EFFECT OF INTRANASAL SUBSTANCE P ON THE PARKINSONIAN SYNDROME

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Anatomical, biochemical, and pharmacologic investigations in recent years have demonstrated close morphological and functional links between dopaminergic (DA-ergic) neurons and neurons synthesizing substance P (SP) in the nigrostriatal system [1, 10, 13, 16, 17]. According to data in [12], strionigral neurons containing SP exert a tonic excitatory action on the DA-ergic neurons of the substantia nigra (SN). Damage to the DA-ergic neurons of SN in the rat brain under the influence of 6-hydroxydopamine leads to lowering of the DA and SP levels in SN and the striatum [9, 11, 17]. These findings suggested that an imbalance of SP in the nigrostriatal system may play a pathogenetic role in the appearance of a parkinsonian syndrome (PS). In the presence of DA deficiency in the caudate nuclei (CN) neurons of these nuclei are disinhibited, with the formation of a generator of pathologically enhanced excitation (GPEE) from them, and which acts as functional trigger of the basic manifestations of PS [2-4]. The writers showed previously that if SP is injected into CN, suppression of the GPEE takes place and is accompanied by reduction of oligokinesia and rigidity [5].

In the investigation described below the possibility of appearance of a similar inhibitory action of SP on PS after intranasal adminsitration was studied.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred rats aged 10-12 months and weighing 500-600 g. SP was induced either by systemic injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a dose of 25 mg/kg at intervals of 12 h for 4-5 days [3, 5] or by a single intraperitoneal injection of reserpine (30 mg/kg). To record electrical activity (EA), nichrome electrodes 0.1 mm in diameter were inserted into the rostral portions of CN of the animals anesthetized with hexobarbital. The reference electrode was fixed in the nasal part of the skull. EA was recorded in unrestrained animals before injection of MPTP and reserpine, and against the background of development of PS. The animals were kept in individual cases under standard animal house conditions and on an ordinary diet. The animals' motor activity was assessed by the "open field" test and expressed as a percentage of the motor activity of intact animals; rigidity was expressed in points, and tremor was recorded by means of a tremorograph, using sensitive piezoelectric cells, responding to mechanical oscillations of low amplitude [3, 4]. SP was instilled into the nasal cavity bilaterally in a dose of 25 μg/kg body weight by means of a "Hamilton" microsyringe with a blunt needle or into the caudal vein in the same dose under superficial open ether anesthesia. Animals of the control group with PS were given physiological saline in the same volume and under the same conditions. The results were subjected to statistical analysis by Student's t-test and by the Wilcoxon Mann—Whitney nonparametric test.

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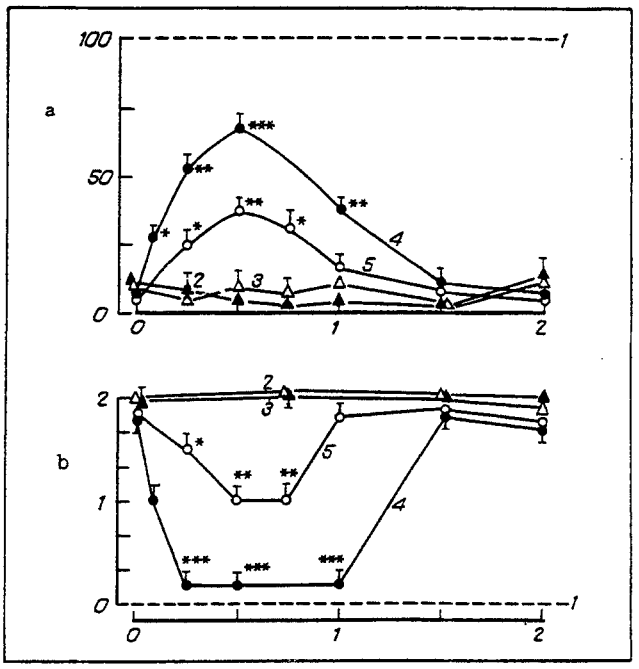

Fig. 1. Effect of intranasal and intravenous injection of SP on oligokinesia and rigidity caused by repeated systemic administration of MPTP. Abscissa, time of observation (in h) after injection of SP; ordinate: a) motor activity (in per cent), number of squares crossed and number of standings by intact animals, taken as 100%; b) rigidity (in points). 1) Intact animals; 2) Animals with PS, receiving physiological saline intranasally (control), 3) Animals with PS, receiving physiological saline intravenously (control), 4) Animals with PS, receiving SP intranasally, 5) Animals with PS receiving SP intravenously. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) significance of differences compared with corresponding control.

EXPERIMENTAL RESULTS

Most (28 of 36) animals developed clear signs of PS 4-5 days after the beginning of injection of MPTP, in the form of oligokinesia and muscular rigidity, accompanied by a marked reduction of the scale and variety of types of motor activity (disappearance of vertical standing, of investigative activity, and grooming). Muscular rigidity was less marked, and was rated at 1.5-2 points. Besides motor disturbances, most animals also revealed loss of body weight, hypothermia, and various autonomic disturbances.

Intranasal injection of SP caused a significant increase in motor activity of the animals with PS. The effect appeared 5-10 min after injection of SP, reached a maximum after 30 min, and continued for 1-1.5 h. (Fig. 1a). The muscular rigidity decreased immediately after intranasal injection of the neuropeptide and remained low for 60 min, after which it began to increase, 80 that after 80-90 min it did not differ from values in the control group (Fig. 1b).

Besides the last-mentioned changes of oligokinesia and rigidity, the experimental animals also showed an increase in other forms of locomotor activity [an increase in the number of vertical movements (rearings), increased investigative activity, the appearance of grooming].

Spontaneous EA (before injection of MPTP in CN was characterized by dysrhythmia, and by the presence of low-amplitude (30-50 μV) fast and slow waves with the periodic appearance of thetalike activity (Fig. 2a). Paroxysmal discharges of high-amplitude fast and slow waves were recorded in CN 4-5 days after the beginning of MPTP injection in animals with the above-mentioned signs of PS. Parallel with this, grouped high-amplitude slow waves with a frequency of 1 Hz and an amplitude of 500-600 μV were observed (Fig. 2b).

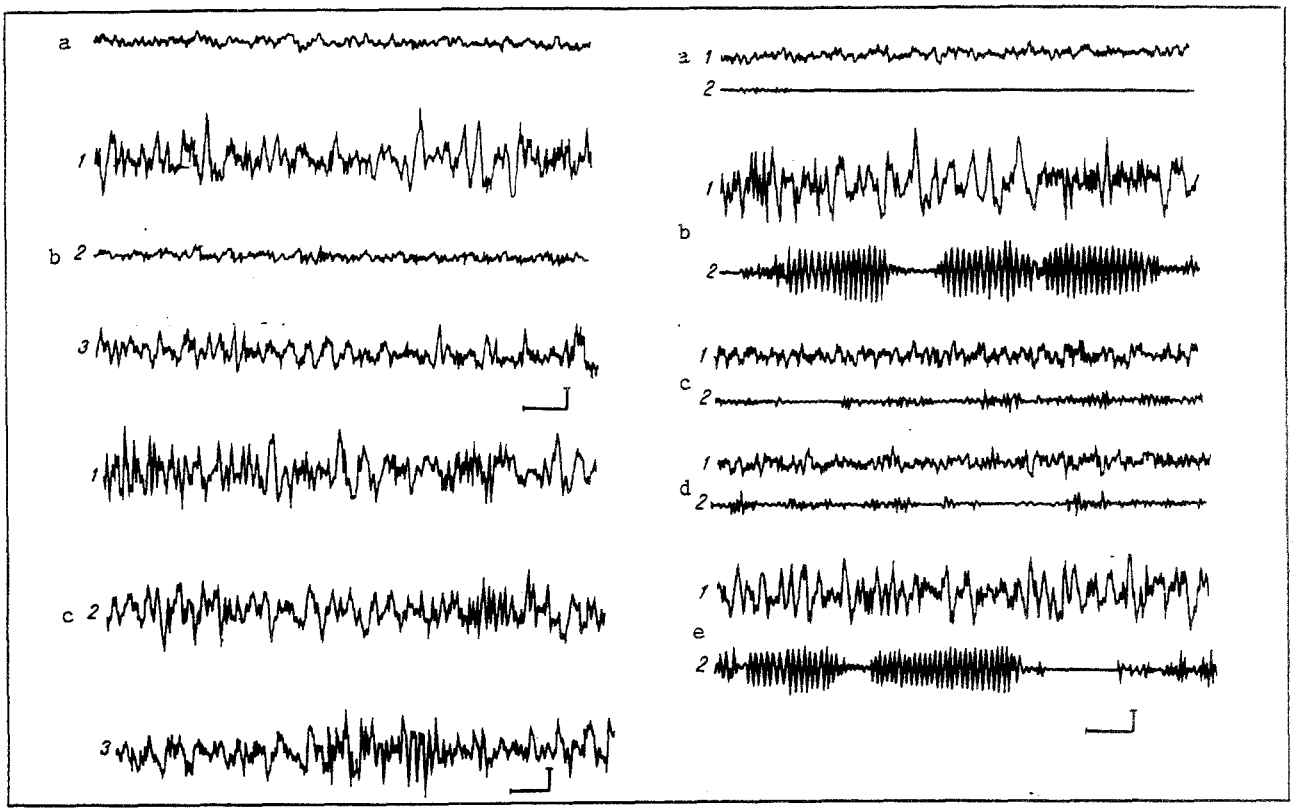

Fig. 2 Fig. 3

Fig. 2. EA in CN before (a) and on 5th day (1) after beginning of MPTP injection (at moment of recording EA, rat showed marked oligokinesia and rigidity), and 30 min (2) and 1.5 h (3) after intranasal injection of SP (b) and physiological saline (control) (c). Calibration: 50 μV, 1 sec.

Fig. 3. EA in CN (1) and tremorogram (2) before (a) and 24 h after intraperitoneal injection of reserpine (b), and 10 min (c), 30 min (d), and 80 min (e) after intranasal injection of SP (25 μg/kg). Calibration as to Fig. 2.

After intranasal injection of SP a marked decrease in EA (frequently to its initial level), a decrease in the number, amplitude, and duration of the paroxysmal discharges, and a decrease in the frequency and amplitude of the slow waves in CN to 100-150 μV took place. These changes appeared 5-10 min after injection of SP and continued for 60-80 min. After 1.5 h increased paroxysmal activity was restored, and high-amplitude waves with an amplitude of up to 500-600 μV and a duration of 1-2 Hz reappeared (Fig. 2b).

In the control group of animals with PS intranasal injections of physiological saline had no significant effect either on oligokinesia and muscular rigidity (Fig. 1a, b) or on EA in CN (Fig. 2c).

Intravenous injection of SP in the same dose (25 μg/kg) caused weakening of oligokinesia and rigidity, but the effect was weaker and shorter in duration (by half) than after intranasal injection (Fig. 1).

The animals developed oligokinesia, rigidity, and tremor 24-48 h after intraperitoneal injection of reserpine. Tremor (mainly of the head and forelimbs) was observed in 8 of the 14 animals. Intranasal injection of SP also weakened oligokinesia (an increase in the number of squares crossed and in the number of vertical movements, Table 1). In animals with manifestations of parkinsonism-paroxysmal discharges and high-amplitude fast and slow waves were recorded in CN. Intranasal injection of SP suppressed this activity and the tremor for 40-50 min (Fig. 3).

Comparison of the effects of intranasal injection of SP on two models of experimental parkinsonism, induced by different chemical agents, reveals the similarity of these effects: pathologically enhanced EA (GPEE), while at the same time the manifestations of parkinsonism (oligokinesia, rigidity, and tremor) disappear or are considerably weakened. After cessation of the action of SP (evidently as a result of its destruction) activity of the GPEE in CN is restored and the manifestations of parkinsonism are intensified. These findings agree with the results of previous investigations [5] in which

TABLE 1. Effect of Intranasal Injection of SP on Reserpine-Induced Oligokinesia in Rats (M ± m)

Experimental conditions	Before injection of SP		After injection of SP	
	number of squares crossed	number of vertical rears	number of squares crossed	number of vertical rears
Intranasal injection CP (n=7)	8.1±2.8	1.8±0.7	23.0±4.0*	3.6±1.0
Intranasal injection of physiological saline (n=6)	9.5±3.0	1.1±0.5	6.5±3.5	0.4±0.2

Legend. *p < 0.05; n) number of animals.

it was shown that direct injection of SP into the region of GPEE caused suppression of the latter and considerable weakening of the parkinsonian syndrome. The fact that SP, injected intranasally, leads to weakening of PS suggests that under pathological conditions SP can pass through the blood-brain barrier into the brain and, in particular, into CN. Meanwhile, when injected intranasally the antiparkinsonian effect of SP was much stronger than after intravenous injection. This result suggests that SP, if injected intranasally, enters the brain in larger amounts and may reach CN.

The mechanism of the observed effects of SP is not clear. We know [7, 8] that the SP level in man is highest in SN and the striatum. The highest density of receptors for SP also is found in the striatum [4]. There is some evidence to show that the SP concentration in SN, determined post mortem in patients with Parkinson's Disease is sharply reduced [6, 18]. Injection of SP into SN in intact animals leads to elevation of the DA level in the striatum [15]. This effect was accompanied by an increase in the number of vertical rears and grooming of the animals and was suppressed after injury to DA-ergic neurons of the strionigral system by the neurotoxin 6-hydroxydopamine [9]. It has been suggested [7] that under physiological conditions SP realizes the integrative role of DA-ergic neurons and that in parkinsonism there is a primary lesion of SP-ergic neurons, which later goes on to secondary degeneration of the DA-ergic neurons of the strionigral system [7].

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