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ALTERED OPEN FIELD BEHAVIOR IN RATS INDUCED BY ACUTE ADMINISTRATION OF 3-NITROPROPIONIC ACID: POSSIBLE GLUTAMATERGIC AND DOPAMINERGIC INVOLVEMENT

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3-nitropropionic acid (3-NP), a substance used for modelling Huntington's disease, was given to male Wistar rats once in 20 mg/kg b.w. dose, and the resulting behavioral alterations in spontaneous locomotor activity were measured after 30 minutes. To detect the involvement of neurotransmitter systems in this immediate effect, the NMDA antagonist MK-801 (0.8 mg/kg); as well as an agonist, quinpirole (QP, 5 mg/kg) and an antagonist, sulpiride (SP, 80 mg/kg) of the dopamine D2 receptors, were given before 3-NP to separate groups of rats. Controls were given saline. All substances were injected ip. 3-NP decreased the rats' locomotor, especially vertical, activity, whereas local activity was increased. Based on the further changes of 3-NP effects in the combination groups it could be concluded that dopaminergic rather than glutamatergic mechanisms were possibly involved in the acute behavioral effect of 3-NP.

Keywords: 3-nitropropionic acid – locomotor activity – open field – rat

INTRODUCTION

The toxin 3-nitropropionic acid (3-NP) is an irreversible inhibitor of succinate dehydrogenase, interfering this way with mitochondrial ATP synthesis [15]. The resulting shortage of ATP causes systemic energy impairment [1] and disturbs central nervous functions [5]. In the affected neurons, reduced ATP supply leads to decreased pumping activity of Na^+/K^+ ATPase, resulting in depolarization, and elimination of the Mg^{2+} block of NMDA channels [15]. Excess activation of NMDA channels results in increase of intracellular Ca^{2+} concentration [8] and cellular damage in neurons expressing this receptor.

3-NP-induced damage is manifested in various behavioural and functional disorders. In rats, systemic administration of 3-NP caused transient motor hyperactivity,

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followed by a longer hypoactive period [6]; or significant hypomotility in pure form [16]. Decreased motor activity is typical also for human Huntington's disease (HD), for modelling of which 3-NP is generally used [1, 27]).

The histological damage observed in 3-NP treated animals (another crucial aspect of the model, [9]) involves important motor and behavioural control centres. The hippocampus, an area rich in NMDA receptors [3], is involved in locomotion and in learning processes, and is susceptible to 3-NP excitotoxicity. Another known site of action of 3-NP is the striatum [12], likewise a motor centre. Here, the dopaminergic system is involved in the damage induced by 3-NP, as reported by Nishino et al. [21]. In the lateral part of the striatum, the centre of tissue damage, D_2 receptors are abundant, and their activation with quinpirole (5 mg/kg) decreased, while inhibition with sulpiride (80 mg/kg) increased tissue damage and motor abnormalities in Nishino's acute model involving repeated injection of 20 mg/kg 3-NP.

Previous results of our laboratory showed that electrophysiological [28] and behavioural [18] alterations appeared within an hour after even a single intraperitoneal injection of a dose of 3-NP equal to Nishino's. This period probably corresponded to the initial ATP level drop (20–25% in 2 hours [22]) and not to the substantial cell death, described to take place later, in 3–48 hours. The rapid action seen in our mentioned works raised the question whether the transmitter-related effects described in longer applications of 3-NP [15, 21] could also be observed in this one-hour time span. A related, wider ranging question could be that of neuroprotection. An antagonist of NMDA receptor, MK-801, was repeatedly mentioned in the literature as a potential neuroprotective agent [1, 8, 13, 24] but its own damaging effect has also been described [11]. Its own behavioural effect was tested in a previous work of us [17]. On the other hand, the role of dopaminergic transmission suggested that an agent acting on dopaminergic receptors also could be of use in counteracting neuronal damage in the 3-NP model.

In the experiments described here, open field behaviour was studied as a continuation of the mentioned work [18, 28], to reveal the role of glutamatergic and dopaminergic transmitter system in the changes seen immediately after application of 3-NP, by means of drugs known to act on the former. In one series, 3-NP was combined with MK-801, and in another, with the above mentioned D_2 receptor agonist quinpirole (QP) and the antagonist sulpiride (SP).

MATERIALS AND METHODS

Animals, drugs and treatments

Adult male Wistar rats (10 weeks old, 180–200 g body weight) were obtained at the University's breeding centre, and were kept under controlled environmental conditions (22–24 °C, 12 h light/dark cycle with light starting at 6.00 am). Standard rodent chow and drinking water was given *ad libitum*.

In the first series of experiments, 10 rats received a single intraperitoneal (ip.) injection of 3-NP (20 mg/kg); while in another 10 rats 3-NP was combined with the NMDA antagonist MK-801 (0.8 mg/kg) in a way that MK-801 was given 30 minutes before 3-NP. In another series, the dopamine D_2 receptor agonist quinpirole (QP, 5 mg/kg) or the antagonist sulpiride (SP, 80 mg/kg) were first injected subcutaneously to 10 rats each, and 3-NP was given ip. 15 min later. Controls (n = 10) were given 1.0 ml/kg b.w. saline. 3-NP, QP and SP were purchased from Sigma Aldrich GmbH (Germany) and were dissolved to 1.0 ml/kg body weight administration volume in saline, except SP which was dissolved in dimethyl sulfoxide (DMSO). The amount of DMSO injected, ca. 1000 mg/kg, had most probably no significant own effect, as 790 mg/kg DMSO daily for 10 days did not alter motor performance [2]. Drug doses and times of injection were based on previous results [18, 28], and on literature data (3-NP: 12,21; MK-801: 13; QP and SP: 21).

Behavioral testing

An automated open field (OF) apparatus (Conducta 1.0 System, Experimetria Ltd., Hungary) was used to investigate the rats' exploratory activity in 10-minute sessions. The rats were placed one by one in the OF box (48 × 48 × 40 cm) where motility parameters – the time spent with horizontal, vertical and local activity – were recorded by the interruption of infrared beams (at floor level and in 12 cm height). A more than 40 mm shift in the location of interrupted floor level beams during the time resolution unit of 1 s was interpreted as ambulatory movement (running); less shift, as local activity (grooming); and no shift, as immobility. Vertical activity (rearing) was recorded if beams at the floor level and at the higher level were interrupted simultaneously. The animals were brought to the testing room immediately after the injection of 3-NP (or the last injection in case of combinations) and were allowed there to acclimate for 30 min. So there was a 30 min interval between drug administration and OF test. The OF investigation was performed during the period of 8.00 am. to 2.00 pm.

Statistical analysis

The distribution of OF data was checked for normality by Kolmogorov-Smirnov test. In case of normal distribution, all data were tested by one-way analysis of variance (ANOVA) (*post hoc* Scheffe test). At non-normal distribution, by Kruskal-Wallis (*post hoc* Mann-Whitney test) was used. Significance was accepted at $p < 0.05$ in all tests. The software pack SPSS 9.0 was used to the statistical analysis.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

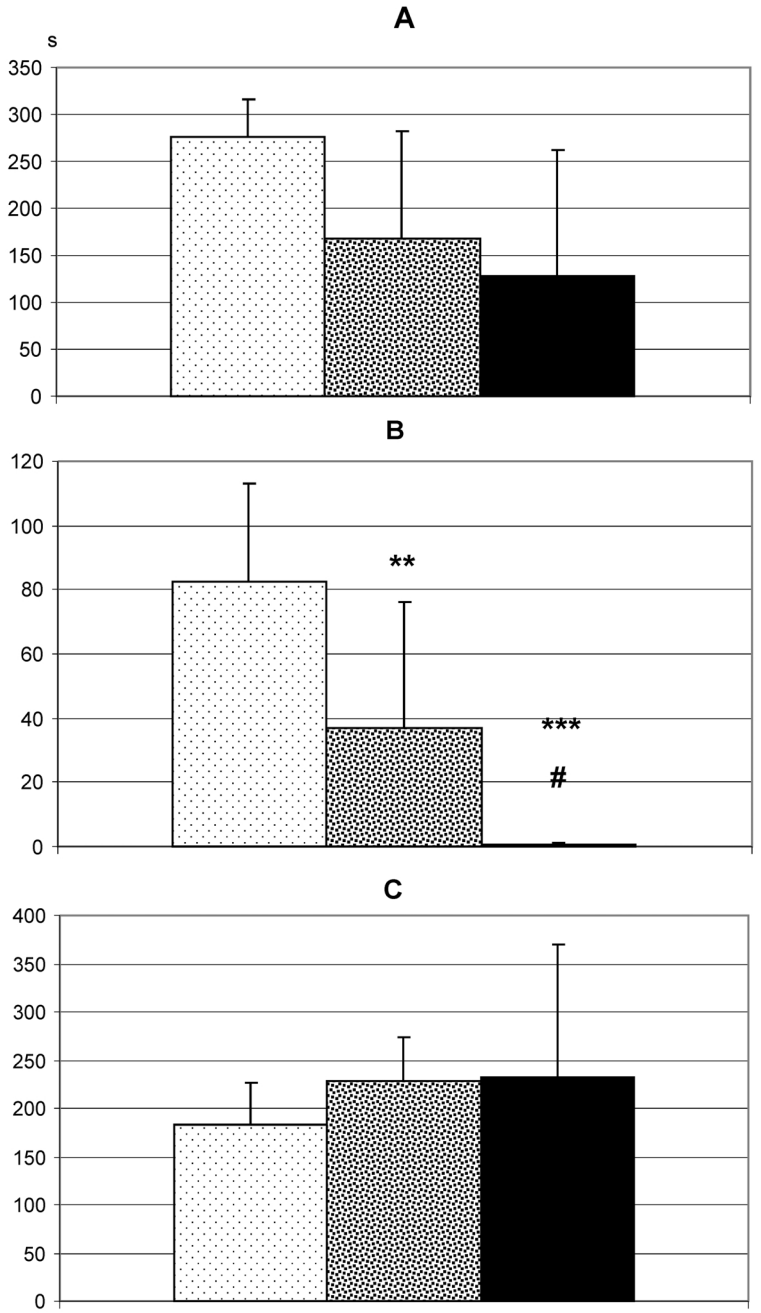

Fig. 1. Spontaneous horizontal (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box, 30 min after the treatments indicated in panel **A** (Control, saline; 3-NP, 20 mg/kg ip.; MK-801+3-NP, 0.8+20 mg/kg ip., Mk-801 first). Ordinate: time (s) spent in the given forms of activity; mean + S.D., n = 10. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group; $^{\#}p < 0.05$ vs. 3-NP-treated group

RESULTS

A single dose of 3-NP decreased – however with no significance – the rats' horizontal activity compared to the control group (Fig. 1A). This diminishing effect of 3-NP was preserved when it was given after the NMDA antagonist MK-801 in the combination group. The vertical activity of the rats was significantly ($F_{2,27} = 20.846$; $p < 0.01$) decreased by 3-NP given alone (Fig. 1B). In the MK-801 combination group, further significant decrease of the vertical activity was seen in comparison to the control ($F_{2,27} = 20.846$; $p < 0.001$) and to the 3-NP treated group ($F_{2,27} = 20.846$; $p < 0.05$). Some increase in the local motility was also observed in the 3-NP and in the MK-801+3-NP group (Fig. 1C).

In the other series, where 3-NP was combined with the dopaminergic agents QP and SP, both caused further decrease of horizontal activity (significant vs. control, QP: $F_{3,36} = 9.757$, $p < 0.01$; SP: $F_{3,36} = 9.757$, $p < 0.001$) when given to the rats before 3-NP administration (Fig. 2A). Like in the first series, 3-NP decreased significantly the vertical activity of the rats (Kruskal-Wallis test: $Chi^2 = 23.81$, $p < 0.01$ vs. control). The effect of QP+3-NP and SP+3-NP was similar (Fig. 2B) but more intense, so that the decrease was significant both vs. control (QP+3-NP: $Chi^2 = 23.81$, $p < 0.001$; SP+3-NP: $Chi^2 = 23.81$, $p < 0.001$) and vs. 3-NP (QP+3-NP: $Chi^2 = 23.81$, $p < 0.05$; SP+3-NP: $Chi^2 = 23.81$, $p < 0.05$). Local activity was slightly altered by 3-NP (Fig. 2C). Compared to that, QP+3-NP caused an increase which was significant vs. control ($F_{3,36} = 43.718$, $p < 0.001$) and vs. 3-NP ($F_{3,36} = 43.718$, $p < 0.001$). SP, on the contrary, had no influence on the local activity.

DISCUSSION

At 30 min after administration, the vertical OF activity of the 3-NP treated rats was significantly lower than the controls'. The decreasing trend of horizontal and increasing trend of local activity, although without significance, was in line with the former, and was consistent with the observation of Seaman [25], who detected similar hypoactivity in rats systemically treated with 3-NP. The relationship of motor activity level and DA concentration [20] and the reduced striatal DA level following intrastriatal injections of 3-NP [4, 7] suggested that hypomotility was due to decreased dopaminergic activity also in our acute model.

First of all vertical motility is a sensitive indicator of striatal dopaminergic activity. In an animal model of Parkinson's disease [26], mice showed decreased rearing activity following administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) a drug selectively affecting dopaminergic neurons. So the significantly reduced rearing in the 3-NP treated rats in our experiments was a strong indicator of its effect on DA concentration; and the results of the combinations with QP and SP were in accordance with that. The acute effect of 3-NP on horizontal and vertical open field activity was significantly strengthened by QP and SP; whereby QP acted selectively on presynaptic D_2 autoreceptors and SP had antagonistic effect on the

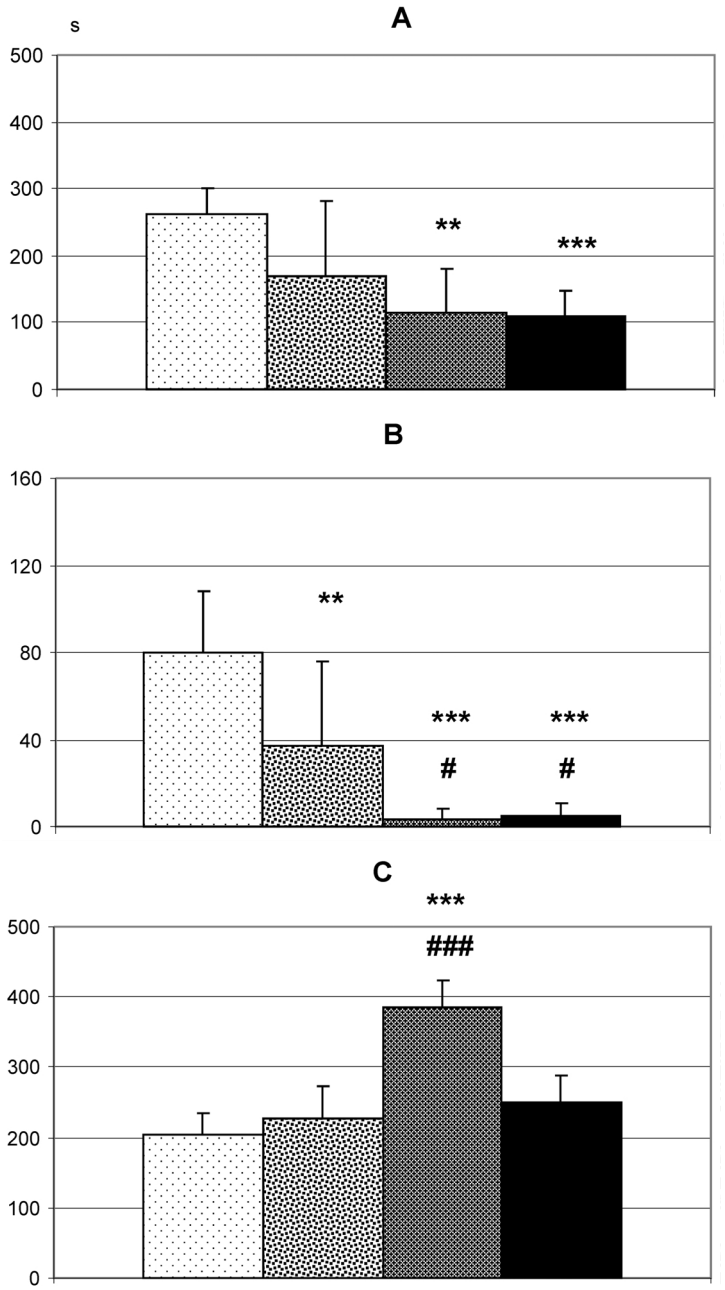

Fig. 2. Spontaneous horizontal (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box 30 min after the treatments indicated in panel **A** (Control, saline; 3-NP, 20 mg/kg ip.; QP+3-NP, 5+20 mg/kg, quinpirole first; SP+3-NP, 80+20 mg/kg, sulpiride first). Ordinate: time (s) spent in the given forms of activity; mean + S.D, n = 10. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. control group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. 3-NP-treated group

postsynaptic D_2 receptors [21], both resulting in decreased release and/or action of DA in the striatum.

3-NP is supposed to indirectly remove the voltage-dependent Mg^{2+} block from the NMDA receptor ion channel [14], causing endogenous glutamate excitotoxicity. This mechanism can theoretically be blocked by antagonists of NMDA receptors such as MK-801, a compound found to be neuroprotective, at least in experimental settings [23]. In our present experiment (and previously, [19]) the effect of MK-801 on OF activity was, in contrast to what could be expected on the basis of literature data, not opposite to that of 3-NP. It was, possibly, independent of central motor control and reflected the action of MK-801 at peripheral NMDA receptors and/or at the neuromuscular junction. Glutamate is a co-transmitter in the neuromuscular junction, acting on NMDA receptors [13] and an NMDA antagonist can inhibit muscle contraction and lead to hypomotility and/or ataxia.

According to our OF results, 3-NP may decrease DA concentration in the striatum. The dorsal striatum, comprising the caudate and putamen, is involved in the regulation of locomotion, and the reduction of DA release here (which is the probable cause of hypomotility) is mediated by an indirect inhibitory mechanism. Corticostriatal axons, mainly from the primary motor cortex, exert a dual presynaptic influence on DA release, because presynaptic D_2 receptors, identified mainly on corticostriatal axon terminals [10], finally decrease the activation of the striatal neurons.

The aim of the work was to understand more of the behavioural aspect of the neurotoxic action of 3-NP, by means of combination with glutamatergic and dopaminergic agents. Our behavioural tests showed rather clearly that 3-NP exerted its immediate toxic effect more through dopaminergic, and less through glutamatergic mechanisms, especially in the striatum. Behavioral methods may contribute to further development of the HD model based on 3-NP; providing an opportunity to monitor the effects of agents, inducing or preventing HD-relevant damage, in a non-invasive way. Beyond modelling HD, the present results also may gain importance in improving the therapy of other chronic neurodegenerative diseases.

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