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Research article

Cyclosomatostatin- and haloperidol-induced catalepsy in Wistar rats: Differential responsiveness to sleep deprivation

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ABSTRACT

Total sleep deprivation (SD) has been found to mitigate motor dysfunctions in Parkinson's disease. Apparently, the similar sensitivity of an animal model for parkinsonism would support the model's validity. Recently, we described catalepsy induced in Wistar rats by somatostatin antagonist, cyclosomatostatin (cSST); this model simulates such a disease-associated abnormality as a fall in brain somatostatin levels. To evaluate the similarity between the cSST model and Parkinson's disease, we assessed here the responsiveness of cSST-induced catalepsy to 1-h and 3-h SD. In parallel, the influence of SD on catalepsy induced by a dopamine receptor antagonist, haloperidol, was examined. It was found that the short-term SD failed to influence cataleptic responses of both types (sleep deprived rats and undisturbed ones displayed a similar duration of immobility, $p > 0.05$). By contrast, 3-h SD suppressed ($p < 0.01$) cSST-induced catalepsy, however, enhanced ($p < 0.01$) cataleptic response to haloperidol. Thus, the anti-cataleptic effect of SD appears to be cSST-specific. These findings support the validity of the cSST-induced catalepsy in Wistar rats as a model for parkinsonian motor dysfunctions.

1. Introduction

Parkinson's disease is characterized by a progressive damage to dopaminergic neurons in the substantia nigra and a few other brain regions. Bradykinesia and other extrapyramidal signs are the major features of the disease; these signs are thought to be linked to an inhibition of the brain dopaminergic system [1–3]. However, the exact mechanism for the extrapyramidal features in Parkinson's disease remains far from fully understood.

A number of animal models are currently in use to identify new potential anti-parkinsonian therapies. Neurotoxic models use toxins causing reversible or irreversible injury to dopaminergic neurons; genetic models are based on genetic mutations that either selectively disrupt nigral neurons or are similar to mutations found in familial parkinsonism [4]. Meanwhile, there is presently no model that would use factors characteristic of idiopathic Parkinson's disease (iPD). As it seems, such models may be of special significance in the search for novel anti-parkinsonian agents.

One of the iPD-associated brain abnormalities is a fall in somatostatin levels (for refs., see [5,6]). These changes may be of pathogenic importance as somatostatin has been found to potentiate dopaminergic neurotransmission [7–9].

We have recently [5,6,10] simulated a decrease in brain somatostatin activity in Wistar rats using intracerebral injections of cyclosomatostatin (cSST), an antagonist of somatostatin receptors [11], and found that cSST induces cataleptic response. Catalepsy, an abnormal behavior that resembles bradykinesia and postural rigidity in iPD, is often linked to an inhibition of central dopaminergic activity [12,13] and thereby is used as a model of extrapyramidal dysfunction. In our experiments, the cSST effect was more pronounced in aged rats than in young [5]. Thus, cSST-induced catalepsy displayed the age-dependence similar to that of iPD. Apparently, this property of the model supports its validity.

Further common features of the cSST-induced catalepsy and iPD would support a pathogenic relevance of the somatostatin deficiency for parkinsonian symptoms. One of the remarkable property of iPD is a beneficial response of parkinsonian patients to sleep deprivation [14–16]. In all likelihood, the similar sensitivity of the cSST-induced catalepsy to sleep deprivation would support the model's predictive validity [17,18].

The present work is aimed to evaluate the effect of sleep deprivation on the cSST-induced model of catalepsy. For comparison's sake, haloperidol-induced catalepsy was also examined. Haloperidol, dopamine D_2 receptor antagonist, causes catalepsy that is frequently considered as a model of parkinsonian motor dysfunctions.

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2. Materials and methods

2.1. Animals

The experiments were conducted with 27–28-month-old male Wistar rats. The average life span for these rats is approximately 25 months [19]; given this, the animals in our study can be deemed to be old.

Rats were housed four per cage in a well-ventilated colony room having a 12-h light/dark cycle (lights on at 7:00 A.M.) and temperature of 22 °C. The animals received standard laboratory rat chow and tap water *ad libitum*. The animals were adapted to these conditions for a minimum of two weeks before the experiments. For use in experiments, animals were divided randomly into groups of eight each. The experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Eighth Edition, National Research Council, Washington, DC, USA, 2011) and received the permission of the local Ethics committee.

2.2. Drugs and doses

Cyclo(7-Aminoheptanoyl-Phe-D-Trp-Lys-Thr[Bzl]) (cyclosomatostatin, cSST), haloperidol (HAL), (±)-ketamine hydrochloride (ketamine), xylazine hydrochloride (xylazine) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gentamicin sulfate (Krka, Slovenia) and Polysporin Triple antibiotic ointment (Johnson & Johnson Inc.) were used as well.

cSST and HAL were dissolved in sterile artificial cerebrospinal fluid [20] and in 1% lactic acid [21], respectively. The drugs were given immediately after the sleep-deprivation period.

cSST was injected intracerebroventricularly at the doses of 0.5, 2.0, and 10.0 μg, HAL was used intraperitoneally at the doses of 0.25, 0.5, and 1.0 mg/kg; these doses were chosen based on previous experiments in our laboratory [5,22]. The drug solutions were prepared just before administration.

2.3. Implantation of an intracerebral cannula

The surgical manipulations were performed as described [23]. Before surgery each rat was injected with gentamicin sulfate, 5 mg/kg intramuscularly. The animal was anaesthetized with intraperitoneal administration of ketamine and xylazine (75 and 7.5 mg/kg, respectively) and placed in a stereotaxic apparatus. A hole was drilled into the skull and a stainless steel guide cannula (22 gauge, 15 mm long) lowered into the right lateral ventricle using the following stereotaxic coordinates: ML = −1.6 mm, DV = −3.6 mm, and AP = −0.8 mm from bregma. Cannula was fixed to the skull with jeweler's screws embedded in dental acrylic cement. After placement, this cannula was sealed with a sterile obturator. Skin in the area of dissection was treated with Polysporin Triple antibiotic ointment. Leakage of fluid from the cannula during implantation was considered as evidence of proper cannula placement [24]. Cannula placements were also verified postmortem by sectioning through the brain. After the surgical procedure, the animals were allowed to recover for 10 days during which they were caged individually.

2.4. Intracerebral injections

Microinjection of cSST solution (5 μl) or vehicle into the lateral ventricle was made as described [23,25]. Briefly, there was used a needle connected by polyethylene tubing to a microsyringe. After removal of the obturator, the needle was protruded 1 mm beyond the cannula tip and solution was injected over 30 s. After the injection, the needle remained in place for 30 s before withdrawal to prevent injection fluid backflow through the cannula.

2.5. Assessment of catalepsy

Catalepsy in rodents is a state of temporal immobility characterized by failure to correct an externally imposed unusual posture [26,27]. Bar test was used as a method for quantitative evaluation of catalepsy. Each rat was placed with its fore limbs on a wooden bar (9.0 cm above the table surface, diameter of 1.5 cm). Animal's hind paws rested on the table surface. The length of time (s) until the animal placed both paws on the table surface, or moved its head in an exploratory manner, or began to climb on the bar [28,29] was measured; this time is herein termed as duration of immobility. In the first two days the rat was placed on the bar daily to adapt to it [27]. On the third day, the animal was injected with drug(s) or vehicle, and experimental session was performed. To complete one test, the animal was placed on the bar three times and the mean of these three periods of immobility was held to be the outcome of this test [28]. The time measurements were performed by an experimenter blind to group status. On each session, these measurements were performed 60, 120, 180 and 240 min after administration of the tested solutions with the animals being kept in their home cages between tests. For all the rats, a tactile stimulation (handling) not related to the sleep deprivation procedure was minimized for a minimum of eighteen hours before the start of the experiments.

Catalepsy was defined as a significant ($p < 0.05$) increase in the duration of immobility compared to that in the control group.

2.6. Sleep deprivation

The procedure was performed during the first 1 or 3 h of the light period of the day, while the animals were resided in their home cages. The rats were inspected continuously and woken up by touching them lightly if they assumed a sleep posture [30]. The duration of sleep deprivation (SD) was chosen based on our preliminary data.

2.7. Outline of experiments

Four separate experiments were performed. In the first and second experiments, dose–response relationship for the effects of cSST and HAL was studied. The lowest effective doses of the drugs were determined; these doses were used in further studies. The next experiments were aimed to assess the effects of SD (1-h and 3-h) on cSST- and HAL-induced catalepsy. Intact and vehicle-treated animals were examined as control groups.

2.8. Statistical analysis

The Shapiro–Wilk *W* test was used to assess the normality of the data distribution. Since the normality assumptions could not be accepted, comparisons were made with non-parametric Mann–Whitney *U* test. Differences between intact and vehicle-treated animals, between vehicle- and cSST/HAL-treated animals, and between animals received cSST/HAL alone or in combination with SD were evaluated by a pairwise comparison. Differences with a *p* value of less than 0.05 were considered statistically significant.

Data are expressed as Me (Q1; Q3), where Me is median and (Q1; Q3) is interquartile range: upper limit of lower quartile (Q1) and lower limit of upper quartile (Q3).

3. Results

Intact male Wistar rat displayed motionlessness of 7–16 s duration that is generally consistent with the published results of others (e.g., [31]).