lesions of the RN in 5 experiments. RN_p: red nucleus parvocellularis; RN_m: red nucleus magnocellularis; PR; RRF: perirubral, retrorubral field; VTA: ventral tegmental area; SN: substantia nigra.

details were according to the procedure described by Turski et al. (23).

Drugs and Treatment

Pilocarpine hydrochloric, kainic acid, isonicotinic acid hydrazide (Isoniazid) and bicuculline were purchased from Sigma Chemical Co. (St. Louis, MO), while pentylenetetrazole and carbachol chloride were obtained from Polfa (Warsaw, Poland). Pilocarpine (200, 300 mg/kg), kainic acid (6, 10 mg/kg) and isoniazid (275 mg/kg) were given intraperitoneally (IP), while pentylenetetrazole (42 mg/kg), and bicuculline (1.45 mg/kg) were injected subcutaneously (SC), and in a volume of 0.5 ml/100 g body weight. Carbachol was administered into the lateral brain ventricles (20 μg/10 μl) of unanesthetized rats by the method of Herman (4). All drugs were freshly diluted in distilled water and administered 7 days after the RN lesions.

MES was expressed as amperage (145 mA) during 0.3 s, which was necessary to cause the hindleg tonic extensor component of the seizure in rats. The electric current was delivered to the animals through the electrode clips applied to the ears 7 days after lesions.

Carbachol-Induced Wet-Dog Shakes (WDS)

For the assessment of the WDS, the rats were placed individually in Plexiglas cages immediately after intracerebroventricular administration of carbachol. WDS were counted for 10-min intervals over a 60-min period.

Biochemical Assays

Acetylcholine assay. Animals were killed by focused microwave irradiation to the head (1.3 kW, 2.45 GHz, 4 s). The brain was removed quickly and dissected into 5 areas: pyriform cortex, substantia nigra, corpus striatum, entorhinal cortex, and cerebellum. After weighing, the tissue was homogenized in a mixture of 15% 1 N formic acid, acetone, and the ACh content was quantified by the radioenzymatic method of Saelens et al. (19), with modifications (12).

GABA assay. The animals were killed and brains dissected as described above. Tissue was homogenized in 80% methanol containing 5-aminovaleric acid as internal standard. Samples were analyzed by the high performance liquid chromatography procedure of Zecca et al. (30) with modifications (29).

EEG Procedure

Three days after the RN lesion electrodes were implanted as described elsewhere (27). Briefly, two screw electrodes were placed bilaterally over the parietal cortex, together with a ground lead positioned over the nasal sinus, and bipolar depth electrodes were implanted in the dorsal, right hippocampus (A = −3.5, L = 2.4, H = 2.9) and left striatum (A = 0.7, L = 2.5, H = 5.5) while rats were anesthetized with sodium pentobarbitone (50 mg/kg IP). The EEG recording for each animal was analyzed visually to detect any activity which was different between sham and lesioned rats. The correct location of the implanted electrodes was confirmed histologically with cresyl violet-stained serial sections.

Statistic

Behavioral and biochemical data were evaluated by Student's *t*-test for groups consisting of 8 animals except where noted.

RESULTS

In each group of 10 rats that received bilateral electrolytic lesions of the RN, 1 or 2 died within 7 days of the operation.

TABLE 1

EFFECT OF RED NUCLEUS LESIONS ON SEIZURES INDUCED BY CHEMICAL CONVULSANTS AND MAXIMAL ELECTROSHOCK IN RATS

Treatment	Dose mg/kg	Drug-Induced Convulsions (clonic or/and tonic) Sham	Lesion
Pilocarpine IP	200	0/10	8/10
	300	2/10	10/10
Kainic acid IP	6	0/10	5/10
	10	5/10	10/10
Isoniazid IP	275	2/10	7/10
Pentylenetetrazole SC	42	0/10	6/10
Bicuculline SC	1.45	2/10	6/10
MES	145 mA	2/10	7/10

Data represents the number of animals showing seizures relative to the total number of animals tested.

No behavioral disturbances were observed in the surviving rats through the resting period and they steadily gained weight at a rate of approximately 5 g per day.

Chemically Induced Clonic-Tonic Convulsions

Table 1 shows that in the RN-lesioned group the percentage of animals with clonic-tonic convulsions induced by pilocarpine in doses of 200 mg/kg and 300 mg/kg was 80% and 100%, respectively, while in sham-operated controls it was 0% and 20%, respectively.

After kainic acid administration at doses of 6 and 10 mg/kg the percentage of convulsing animals in the lesioned group was 50% and 100%, respectively, while in sham-operated controls it was 0% and 50%, respectively.

Isoniazid (275 mg/kg) induced convulsion in 70% of animals in the lesioned group, but only in 20% of the sham-operated controls. When 42 mg/kg of pentylenetetrazole was given to rats, it induced convulsions in 60% of the lesioned animals and 0% of the sham-operated controls.

Bicuculline (1.45 mg/kg) induced clonic-tonic seizures in 60% of the lesioned rats and in 20% of the sham control group.

MES-Induced Convulsions

A similar potentiating effect to that found with chemically induced seizures was observed in the MES test. The tonic hindlimb extention response to electroshock was increased in the lesioned group (70% of convulsing rats) in comparison with sham-operated rats (20%) (Table 1).

Carbachol-Induced WDS

Carbachol-induced WDS were clearly increased in lesioned rats when compared to the sham-operated controls (Fig. 2). The maximum effect of carbachol was observed 20–40 min after administration in both control and lesioned groups.

Brain ACh and GABA Concentrations

Table 2 indicated that after the RN lesion the ACh level was significantly decreased in the striatum in comparison with sham-operated rats, while there were no changes in other areas investigated (pyriform cortex, substantia nigra, entorhinal cortex and cerebellum). The brain GABA level is shown in Table 3. In the lesioned group the GABA level was decreased in substantia nigra only.

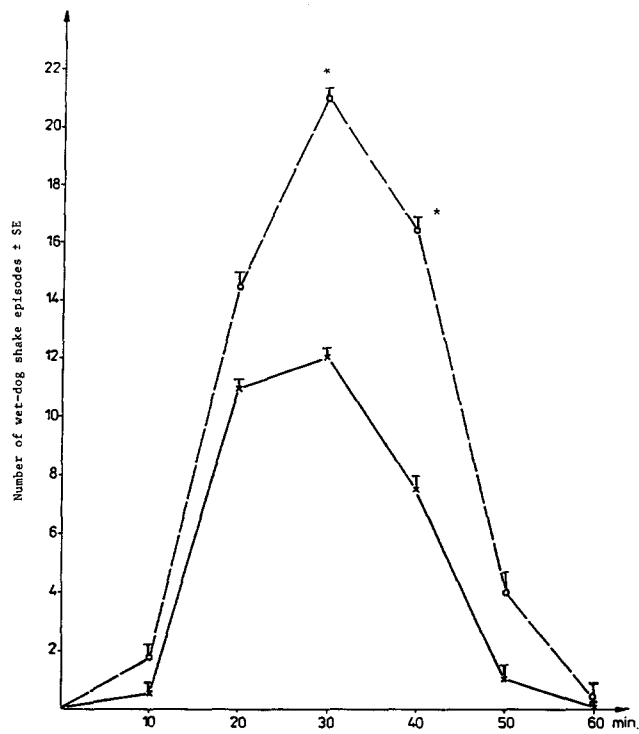

FIG. 2. The influence of red nucleus lesions on carbachol-induced (20 μg ICV) wet-dog shake episodes (means ± SE, n = 8). *$p<0.05$. ×: Control; ○: RN-L.

The EEG Recording

There were no changes in the EEG recording after lesions as compared with sham-operated rats.

Morphological Evaluation

Histological examination revealed the bilateral destruction after electrolytic lesion (Fig. 3).

DISCUSSION

The present study shows that electrolytic lesions of the RN potentiate convulsions and MES induced by all the chemical

TABLE 2

EFFECT OF RED NUCLEUS LESION ON THE ACETYLCHOLINE CONCENTRATION (μmol/g WET TISSUE) IN RAT BRAIN REGIONS, 7 DAYS AFTER OPERATION

Brain Area	ACh Level (μmol wet wt. ± SE) Sham	Lesion
Pyriform cortex	18.4 ± 1.5	17.67 ± 4.48
Substantia nigra	61.1 ± 4.3	54.07 ± 7.75
Corpus striatum	80.1 ± 3.3	62.81 ± 3.12*
Entorhinal cortex	14.4 ± 1.7	17.41 ± 1.54
Cerebellum	18.1 ± 1.2	21.98 ± 2.79

Values are mean ± SE (n = 8). *$p<0.05$.

TABLE 3

EFFECT OF RED NUCLEUS LESIONS ON THE GABA CONCENTRATION (μmoles/g WET TISSUE) IN VARIOUS AREAS, 7 DAYS AFTER RED NUCLEUS LESIONS

	GABA Level (μmol wet wt. ± SE)	
Brain Area	Sham	Lesion
Pyriform cortex	1.88 ± 0.04	1.86 ± 0.02
Substantia nigra	4.04 ± 0.30	2.91 ± 0.26*
Corpus striatum	2.23 ± 0.09	2.17 ± 0.09
Entorhinal cortex	1.16 ± 0.20	1.20 ± 0.09
Cerebellum	1.86 ± 0.15	1.79 ± 0.05

Values are mean ± SE (n=8). *$p<0.05$.

convulsants used and by MES. Increase of tonic hindlimb extension in the MES test and potentiation of clonic and tonic seizures produced by pentylenetetrazole, bicuculline and isoniazid indicate that GABA-ergic activity is probably involved in the RN mechanism.

Among the seizure models which are currently being used in epilepsy research (23), the pilocarpine model of limbic convulsions in rodents has been the subject of great interest (21). There is evidence that the threshold for seizures produced by pilocarpine is subject to regulation by GABA-ergic activity within the SN (24,25), although cholinergic participation in this kind of seizures cannot be excluded (22). In fact, in our work, an increased number of convulsive episodes, in addition to decreased GABA (SN) and ACh (striatum) concentrations, was observed in lesioned animals. Moreover, the present results show that carbachol-induced WDS, which are mediated through the muscarinic cholinergic receptor, are increased in the RN-lesioned group.

RN sends projections to various regions, indeed we found after lesioning the GABA-ergic output to SN and the cholinergic output to striatum, GABA and ACh concentrations are decreased in the corresponding areas. It is well known such decreases being related to convulsive behavior (7, 8, 26) and we have practically observed an increased susceptibility to seizures by various agents and MES in lesioned rats.

Our data in accordance with histochemical evidence of neuronal links between RN, RRF and mesolimbic-striatal areas (3) strengthen the proposal of a functional intercommunication between the RN, striatum and substantia nigra. From this point of view it is possible that the RN affects striato-nigral GABA-ergic feedback that is important in the control of nigro-striatal dopamine system, since the existence of nigro-striatal trineuroal link consisting of GABA-ergic-dopaminergic-cholinergic neurons, terminating in the striatum, is well documented (12).

Based on the present results we propose the involvement of the RN in the generalization and acceleration of seizure activity within the limbic and motor centers in rats.

The RN can be considered as a site at which pathology could alter the susceptibility to generalized convulsions and that GABA-ergic and cholinergic neurons might be involved in the mechanism of action of this nucleus.

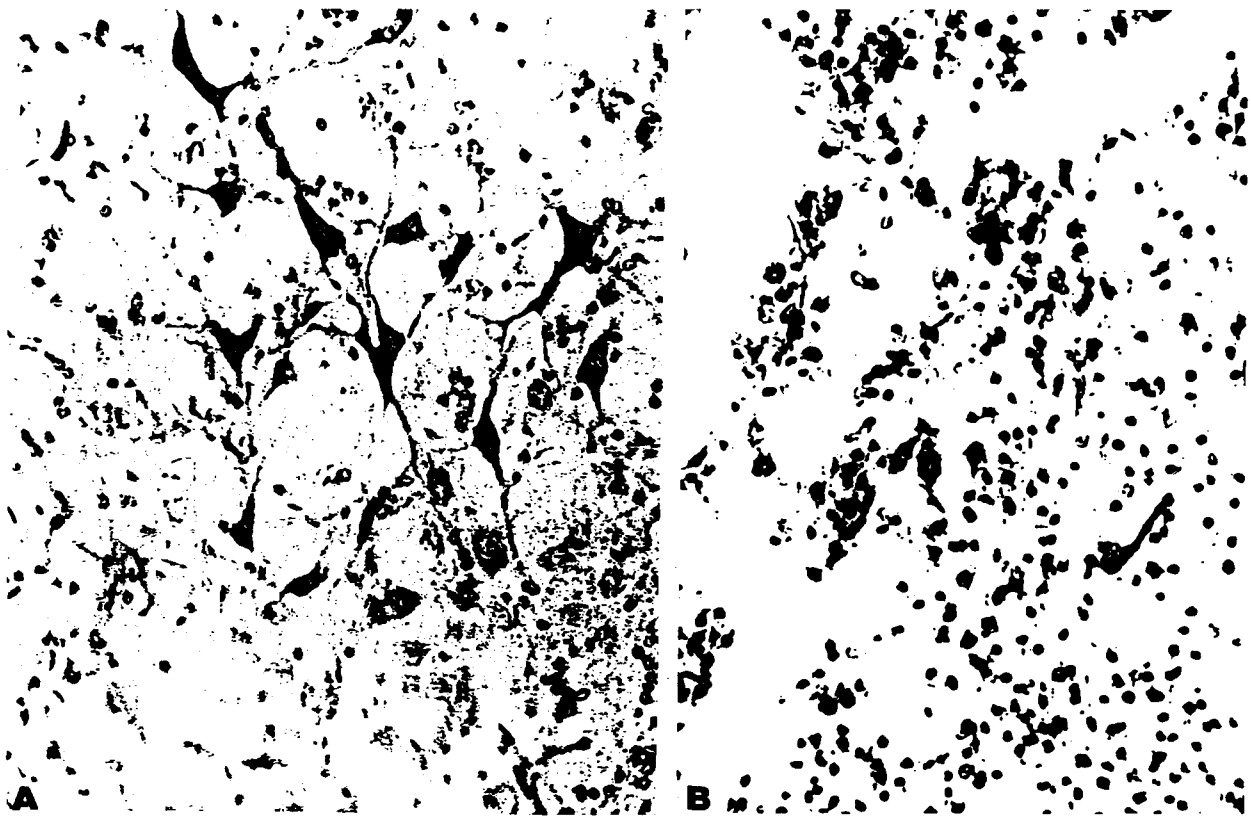

FIG. 3. Morphology of RN from (A) control and (B) lesioned rats (magnification ×200).

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