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Behavioral and Biochemical Changes After Bilateral Electrolytic Lesions of the Red Nucleus of Rat

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KOLASA, K., S. CONSOLO, P. COSTI, Z. KLEINROK AND L. ZECCA. *Behavioral and biochemical changes after bilateral electrolytic lesions of the red nucleus of rat.* PHARMACOL BIOCHEM BEHAV 51(1) 29–35, 1995.—Bilateral electrolytic lesions of the red nucleus (RN) of rat decreased apomorphine-induced stereotypy, increased haloperidol-induced catalepsy, reversed apomorphine-induced hypothermy, decreased spiroperidol-induced hypomotility, and BHT-920–induced yawning and penile erection episodes. Moreover, apomorphine antagonized haloperidol-induced catalepsy in the RN-lesioned group. The lesioned animals revealed depleted levels of dopamine and its metabolites in brain areas as well as serotonin and its metabolite. The brain areas analyzed were pyriform cortex, substantia nigra, striatum, enthorinal cortex, and cerebellum. Based on these results, it is very likely that the RN has a complex role in the behavior of rats as a consequence of dopaminergic-serotoninergic changes in the central nervous system.

Red nucleus lesions Behavior DA and metabolite levels 5-HT and metabolites levels Rats

THE RED NUCLEUS (RN) neurons is a brain stem structure receiving two main inputs from the ipsilateral motor cortex and the contralateral nucleus interpositus of cerebellum. These cerebellar and cortical structures are activated very early in the initiation of movement (19). The RN appears to be involved in the motility of limbs because it exists only in tetrapods (18). The RN and the retrorubral field (RRF) are considered as part of the ventral midbrain tegmentum (VMT).

The A8 cell group, which contains dopamine (DA) (5,6), is found in this region. The cerebellar projection to A8 cells in the VMT could represent a pathway through which the cerebellum influences the extrapyramidal system (21). Nauta et al. (16) noted that neurons situated within the A8 cell group region project to the nucleus accumbens as well as to the striatum. The A8 neurons were shown also to contribute to the dopaminergic innervation of the olfactory tubercles, amygdala, pyriform, and entorhinal cortices (7). The A8 cells appear rostrally as a small group of neurons forming a cell bridge, joining the dopaminergic neurons of A10 cells of the ventral tegmental area (VTA) and A9 neurons of the substantia nigra (SN). Fibers were observed to traverse the A10 cell group, to innervate the contralateral VTA, the contralateral SN pars compacta, and, finally, to terminate in the RRF (5,6).

Measurement of neurotransmitter biochemical markers and study of their specific localizations using morphological techniques in lesioned rats and the deafferentation of the RN indicate the participation of a variety of putative neurotransmitters in this pathway (15).

In rats and humans, the concentration of DA in the RN is remarkably high. This high concentration in humans decreases in Parkinson disease. A nigrorubral projection that recently has been investigated electrophysiologically may be the origin of dopaminergic innervation of the RN (17).

In view of the fact that the A8 dopamine cell group is uniquely situated to modulate functional activity within the nigrostriatal and mesocorticolimbic regions, it was of interest to study the behavioral and biochemical changes in rats after bilateral electrolitic lesions of RN.

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METHOD

Animals

Male Wistar rats weighing 260–300 g were used for behavioral experiments and Charles River rats for biochemical experiments. Upon their arrival to the laboratory, the rats were housed at controlled room temperature (22 ± 1°C) with free access to food and water.

Drugs and Treatments

Apomorphine hydrochloride and 6-hydroxydopamine (6-OHDA) were purchased from Sigma Chemical Co. (St. Louis, MO); spiroperidol and haloperidol were obtained from Jannsen (Dusseldorf, Germany); and BHT-920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d)azepine dihydrochloride) from Boehringer-Ingelheim (Burlington, Ontario, Canada).

Apomorphine (0.1, 3, 16 mg/kg) was administered subcutaneously (SC), while spiroperidol (0.1 mg/kg), BHT-920 (0.1 mg/kg), haloperidol (1 mg/kg) were given intraperitoneally (IP) in a volume of 0.5 ml/100 g body weight. All drugs were freshly diluted in distilled water and administered 7 days after the RN or sham lesions. The 6-OHDA was administered intracerebroventricularly (250 μg) on the eighth and ninth day prior to the experiment (14).

Surgery for the RN Lesion

Three to 7 days after arrival the rats were anesthetized with methohexital sodium (Brietal) (Lilly, France) 12.5 mg/kg IP and mounted in the stereotaxic frame, positioned 5 mm above the horizontal line. 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 for 0.5 mm at the top. 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 according to atlas of Paxinos and Watson (20) as shown in Fig. 1.

The same procedure was applied to another group of rats as a sham operation; in fact, they did not receive the RN lesion. Control rats and nonoperated rats treated with drugs were also employed.

Morphological Techniques

The brains were processed for morphological examination by light microscopy 7 days after electrolytical lesion into the RN. The details for histological processing are described elsewere (23). The rats were anesthetized with an overdose of sodium pentobarbitone and perfused with the fixative containing 10% acetic acid, 10% formaldehyde, and 80% methanol. The brains were allowed to fix in situ at 4°C for 24 h, then removed and processed for paraffin embedding. Subsequently, serial sections of the entire brain were cut coronally at 10 μm. The first section of every 10 was mounted on a glass slide and stained with Cresyl fats violet or according to the Fink and Heimer technique (12).

Behavioral Observations

The effect of the RN lesion on stereotypy induced by apomorphine was scored by direct observation 15, 30, 45, and 60 min after drug injection, according to the Del Rio et Fuents method (8). Each stereotypy pattern was scored as: 1—periodical sniffing; 2—continous sniffing, periodical licking; 3—permanent licking, periodical biting; 4—permanent biting; every positive test was scored as 1 point (total score: 0–4 points).

The effect of the RN lesion on haloperidol-induced catalepsy was scored as described by Dunstan et al. (9). During evaluation of catalepsy rats were maintained in their home cages, but not given access to food or water. The degree of catalepsy is expressed as a total score (0–4), which was obtained by summing the individual scores given in the four tests

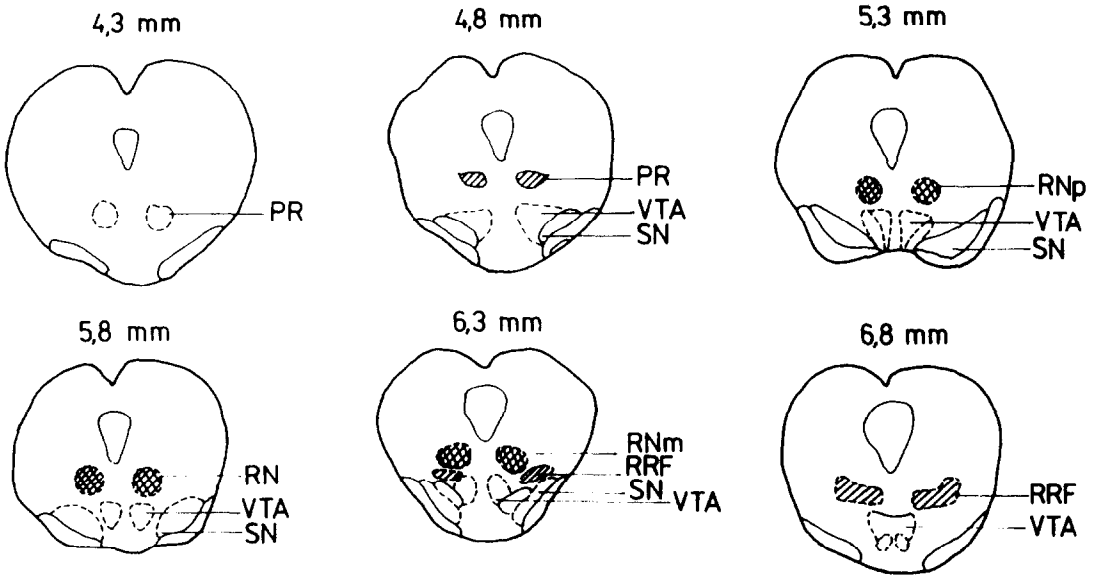

FIG. 1. Brain sections at the RN according to Paxinos and Watson. The areas with crossed and slashed lines indicate position and extension of RN lesions. RNp: red nucleus parvocellularis; RNm: red nucleus magnocellularis; PRF: perirubral, retrorubral field; VTA: ventral tegmental area; SN: substantia nigra.

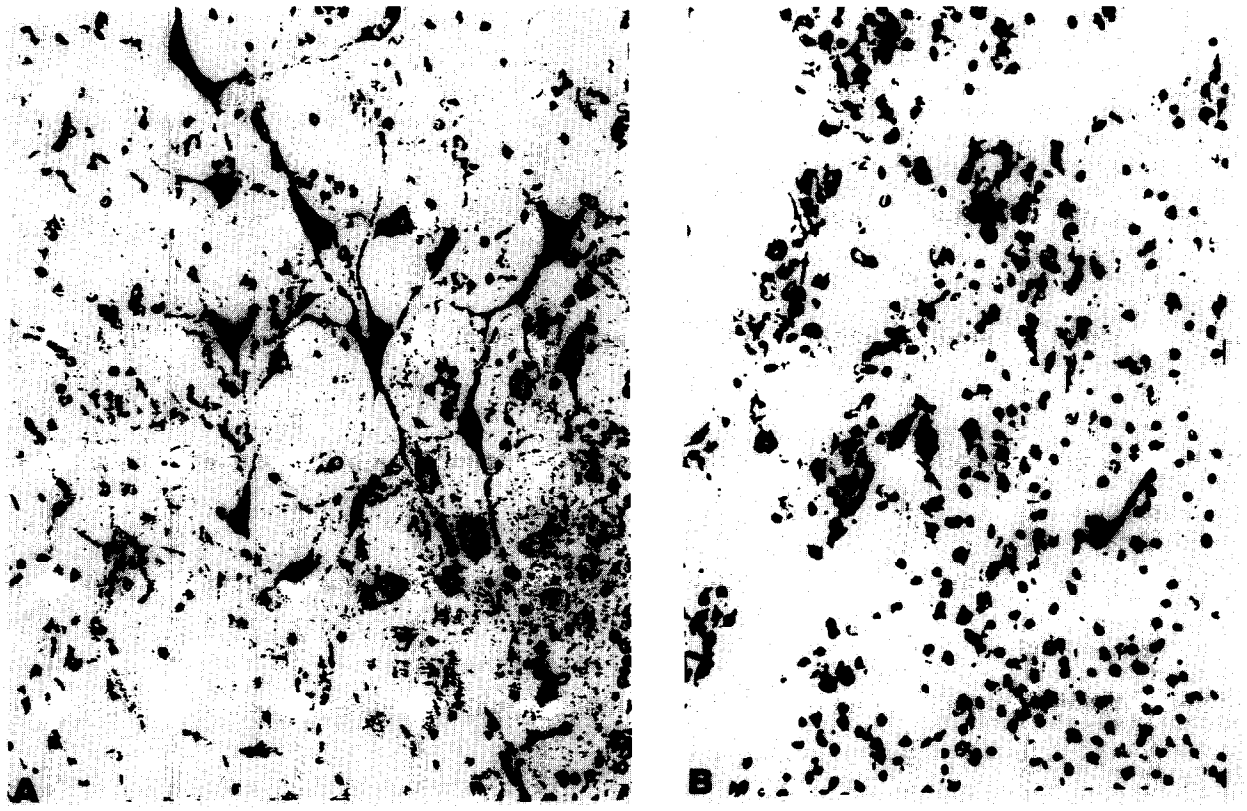

FIG. 2. Morphological examination of RN in control (A) and lesioned rats (B) by using ×200 magnification.

(1—work bench; 2—sloping grid; 3—front paws resting on a bar; 4—Buddha position).

The effect of the RN lesion on the apomorphine-induced hypothermy was tested by measuring the rectal temperature of rats with a thermistor thermometer. The measurements were taken three times before the apomorphine administration and then 15, 30, 45, and 60 min after the injection of the drug.

The effect of the RN lesion on spiroperidol-reduced motility was measured by using individual motility cages (42 × 26 cm) equipped with four sets of infrared light sources and photocells. The light beams crossed horizontally 4 cm above the cage floor. Recording of motility counts required interruption of consecutive light beams. Motility was measured for 60 min and readings were taken 15, 30, 45, and 60 min after spiroperidol injection.

To assess the BHT-920-induced yawning and penile erection in the RN-lesioned animals, the rats were placed into the individual Plexiglas boxes immediately after the drug injection. The number of yawning and penile erection episodes was counted during the 30-min test.

Biochemical Experiments

The animals were killed 7 days after lesions by focused microwave irradiation of the head (1.3 kW, 2.45 GHz, 4 s). The brain was removed quickly, dissected, and separated according to specific areas: pyriform cortex, substantia nigra, corpus striatum, enthorinal cortex, and cerebellum. After weighing, the tissues were homogenized with 10–30 ml/g of 0.1 M $HClO_4$ containing 1–2 μg/ml of isoproterenol as an internal standard and 10 μg/ml of ascorbic acid, then centrifuged at 1500 × *g* for 10 min. Dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindolacetic acid (5-HIAA) concentration in each brain area was measured by high-performance liquid chromatography (HPLC) with electrochemical detection. A 10–20 μl volume of supernatant was injected for each HPLC measurement (11).

Statistics

Statistical analysis was performed by using Student's *t*-test and Mann–Whitney *U*-test.

RESULTS

Of the 10 rats per group that received bilateral electrolytic lesion of the RN, one or two rats in each group died in the 7 days after operation. In all other rats no behavioral disturbances were observed throughout the 3-week testing period; the animals steadily gained weight during this period at a rate of aproximately 20 g per week.

Morphological Evaluation

Histological examination revealed the bilateral RN destruction after electrolytic lesion (Fig. 2).

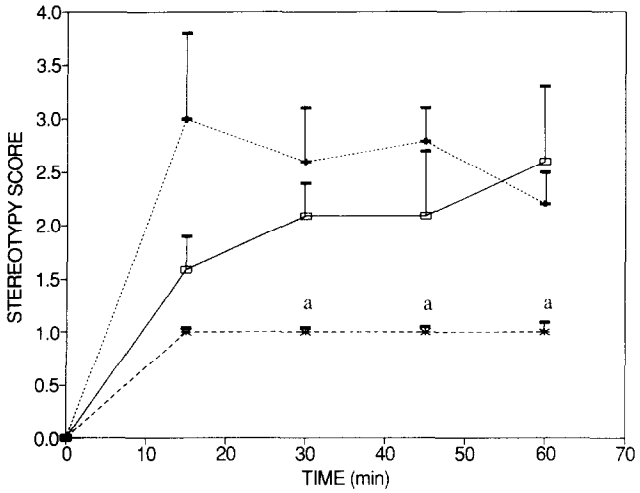

FIG. 3. The influence of RN lesion (RN-L) on apomorphine-induced stereotypy. □ apo (3 mg/kg); * apo + RN-L; + apo + 6-OHDA. $^{a}p < 0.05$ vs. apo. Values shown are means ± SE (n = 8 per treatment group).

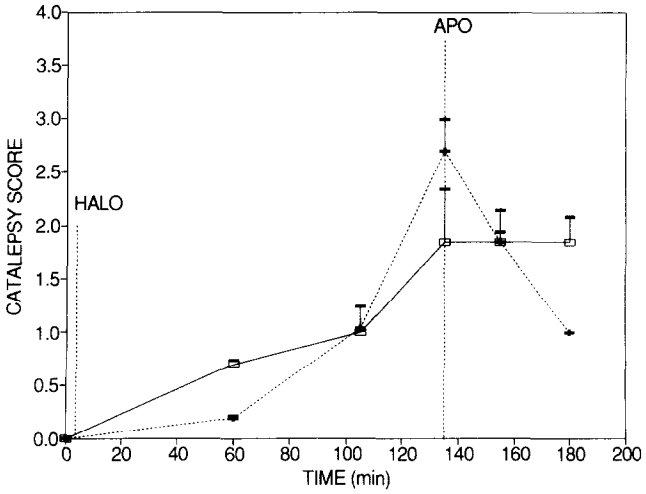

FIG. 5. The influence of apomorphine on haloperidol-induced catalepsy in RN-lesioned rats. □ halo (1 mg/kg) + apo (0.1 mg/kg); + halo + apo + RN-L. Values shown are means ± SE (n = 8 per treatment group). At 180 min the scores were significantly different ($p < 0.05$) between control and experimental group.

Apomorphine-Induced Stereotypy

The effect of apomorphine (3 mg/kg SC) and apomorphine + 6-OHDA-induced stereotypy is shown in Fig. 3. In the group with the RN lesion, the stereotypy scores were significantly decreased when compared to the sham-operated group, given apomorphine + 6-OHDA.

Haloperidol-Induced Catalepsy

Catalepsy score was significantly increased in the RN-lesioned group in comparison with sham-operated rats. This effect was especially marked 60–120 min after haloperidol treatment (Fig. 4).

Effect of Apomorphine on Haloperidol-Induced Catalepsy in RN-Lesioned Rats

Apomorphine, injected at a dose 0.1 mg/kg SC, antagonized haloperidol-induced catalepsy (1 mg/kg IP) in RN-lesioned rats (Fig. 5). The apomorphine effect could be seen 20, 30, and 45 min after its injection.

Effect of RN Lesion on Apomorphine-Induced Hypothermy

Apomorphine, when injected at a dose of 16 mg/kg IP, induced hypothermy in sham-operated rats. This effect was reversed in RN-lesioned rats 15, 30, and 45 min after apomorphine injection. Maximum effect (more than 2°C) was observed 30 min after drug treatment (Fig. 6).

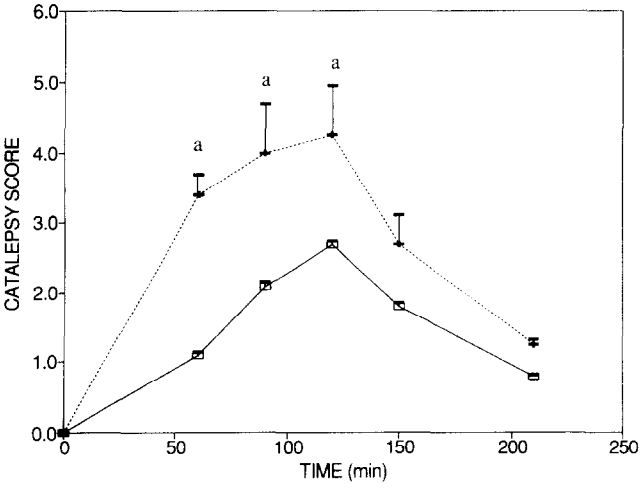

FIG. 4. The influence of RN lesion (RN-L) on haloperidol-induced catalepsy. + halo (1 mg/kg); □ halo + RN-L. $^{a}p < 0.05$. Values shown are means ± SE (n = 8 per treatment group).

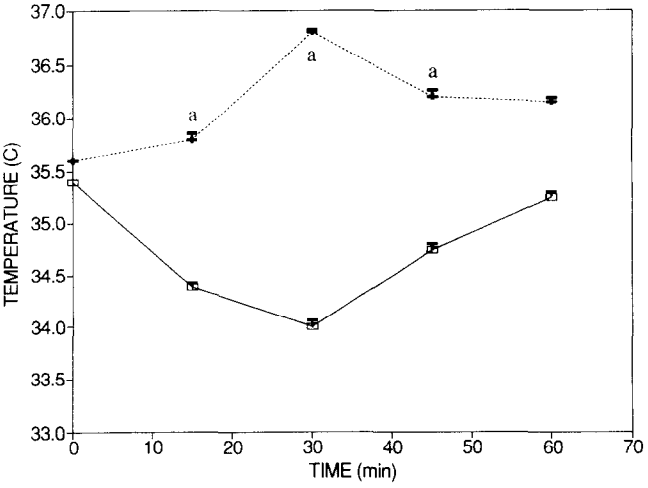

FIG. 6. The influence of RN lesion on apomorphine-induced hypothermy. □ apo (16 mg/kg); apo + RN-L. $^{a}p < 0.05$. Values shown are means ± SE (n = 8 per treatment group).

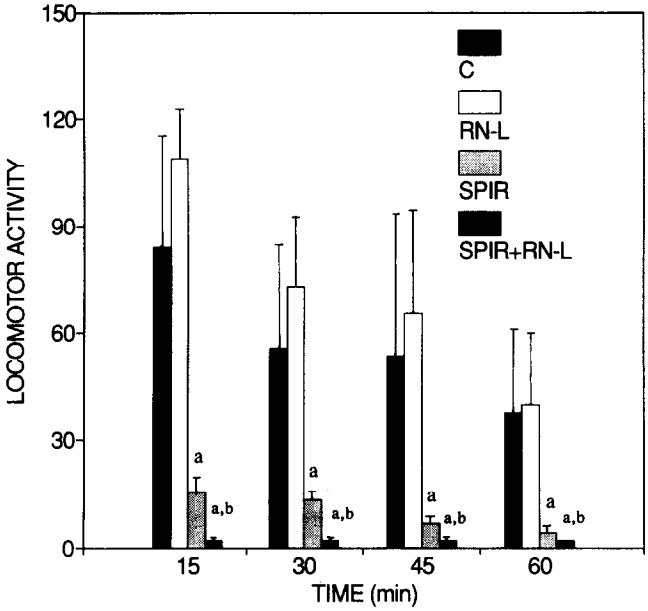

FIG. 7. The influence of RN lesion on spiroperidol-induced locomotor hypoactivity. [a]$p < 0.05$ vs. control and RN-L; [b]$p < 0.05$ vs. spiroperidol. Values shown are means ± SE ($n = 8$ per treatment group).

Effect of RN Lesion on Spiroperidol-Induced Locomotor Hypoactivity

The effect of spiroperidol (0.1 mg/kg IP) on motility in RN-lesioned animals is shown in Fig. 7. At 15, 30, 45, and 60 min after spiroperidol administration, the significant decrease of motility was seen in sham-operated (SPIR) and RN-lesioned groups (SPIR + RN-L). There was also significant decrease of motility in lesioned SPIR + RN-L group in comparison with SPIR alone.

Effect of RN Lesion on BHT-920-Induced Behavior

BHT-920-induced yawning and penile erection episodes were reduced significantly in the RN-lesioned group during 30-min observation (Fig. 8).

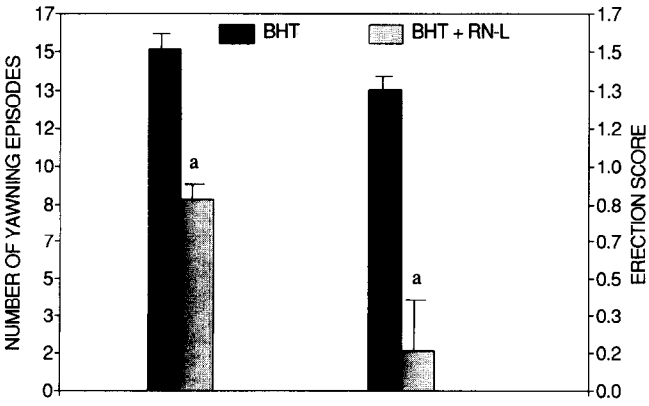

Fig. 8. The influence of RN lesions on BHT-920-induced behavior. [a]$p < 0.05$. Values shown are means ± SE ($n = 8$ per treatment group).

TABLE 1

EFFECT OF RN LESIONS ON REGIONAL LEVELS OF DA, DOPAC, AND HVA IN RATS

Regions	DA	DOPAC	HVA
Pyriform cortex			
Control	4.33 ± 1.61	0.52 ± 0.03	1.26 ± 0.21
Lesion	0.45 ± 0.14*	< 0.05*	0.74 ± 0.3
Substantia nigra			
Control	6.37 ± 0.75	1.09 ± 0.09	0.07 ± 0.01
Lesion	4.02 ± 0.34*	0.38 ± 0.16*	0.006 ± 0.0005*
Striatum			
Control	98.42 ± 1.97	10.45 ± 0.47	1.72 ± 0.09
Lesion	22.29 ± 1.57*	3.23 ± 0.67*	0.64 ± 0.08*
Entorhinal cortex			
Control	0.36 ± 0.06	0.116 ± 0.04	0.77 ± 0
Lesion	0.26 ± 0.03	< 0.05*	0.53 ± 0.03
Cerebellum			
Control	0.45 ± 0.10	0.055 ± 0.005	0.264 ± 0.03
Lesion	0.13 ± 0	< 0.05	0.48 ± 0.14

Values are means ± SE as nmol/g wet tissue. Levels of significance (*)$p < 0.05$.

Effect of RN Lesion on the Level of DA and Its Metabolites in Rats

RN-lesioned animals revealed a 90% depletion of DA in pyriform cortex, 37% in substantia nigra, 78% in striatum, and 72% in cerebellum. Compared to sham-operated animals, there was more than 90% depletion of DOPAC in pyriform cortex, 65% in substantia nigra, 70% in striatum, and more than 57% in entorhinal cortex. Also, the level of HVA was significantly decreased in substantia nigra (−92% of depletion) and in striatum (−63% of depletion) (Table 1).

Effect of RN Lesion on 5-HT and Its Metabolite Level in Rats

There was 68% depletion of 5-HT in pyriform cortex, 63% in substantia nigra, 49% in striatum, 48% in entorhinal cortex, and 70% depletion of 5-HIAA in pyriform cortex, 46% in substantia nigra, 61% in striatum, and 68% in entorhinal cortex (Table 2).

DISCUSSION

Neurochemical analysis of the RN-lesioned animals revealed significant depletion of DA and its metabolites in the analyzed brain areas. The ability of the RN lesion to decrease the concentration of DOPAC and HVA suggests that RN contributes to the control of DA synthesis and release or interferes with impulse flow in dopaminergic terminals of the mentioned areas. Therefore, the observed changes in DA and its metabolites in pyriform cortex, substantia nigra, and striatum, are consequences of lesions of the RN. This situation is similar to that occurring in Parkinson's disease, in which DA and HVA levels are decreased due to a degeneration of DA terminals innervating putamen, caudatum, accumbens, and hypothalamus. Lesions of the substantia nigra in cats causing marked DA decrease in the ipsilateral nucleus accumbens have been described (22). In the RN-lesioned rats, a large decrease in

TABLE 2

EFFECT OF RN LESIONS ON REGIONAL LEVELS OF 5-HT AND 5-HIAA IN RATS

Regions	5-HT	5-HIAA
Pyriform cortex		
Control	5.91 ± 0.22	5.20 ± 0.30
Lesion	1.91 ± 0.16*	1.57 ± 0.22*
Substantia nigra		
Control	0.97 ± 0.14	9.19 ± 0.75
Lesion	0.36 ± 0.04*	5.02 ± 0.51*
Striatum		
Control	0.58 ± 0.01	5.71 ± 0.21
Lesion	0.44 ± 0.01*	2.25 ± 0.17*
Entorhinal cortex		
Control	6.02 ± 1.19	9.66 ± 1.12
Lesion	3.12 ± 0.25*	3.14 ± 0.31*
Cerebellum		
Control	1.30 ± 0.32	1.19 ± 0.18
Lesion	1.30 ± 0.17	2.01 ± 0.31

Values are means ± SE as nmol/g wet tissue. Levels of significance (*)$p < 0.05$.

5-HT and 5-HIAA levels was observed in all areas except the cerebellum. In this case, the same consideration as above can be made for the RN control on the 5-HT terminals in all areas except the cerebellum.

The observed behavioral effects of RN lesions are likely to be mediated through a DA receptor rather than other mechanisms. Most dopaminergic antagonists bind to D_2 receptors, and their affinity correlate well with apomorphine antagonism-induced stereotype behavior (1). The apomorphine-induced stereotypy is caused by stimulation of postsynaptic D_1 and D_2 receptors (2,4). Considering that RN lesions antagonized apomorphine stereotipy and reversed apomorphine hypothermy, this is possibly occurring because of the receptor supersensitivity following the decrease of dopamine content in forebrain. The inhibition of spiroperidol-induced hypoactivity demonstrates an impairment of D_2 receptors following RN lesions. The observation that apomorphine antagonized the haloperidol-induced catalepsy may be due to competition for common receptor sites. It seems also possible that the potentiation of behavioral effects of haloperidol, spiroperidol (DA antagonists), and the antagonism generated by the RN lesion on BHT-920 and apomorphine (DA agonists) results from the decrease of DA levels and inhibition of its metabolism. Cholinergic neurons may also be involved in the observed changes of striatal dopaminergic trasmission and related behavioral changes. It was, indeed, shown that haloperidol (D_1/D_2 antagonist) and sulpiride (D_2 antagonist) reduce striatal Ach levels, increasing its turnover and Ach neuron activity (13). This increase in striatal cholinergic tone plays a major role in the expression of the DA-related motor pattern (10). The potentiation of haloperidol-induced catalepsy and the observed antagonism after apomorphine treatment, once more indicate the participation of dopaminergic function in the RN mechanism of action. This effect could also suggest that the greater effect observed on dopamine receptor, by dopamine receptor blockers, is a consequence of the lack of DA in RN-lesioned rats. In fact, Costall and Naylor (5) reported that large doses of alpha-MT greatly potentiated the behavioral action (i.e., catalepsy) of the dopamine receptor blockers; however, alpha-MT has no effect until severe catecholamine depletion occurs. The same consideration can be proposed about the reduction in yawning and penile erection episodes in RN-lesioned rats after treatment with BHT-920 (dopamine agonist).

In conclusion, from these results we propose a role for the RN in the mechanisms by which DA regulates the extrapiramidal system and other motor pathways. Furthermore a dopaminergic-cholinergic-serotoninergic link is also important in regulating dopamine-mediated behavior.

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