Effect of Galanin on Experimental Parkinson's Syndrome Provoked by Intrastriatal Injection of Kainic Acid

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Currently, a neuropeptide galanin is considered to play an important role in the mechanisms of mnestic disturbances in various diseases (Alzheimer's disease, parkinsonism) [5-7]. In particular, activation of galaninergic mechanisms is thought to occur in specific pathways of the brain, this in turn leading to suppression of the acetylcholinergic system in the above structures, the most important mechanism of mnestic disturbances. It seemed of interest to elucidate whether galanin just disturbs the functions of learning and memory in these diseases, or whether it may also participate in the development of other disturbances typical of these neuropathological syndromes. In the present work we studied the effect of galanin on experimental Parkinson's syndrome induced by intrastriatal injections of kainic acid in rats [2], leading to the formation of a generator of pathologically enhanced excitation (GPEE) in the above-mentioned structures [1], which is behaviorally manifested as the akinetic-rigid form of parkinsonism [2].

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats weighing 250-300 g. The animals were kept under conditions of food and water given ad libitum and of artificial 12-h cycles of dark and light. The experiments were performed during the period between 11:00 and 14:00 h. Guide cannulas were bilaterally implanted into the caudate nuclei of nembutal-narcotized (35 mg/kg, Serva) animals according to stereotaxic coordinates AP=+0.2, L=4.5, H=4.5 [12]. The cannulas were fixed to the cranial bones with the aid of dental cement (SPOFA, Czechoslovakia). By the end of the postoperative period (7-10 days) kainic acid (Sigma, USA) in a dose of 10 or 20 ng/side in 2 μl of phosphate buffer (pH 7.4) was injected with the aid of a microinjector (Mauser, Germany) via the guide cannulas in the animals under conditions of free behavior [2]. Galanin (Bachem, Switzerland) was introduced under similar conditions (separately or 10 min after the injection of kainic acid in a dose of 20 ng) in doses of 2, 10, or 50 ng/side in 2 μl of 0.9% NaCl. The animals of the control groups received the corresponding solvent. Directly before and 3 h after the microinjections, the behavioral changes of the animals were studied using the following tests: 1) "open field," in which the animals were observed over 2 min beginning with the moment of their placement in the center of the field, the initiation of the movement (the time, required to cover a distance equal to the length of the animal's body after placing it in the center of the field, sec) and the number of squares crossed and the number of upright postures being registered; 2) ptosis; 3) arching (1 di-

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TABLE 1. Behavioral Disturbances for Intrastriatal Injection of Kainic Acid (KA) ($M \pm m$)

Group, number of animals	Initiation of movement, sec	Number of squares crossed	Number of upright postures	Arching parameter	Ptosis, number of rats
Control, $n=15$	2.2±0.4	19.5±3.3	6.3±1.8	6.8±0.3	0
	1.4±0.4	16.7±2.5	4.8±2.2	6.9±0.2	0
KA, 20 ng, $n=11$	1.9±0.4	23.5±3.8	7.5±2.8	7.0±0.1	0
	1.3±0.9	18.0±6.5	5.4±1.3	7.0±0.1	0
KA, 100 ng, $n=17$	2.0±0.2	25.5±3.7	6.8±1.1	6.3±0.1	0
	97.4±18.5*	5.7±1.8*	0.4±0.2*	7.3±0.1*	17*

Note. The first value is the parameter before injection of kainic acid (or of control solvent); the second number is the same parameter after injection of kainic acid (or of control solvent). Here and in Tables 2 and 3: an asterisk indicates reliable differences as compared with the initial values ($p<0.05$). All tests (excepting ptosis) are processed using the rank paired Wilcoxon test; for ptosis the precision Fisher method for a 4−field table was used.

vided by the distance in centimeters from the intra-aural line to the base of the tail). The experiments were performed according to the double-blind method. After their completion, the animals were killed with an overdose of nembutal (100 mg/kg); frontal sections were prepared and the zones of implantation were marked according to the tracks of the cannulas, these tracks being compared to the pictures of the atlas [12]. The results were processed using nonparametric methods of statistical analysis.

RESULTS

Intrastriatal administration of kainic acid in a dose of 10 ng/side caused behavioral disturbances in the rats, manifested in an increase in the time of initiation of movement in the open field, a reliable decrease of the number of squares crossed in the "open field," ptosis in all animals, arching, and an increased muscle tone (Table 1).

Bilateral injection of kainic acid in a dose of 20 ng/side in the caudate nuclei did not cause any behavioral changes in the animals (Table 1).

Comparison of the parameters of behavior before and 3 h after the injection of galanin in a dose of 2 ng/side showed that peptide in the above dose did not cause any behavioral disturbances (Table 2). When galanin was used in doses of 10 or 50 ng, a reliable decrease (vs. the initial values) of the number of squares crossed and of upright postures was registered in the animals during the "open field" test. The rest of the behavioral parameters remained unchanged (Table 2).

Under conditions of a combined injection of kainic acid (20 ng/side) and galanin in a dose of 2 ng/side in the caudate nuclei, a reliable decrease (vs. the initial value) of the number of squares crossed, as well as of the number of upright postures was noted in the animals in the "open field." The other parameters studied remained unchanged (Table 3).

After intrastriatal injection of kainic acid (20 ng) followed by subsequent microinjection of galanin (10 ng), arching was observed in the animals along with a decreased number of squares crossed and of upright postures in the "open field," the former parameter being reliably increased as compared with that before the use of kainic acid and galanin. In addition, ptosis developed in some animals (Table 3).

Combined administration of kainic acid (20 ng) and galanin in a dose of 50 ng caused an increase of the time of initiation of movement in

TABLE 2. Behavioral Disturbances for Intrastriatal Injection of Galanin (G) ($M \pm m$)

Group, number of animals	Initiation of movement, sec	Number of squares crossed	Number of upright postures	Arching parameter	Ptosis, number of rats
Control, $n=10$	2.7±0.3	22.5±4.8	5.6±1.2	6.4±0.1	0
	2.2±0.3	18.8±5.6	4.4±2.0	6.7±0.1	0
G, 2 ng, $n=11$	3.2±0.4	26.5±5.3	6.2±2.5	6.9±0.2	0
	2.5±0.5	17.9±8.2	5.0±2.4	6.4±0.2	0
G, 10 ng, $n=10$	3.8±0.5	19.2±3.7	5.3±1.1	7.0±0.1	0
	2.8±0.6	5.6±1.1*	0.2±0.2*	7.2±0.1	0
G, 50 ng, $n=10$	4.4±0.7	20.5±4.1	7.8±1.2	6.6±0.2	0
	2.2±1.1	7.2±1.3*	1.9±0.6*	6.5±0.1	0

Note. The first value is the parameter before injection of galanin (or of control solvent); the second number is the same parameter after injection of galanin (or of control solvent).

TABLE 3. Behavioral Disturbances for Combined Injections of Kainic Acid (KA) (20 ng) and Galanin (G) in the Caudate Nuclei of Rats ($M \pm m$)

Group, number of animals	Initiation of movement, sec	Number of squares crossed	Number of upright postures	Arching parameter	Ptosis, number of rats
Control, $n=8$	1.5±0.5	27.2±6.3	8.2±3.9	7.3±0.1	0
	0.9±0.3	19.0±7.7	5.8±2.7	7.3±0.2	0
KA + G, 2 ng, $n=11$	1.7±0.5	30.4±6.2	9.9±3.8	6.7±0.1	0
	1.1±0.7	6.7±1.3*	1.0±0.5*	6.5±0.1	0
KA + G, 10 ng, $n=10$	2.5±0.3	27.5±4.1	7.2±2.3	6.3±0.177	0
	3.5±0.7	8.9±2.2*	0.3±0.3*	7.2±0.1*	0
KA + G, 50 ng, $n=11$	2.7±0.6	29.0±6.6	9.5±2.1	6.8±0.1	0
	24.5±1.8*	3.8±1.7*	0.8±0.5*	7.9±0.2*	11*

Note. The first value is the parameter before injection of kainic acid and galanin (or of control solvent); the second number is the same parameter after injection of kainic acid and galanin (or of control solvent). Other symbols as in Table 1.

the "open field," a decrease of the number of squares crossed and of upright postures, arching, an increase of muscle tone, and variously marked ptosis in all rats (Table 3).

Thus, our studies showed that intrastriatal injection of kainic acid in a dose of 100 ng/side caused behavioral changes resembling the akinetic-rigid form of Parkinson's syndrome in the animals, specifically bradykinesia, arching, and ptosis. It is known that today, degeneration of the nigrostriatal dopaminergic pathway, followed by disinhibition of the cholinergic neurons of the striatum, is regarded as the leading mechanism of Parkinson's syndrome [8]. However, in recent studies it has also been shown that activation of the corticostriatal glutamatergic pathway may participate in the mechanisms of parkinsonism as well [13]. In this connection, the formation of a GPEE in the striatum caused by a direct and indirect agonist of excitatory amino acids (kainic acid) reflects the above-mentioned mechanism of Parkinson's syndrome.

Intrastriatal injection of galanin caused a dose-dependent decrease of the parameters of locomotor activity in the "open field," in particular, a decrease of the number of squares crossed and of the number of upright postures, without affecting the rest of the parameters studied. At the same time, combined administration of kainic acid in a dose of 20 ng (not effective in provoking any behavioral disturbances) and galanin caused the development of behavioral disturbances not observed for separate administration of the preparations in the corresponding doses. For instance, a decrease of the number of squares crossed and of upright postures in the "open field" was observed for galanin injection in a dose of 2 ng following microinjection of kainic acid, while galanin was injected in high doses (10 and 50 ng) the disturbances of behavior also included (along with the above-mentioned) an increased time of initiation of movement, arching, and ptosis. The fact that the last two symptoms were not reproduced after separate galanin administration, permits us to assume that in this case we are dealing with galanin-mediated potentiation of kainate-induced parkinsonism-like behavioral disturbances.

The possible mechanisms of the established interaction between galanin and kainic acid in the striatum are of interest. It is known that one of the effects of excitatory amino acids and their structural analogs is stimulation of acetylcholine release in the striatum [9]; this mechanism may also contribute to the development of Parkinson's syndrome. It should be mentioned that while suppressing acetylcholinergic transmission in the majority of brain structures (in particular, in the Meinhert nucleus in the limbic system [5,7]) galanin facilitates acetylcholine release in the caudate nuclei [11]. Possibly, activation of acetylcholine release occurs at the site of interaction between kainate and galanin in the striatum. Another known effect of agonists of excitatory amino acids is an enhanced release of endogenous opioids [15]; furthermore, potentiation of the analgetic effects of morphine has been shown for galanin in experimental tests [14]. It should also be mentioned that a typical behavioral symptom of the effect of endogenous opioids on the CNS is the development of muscle rigidity - a syndrome characteristic of parkinsonism. Hence, this mechanism may also underlie the interaction between galanin and the system of excitatory amino acids. And, finally, a direct effect of galanin on the activity of the system of excitatory amino acids is possible, although such effect has not been described previously.

Thus, the present studies have shown that galanin injected in the caudate nuclei of rats potentiates behavioral manifestations of Parkinson's syndrome caused by intrastriatal injection of kainic acid. One may assume that activation of galaninergic

mechanisms in the striatum is one of the factors underpinning the activation, stabilization, and maintenance of the activity of the GPEE in this structure, this promoting the development of parkinsonism.

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The Effect of Synthetic Analogs of Enkephalins on the Development of Traumatic Cerebral Edema

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Cerebral edema (CE) is the most grave complication after cranio-cerebral trauma. According to current views [6], pathophysiological and biochemical changes in the brain after trauma should be regarded as a response of the organism to severe stress. In view of this, timely correction of the stress reaction and preventing compensatory changes from becoming pathological are of utmost importance for prophylaxis and therapy of CE.

Special attention has been paid lately to the discussion of the role of endogenous opioid peptides as regulators of the biochemical processes and functions of the organism under extreme conditions. It is noted that opioid peptides attenuate the stress reaction and protect the organism from stress injury [1,3]. Earlier, we demonstrated the involvement of the endogenous opioidergic system in CE formation [5]. The present study was undertaken to study the effect of synthetic analogs of enkephalins on the development of traumatic CE.

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MATERIALS AND METHODS

The experiments were performed on 179 nonpedigree albino rats of both sexes weighing 160-220 g. The animals were subjected to a trauma, and the development of CE was assessed from the content of total water and the density of the cerebral

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