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Parkinson`s disease: influence of cannabinoid and peptidergic systems on pain

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Parkinson's disease (PD) results primarily from the death of dopaminergic neurons in the substantia nigra, but it is now clear that its pathogenesis is underlined by interaction of different mediatory systems. The endocannabinoid system (ECS) is vastly distributed in the central nervous system and represents a potential therapeutic approach for a number of neurologic diseases, PD among them. MIF-1 and Tyr-MIF-1`s modulating action on ECS is also of interest as well as ECS and peptides combined effect on pain perception in PD.

Cannabinoids` and neuropeptides` interactions were estimated in a rat model of 6-hydroxydopamine hemiparkinsonism by Paw pressure test.

Anandamide and AM251 influenced pain perception in control animals as well as in animals with experimental PD. MIF-1 and Tyr-MIF-1 modulated ECS in PD while naloxone changed nociception in PD animals compared to controls.

MIF-1and Tyr-MIF-1 neuropeptides interact with ECS and modulate pain perception.

Keywords: Parkinson`s disease, pain perception, cannabinoid system, MIF-1, Tyr-MIF-1

INTRODUCTION

Parkinson`s disease (PD) first described by James Parkinson in 1817 represents a chronic incurable progressive neurodegenerative condition characterized by predominantly motor disturbances – tremor, rigidity, bradykinesia, and postural disorders [1]. It affects between 1 and 3% of the population over 50 years of age. Its pathological hallmark is specific degeneration of dopaminergic neurons in the substantia nigra pars compacta [2, 3]. The complex integrative system of the basal ganglia in the central nervous system (CNS) comprises substantia nigra, putamen, nucleus caudatus, nucleus accumbens, and globus pallidus. The effectiveness of the system described depends on the synaptic transmission that represents itself the outcome of interaction (and integration) of different neurotransmitters and neuromodulators [4, 5]. Animal studies suggested that basal ganglia play also a role as a sensory analyzer integrating and focusing adequate sensory impulses, and finally modulating motor performance [6]. Such a sensorimotor integration links sensory input to the motor output producing adequate voluntary movements [7], and probably accounts for the pathogenesis of bradykinesia in PD.

Over the last decade researchers have focused their interest on purely sensory functions in PD. Along with motor dysfunctions 75% of PD patients manifest also sensory disorders with pain among them [8]. Living organisms possess a complex mechanism to control pain sensations. The antinociceptive pathways integrate two interrelated components – an opioid and a non-opioid one [9].

The first component is connected with the opioid system, which comprises the opioid receptors (μ-, δ-, κ-, λ-, σ-) and their endogenous ligands (β-endorphins, enkephalins, and dynorphin) [10]. The non-opioid component of analgesia integrates different neuromodulator/neurotransmitter systems - the adrenergic, the serotoninergic, the nitric-oxide, the endocannabinoid systems.

Experimental data support the importance of the endocannabinoid system (ECS) in CNS and the peripheral nervous system. The ECS consists of two types of cannabinoid receptors (CB1 and CB2), their endogenous ligands and the enzyme systems involved in their synthesis and degradation [11].

CB1 predominates in the brain and especially in the basal ganglia. In the last years several experiments proved that the endocannabinoids exerted an important role in the striatum: they influence its normal functions, interact with dopamine and mediate the changes after dopamine depletion [5, 12]. It has been proved that endocannabinoid levels in the striatum increase after dopamine depletion [5, 13]. The role of endocannabinoid and peptidergic neurotransmissions in the pathogenesis of motor dysfunctions in PD has also been confirmed [14, 15].

The peptides of the Tyr-MIF-1 family exert opioid as well as anti-opioid effects [16-18]. MIF-1 and Tyr-MIF-1 have also modulating effect on the dopaminergic neurotransmission [19-23].

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Changes in dopaminergic neurotransmission are undoubtedly crucial to the pathogenesis of motor dysfunctions in PD, and it is also important in modulating pain perception and natural analgesia within supraspinal striatal and extra-striatal regions. Yet there are some evidences questioning the dopaminergic transmission role in pain processing [24]. It is then possible that other non-dopaminergic basal ganglia neurotransmitter systems may account for the sensory abnormalities in PD and thus influence the sensorimotor integration.

In the present study, we evaluated the changes in pain thresholds after injection of: 1) CB-1 agonists and antagonists; 2) MIF-1 and Tyr-MIF-1 neuropeptides; 3) MIF-1 or Tyr-MIF-1 after CB1 agonist. The experiments were performed in a rat model of 6-hydroxydopamine (6-OHDA)-induced Parkinsonism which is one of the most common animal models of PD. 6-OHDA is a hydroxylated analogue of natural dopamine that selectively destroys catecholamine neurons. It also leads to production of reactive oxygen species (ROS) which damage proteins, lipids and DNA, causes mitochondrial inhibition and impairment, and ATP deficiency [25-27].

EXPERIMENTAL

Animals

The experiments were carried out on male Wistar rats (200-240 g at the beginning of study), housed individually in polypropylene cages (40 × 60 × 20 cm) at a temperature-controlled colony room maintained at 21 ± 3 °C under 12:12 h light/dark cycle with lights on at 6:00 a.m. The animals were given free access to tap water and standard rat chow. All procedures were carried out according to the ‘‘Principles of laboratory animal care’’ (NIH publication No. 85_23, revised 1985), and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences.

Stereotaxic drug injection into the ventrolateral striatum

Rats were anesthetized with intraperitoneal injection of a mixture of ketamine (75 mg/kg), acepromazine (0.75 mg/kg) and rompun (4 mg/kg). The animals were placed in a stereotaxic apparatus (Stoelting, USA). 8 μg (free base weight) 6-OHDA (RBI) was dissolved *ex tempore* in 2 μl of 0.2% ascorbic acid with 0.9% normal saline and 2 μl of the solution was microinjected trough Hamilton micro-syringe (Hamilton, Reno, NV) at the following coordinates: AP "4.4 mm, ML 1.2 mm relative to bregma, and DV "7.5 mm from the dura over a period of 2 min (rate 0.5 μl /min) and the injection cannula was left in place for additional 30 seconds.

The control group was microinjected with 2 μl saline into the same area.

Immediately prior to sacrificing, the animals were injected with 1 ml 2% Fastgreen dye through the injection cannula.

Injection sites were then anatomically verified post-mortem in 25 mm coronal brain sections cut through the hippocampus by an investigator, blind to the behavioural results. Results from animals with cannulas` placements outside the ventrolateral striatum area were excluded from the statistical analysis.

Drugs and treatment

All drugs were obtained from Sigma. Anandamide (arachidonoyl ethanolamide, AEA) at a dose 1mg/kg, and AM251 at a dose 1,25mg/kg dissolved in DMSO were injected intraperitoneally (i.p.). MIF-1 and Tyr-MIF-1 were dissolved in sterile saline solution (0.9% NaCl) and injected i.p. at a dose 1mg/kg. When evaluating the neuropeptides` effect on cannabinoids MIF-1 and Tyr-MIF-1 were administered 10 min after anandamide or AM251.

Nociceptive test

Paw-pressure test (Randall-Selitto test). The changes in the mechanical nociceptive threshold of the rats were measured by analgesiometer (Ugo Basile). Increasing pressure (g) was applied to the hind-paw and the value required to elicit a nociceptive response (a squeak or struggle) was taken as the mechanical nociceptive threshold. A cut-off value of 500 g was observed in order to prevent damage of the paw.

Statistical analysis

The results were statistically assessed by one-way analysis of variance ANOVA followed by t-test comparison. Values are mean ± S.E.M. Values of $p \leq 0.05$ were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Left-sided injection of 6-OHDA led to right-sided hemiparkinsonism (RSHP). The right paws of the animals were regarded as RSHP-paws, while the homolateral to the lesion ones were regarded as auto-controls (AC). Animals with saline microinjection were taken in consideration as controls.

The evaluations started 10 min after drugs` administration

Estimation of pain thresholds of the control animals, the AC, and the RSHP without any substances administrated showed that AC and RSHP had higher values than controls with RSHP being the highest (Fig. 1).

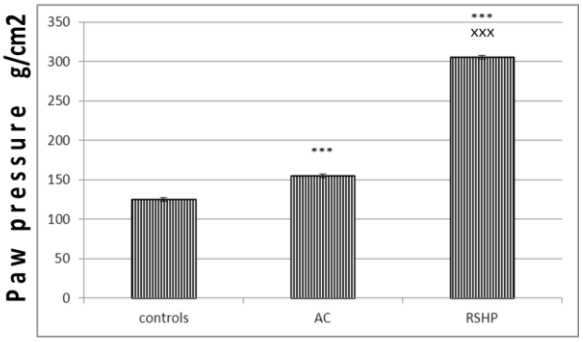

Fig. 1.Pain thresholds of control animals, left auto-control-paws (AC) and right 6-OHDA-hemiparkinsonian paws (RSHP) before evaluated substances administration. The results are represented as mean values ± S.E.M. AC and RSHP were compared to controls (***p<0.001); RSHP were compared to AC (^{xxx}p<0.001).

After AEA injection the pain thresholds of AC and RSHP increased in respect to the control values. AC+AEA values were higher than AC on the 10th min (Fig. 2), and similarly RSHP+AEA were higher than RSHP (Fig. 3).

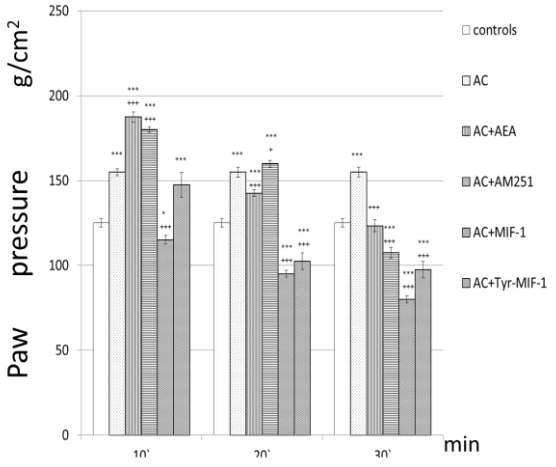

Fig. 2. Effects of AEA (1.0 mg/kg, i.p.), AM251 (1.25 mg/kg, i.p.), MIF-1 (1.0 mg/kg, i.p.), and Tyr-MIF-1 (1.0 mg/kg, i.p.) on the pain threshold of the auto-control (AC) paws in animals with experimental 6-OHDA-RSHP. The results are represented as mean values ± S.E.M. AC, AC+AEA, AC+AM251, AC+MIF-1, and AC+Tyr-MIF-1 were compared to controls (***p<0.001; **p<0.01; *p<0.05); AC+AEA, AC+AM251, AC+MIF-1 and AC+Tyr-MIF-1 were compared to AC ($^{+++}$p<0.001; $^{+}$p<0.05).

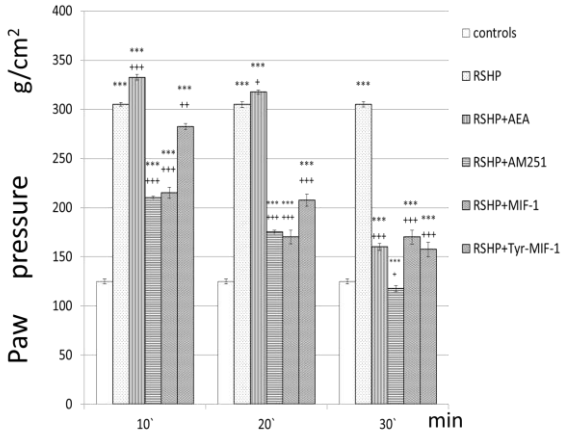

Fig. 3. Effects of AEA (1.0 mg/kg, i.p.), AM251 (1.25 mg/kg, i.p.), MIF-1 (1.0 mg/kg, i.p.), and Tyr-MIF-1 (1.0 mg/kg, i.p.) on the pain thresholds of the lesioned paws in animals with experimental 6-OHDA-RSHP. The results are represented as mean values ± S.E.M. RSHP, RSHP+AEA, RSHP+AM251, RSHP+MIF-1, and RSHP+Tyr-MIF-1 were compared to controls (***p<0.001); RSHP+AEA, RSHP+AM251, RSHP+MIF-1, and RSHP+Tyr-MIF-1 were compared to RSHP ($^{+++}$p<0.001;$^{++}$<0.01; $^{+}$p<0.05).

In a second series of experiments the effects of MIF-1 and Tyr-MIF-1 neuropeptides on nociception in rats with 6-OHDA-RSHP were estimated.

Administration of neuropeptides decreased pain thresholds of both AC- and RSHP- paws compared to values of animals without the substances (Fig. 2 and 3).

In AC-paws the effect was statistically relevant on the 20th and the 30th min of the experiment. Both peptides increased nociception in comparison to AC as well as to controls (Fig. 2).

In RSHP-paws the pronociceptive effect after MIF-1 and Tyr-MIF-1 administration was obvious as soon as the 10th min and remained visible for the whole experimental time. Pain thresholds were lower compared to RSHP without substances, but higher than control values (Fig. 3).

In the next series of experiments the modulating effect of the two neuropeptides on the cannabinoid system in animals with experimental hemi-parkinsonism was evaluated.

MIF-1 and Tyr-MIF-1 administration after AEA in animals with experimental RSHP led to a statistically relevant decrease in pain thresholds of both AC- and lesioned paws compared to AC- and lesioned paws in animals with AEA without the peptides (Fig. 4 and 5).

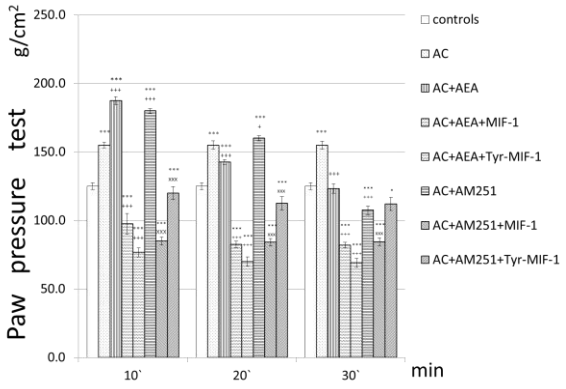

Fig. 4. Effects of MIF-1 and Tyr-MIF-1 (both at 1.0 mg/kg, i.p.) on auto-control (AC) paws pain thresholds of animals with experimental 6-OHDA-RSHP injected with AEA (1mg/kg, i.p.) or AM251 (1.25 mg/kg, i.p.). Results are represented as mean values ± S.E.M. All thresholds of experimental animals were first compared to controls (***p<0.001; *p<0.05); AC+AEA, AC+AEA+MIF-1 and AC+AEA+Tyr-MIF-1 were compared to AC ($^{+++}$p<0.001, $^{+}$p<0.05); AC+AM251+MIF-1 and AC+AM251+Tyr-MIF-1 were compared to AC+AM251(^{xxx} p<0.001).

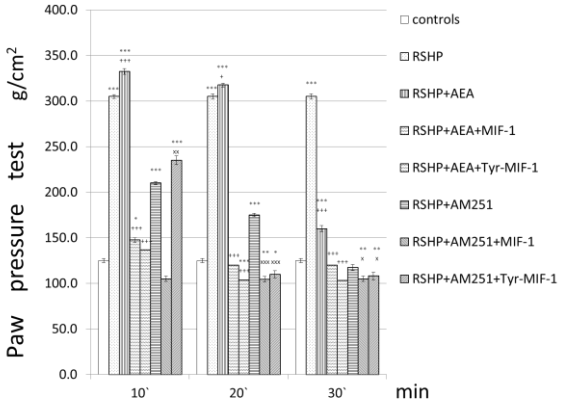

Fig. 5. Effects of MIF-1 and Tyr-MIF-1 (both at 1.0 mg/kg, i.p.) on lesioned paws pain thresholds in animals with experimental 6-OHDA-RSHP injected with AEA (1mg/kg, i.p.) or AM251 (1.25 mg/kg, i.p.). Results are represented as mean values ± S.E.M. All thresholds of experimental animals were first compared to controls (***p<0.001; **p<0.01; *p<0.05); AC+AEA, AC+AEA+MIF-1 and AC+AEA+Tyr-MIF-1 were compared to AC ($^{+++}$p<0.001, $^{+}$p<0.05); AC+AM251+MIF-1 and AC+AM251+Tyr-MIF-1 were compared to AC+AM251(^{xxx} p<0.001; ^{xx} p<0.01; ^{x} p<0.05).

AC+peptides-thresholds decreased for the whole estimated period and were lower than controls, AC, and AC+AEA. A tendency toward hyperalgesia was observed (Fig. 4).

RSHP-paws thresholds showed a statistically relevant decrease in respect to RSHP and RSHP+AEA for the whole experimental time. Compared to controls a slight tendency toward hyperalgesia was detected only for Tyr-MIF-1 on the 20th and 30th min (Fig. 5).

Administration of the CB1 receptor antagonist AM251 increased the pain thresholds of AC-paws compared to animals without the substance on the 10th and 20th min. Compared to the agonist the CB1 antagonist led to comparable thresholds on the 10th min, and even higher thresholds on the 20th min (Fig. 2).

Vice versa CB1 receptor antagonist decreased the pain thresholds of RSHP+AM251 compared to RSHP and RSHP+AEA for the whole estimated time (Fig. 3).

Administration of MIF-1 or Tyr-MIF-1 after CB1 antagonist AM251 decreased the pain thresholds of the AC-paws (AC+AM251+peptides) on the 10th and the 20th min compared to animals with AM251 but without the peptides (AC+AM251). The effect was more pronounced for MIF-1, and a tendency toward hyperalgesia was observed (fig. 4).

The pain thresholds of RSHP-paws after both AM251 and MIF-1 were lower than controls, RSHP, and RSHP+AM251 without peptides. Tyr-MIF-1 led to increase in the pain thresholds on the 10th min, while for the remaining time the values were comparable to MIF-1`s (fig. 5).

The aim of the present study was not to delimitate changes in pain perception from pure motor dysfunctions. Such discrimination would be difficult given the complex interconnection and interrelation between sensory input and motor output underlying motor activity. The purpose was more to establish whether the simultaneous activation of different systems would exert an effect different from the individual effects of each of the systems.

Separately administrated AEA, MIF-1 and Tyr-MIF-1 increased pain thresholds of RSHP-paws of 6-OHDA-hemiparkinsonian animals compared to the controls. Injection of the neuropeptides 10 min after AEA didn`t lead to a cumulative effect, but instead decreased the thresholds toward values equal to the controls`. Paradoxically injection of the neuropeptides 10 min after the antagonist AM251 led to comparable effects. This is substantial with findings of other trials searching relief of bradykinesia using CB1 receptor antagonists – the effects were similar to those described also after CB1 receptor agonists and the inhibitors of the endocannabinoid inactivation, the so-called indirect agonists [28-32]. The presence of CB1 receptors in multiple sites, both in excitatory and inhibitory synapses within the basal ganglia circuitry, might explain such controversial findings.

The AC-paws` thresholds showed statistically relevant differences compared to control values even though, being ipsilateral to the 6-OHDA lesion, they should not be affected by changes. We assume that sensorimotor integration accounts for such findings, since sensorimotor actions demand the synchronized activity of medullar, subcortical and cortical levels, making circuits in series and parallel [33].

Comparison between AC- and RSHP-paws showed that the increase in pain thresholds of individually administered AEA and the peptides was more expressed in RSHP-paws than in AC-ones. The decrease in the thresholds after the combined administration of AEA and the peptides was more pronounced for the AC-paws than for RSHP-ones. A possible explanation may be that the cannabinoid signaling trough the CB1 receptor type is are altered during the course of nigral degeneration in PD [34], changing the impact of the receptor activation. Statistically relevant differences in pain thresholds of AC- and RSHP-paws have also been observed after antagonizing CB1 receptors by AM251. Additional complication for the results` explanation arises from the implication of MIF-1 and Tyr-MIF-1`s receptors. MIF-1 does not interact with opioid receptors and has its own non-opioid receptor [35] and it has been demonstrated that it can modulate the dopaminergic neurotransmission in the nigrostriatal pathways [36]. Tyr-MIF-1 interacts with μ-opioid receptors [35], and AM251 has been demonstrated to act as a μ-opioid receptor antagonist as well as CB1`s one [34]. Such complex interactions account for the final effect.

In conclusion**,** Parkinson`s disease is characterized by a complex pathogenesis with derangement in many of the mediating and modulating systems. Beside the dopaminergic, the cannabinoid, and the opioidergic, other systems (utilizing adenosine, glutamate, GABA, serotonin) also take part in the basal ganglia circuits [37, 38]. Such a constellation complicates the interpretation of experimental data but gives the opportunity for differential approaches to Parkinson`s disease by targeting the different mediatory systems alone and in combinations.

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ПАРКИНСОНОВА БОЛЕСТ: ПОВЛИЯВАНЕ НА БОЛКАТА ОТ КАНАБИНОИДНАТА И ПЕПТИДЕРГИЧНАТА СИСТЕМИ

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(Резюме)

Паркинсоновата болест (ПБ) е резултат от дегенерация на допаминергичните неврони в substantia nigra, като е изяснено, че в патогенезата на заболяването участват множество взаимодействащи си системи. Ендогенната канабиноидна система (ЕКС) е широко разпространена в централната нервна система и повлияването ѝ представлява потенциален терапевтичен подход при различни патологични неврологични състояния, в т.ч. и ПБ. Модулаторният ефект на пептидите MIF-1 и Tyr-MIF-1 върху ЕКС също представлява интерес, като е ясно и съвместното им влияние върху болковата чувствителност при ПБ.

Съвместното повлияване на болката от страна на канабиноидите и невропептидите MIF-1 и Tyr-MIF-1 бе изследвано върху 6-хидроксидопаминов модел на паркинсонизъм у плъх посредством метода Paw pressure test.

Резултатите показаха, че анандамидът и AM251 повлияват болковата перцепция, а MIF-1 и Tyr-MIF-1 модулират ефекта на канабиноидите при ПБ. След антагонизирането на действието на пептидите чрез налоксон болковата перцепция на експерименталните животни се изравни с тази на контролните.