BEHAVIOURAL ALTERATIONS INDUCED BY ACUTE AND SUBACUTE ADMINISTRATION OF 3-NITROPROPIONIC ACID IN RATS*

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ABSTRACT: Animals treated with the succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NP) mimic the brain lesions and dysfunctions seen in Huntington's disease and, consequently, treatment with 3-NP can serve to model this disease. In rats, acute and subacute systemic administration of 3-NP caused decreased locomotor activity and altered pre-pulse inhibition. In the present study, male Wistar rats received systemically 10 or 20 mg/kg 3-NP; as a single dose (acute treatment) or in six consecutive injections (subacute treatment). Controls were given saline. Before the treatment, 30 minutes after the single administration, and after the sixth injection in the subacute treatment, behavioural investigations (open field activity, acoustic startle response, and rota-rod) were done. In acute treatment, low dose of 3-NP significantly decreased the horizontal activity and caused significant local hyperactivity in the open field test. In the acoustic startle response, the number of „noise-positive responses" displayed nosignificant change. Low dose of 3-NP reduced the time spent on the rod. In subacute treatment, decreased horizontal and local activity was measured; the vertical activity significantly decreased in the high-dose group compared to the low-dose one. The differences in the number of acoustic startle responses were below the level of significance. 3-NP had a negative effect on the time spent on the rod. Our results showed that 3-NP had an effect on the locomotor activity and on the pre-pulse inhibition of acoustic startle. The results can contribute to the understanding of the mechanism of 3-NP toxicity and thereby they might prove useful in developing neuroprotective strategies in models based on the neurotoxic effect.

KEY WORDS: 3-nitropropionic acid, behavioural alterations, rat

INTRODUCTION

The succinate dehydrogenase inhibitor 3-nitropropionic acid (3-NP) – a mitochondrial toxin – is produced by *Arthrinium* fungi and numerous plant species of *Leguminosae*. *Arthrinium* spp. can infest sugar cane, soybeans, and peanuts. Consumption of mouldy foods can, therefore, lead to human poisoning (Johnson et al., 2000). Victims of 3-NP poisoning developed acute encephalopathy and late-onset dystonia. After high dose acute and chronic intoxications, permanent brain damage was observed (Ludolph et al., 1991).

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In mice and rats, high dose of 3-NP (20 mg/kg) caused symmetric degeneration primarily in the striatum, with concomitant damage in the hippocampus, thalamus, and cortex (Kodsi and Swerdlow, 1997). In rats, 3-NP-induced striatal damage leads to specific motor and behavioural alterations, similarly to those observed in human Huntington's disease. These included hypoactivity (Borlongan et al., 1997) and altered sensorimotor inhibition (Seaman, 2000), the latter being a symptom observed in several human diseases involving the striatum (Braff and Geyer, 1990).

In this study, our aim was to see the acute and subacute systemic neurobehavioural effects of 3-NP in open field, rota-rod, and startle tests.

MATERIALS AND METHODS

Male Wistar rats (150–160 g) were obtained from the University's breeding centre and kept under standard conditions. In acute treatment, three groups of 10 rats were treated intraperitoneally with 10 mg/kg (low dose), 20 mg/kg (high dose) 3-NP (purchased from Sigma Aldrich Ltd.), or saline (control), respectively. In subacute treatment, the groups of rats (again 10/group) were injected, with the same doses of 3-NP as in the acute treatment, every other day, altogether 6 times. Controls were given saline. The behavioural investigations included spontaneous open field activity, acoustic startle response, and rota-rod test that were performed before the treatment, 30 minutes after the single injection, or after the sixth injection in the subacute experiment.

The rats' motility and spontaneous locomotor activity was measured in an automated open-field (OF) apparatus (Conducta 1.0 System, Experimetria Ltd.), in 10-minute sessions. Locomotor activity of the rats was detected by two arrays of infrared light gates (at floor level and in 12 cm height) in the OF box, and the time spent by horizontal, vertical, and local activity, and immobility was computed on the basis of infrared beam interruptions. If there was more than 40 mm shift in the location of interrupted beams at the floor level during a time unit of 1 s, locomotion was recorded, if there was less shift, local activity, and if there was no shift at all, immobility. Rearing was recorded if beams at the floor level and at the higher level were simultaneously interrupted.

The acoustic startle response (ASR) is a contraction of body muscles evoked by a sudden beep (in this study: 110 dB, 5000 Hz, 200 ms) (Koch, 1999), which, in our studies, was recorded on the bottom of the cage by a piezo force transducer. A muscle twitch producing more than 50 g force on the bottom was accepted as "noise-positive response", of which the latency, time to peak, and peak amplitude were calculated (Responder X System, Columbus Instruments, Ohio, USA). Following 15 min rest in the test cage, pre-pulse inhibition (PPI), which is the normal suppression of ASR by a preceding stimulus (73 dB, 1000 Hz, 500 ms) (Braff and Geyer, 1990), was tested.

As an indicator of nigrostriatal lesions, we measured the time that the rats were able to spend on the rod, rotating from 1 to 10 r.p.m. (Ugo Basile, Italy), in sessions of 5 min (Sedelis et al., 2001). The rats received first a preliminary training on the rota-rod on 5 consecutive days, then they were able to stay on the rod. Drugs acting on the dopaminergic system are known to cause decreased motor skills in the rota-rod test (Rozas et al., 1998).

The number of "noise-positive responses" of the ASR and PPI tests was evaluated by χ^2 test ($p<0.05$). Distribution of OF, rota-rod, and other ASR/PPI data was checked for normality by Kolmogorov-Smirnov test. In case of normal distribution, data were analysed by one-way ANOVA and post hoc Scheffe test ($p<0.05$). In case of non-normal distribution, Kruskal-Wallis test was done. For the statistical analysis SPSS 9.0 for Windows was used.

RESULTS

In the OF test, significantly decreased horizontal activity (*Fig. 1A*) and increased local motility (*Fig. 1B*) was observed in the low-dose group 30 min after 3-NP administration in the acutely treated rats. In the high-dose group, changes in the horizontal and local OF activity were below the level of significance compared to the controls. The rearing activity was apparently reduced in both groups, maybe, in a dose-dependent way (*Fig. 1C*). The time spent in immobility significantly increased in the low-dose group compared to the controls (*Fig. 1D*).

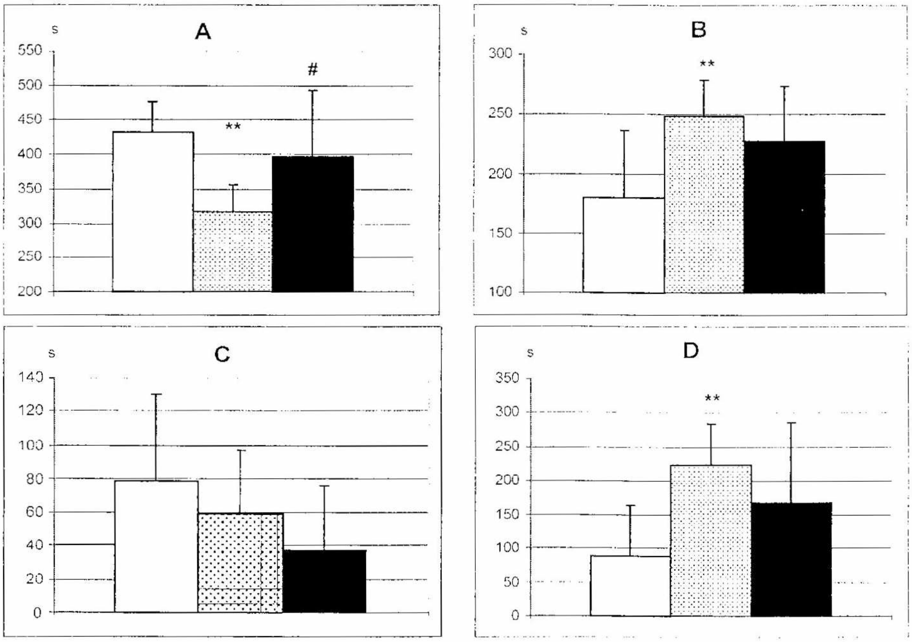

*Fig 1. Horizontal (A), local (B), vertical (C) open-field activity, and time spent in immobility (D) 30 minutes after acute 3-NP treatment. Ordinate: time (s) spent in the given forms of activity, mean+SD. ** p<0.01 vs. control, # p<0.05 vs. low dose.* □ – *control,* ⊡ – *low dose,* ■ – *high dose*

In the subacute treatment, 3-NP seemed to induce horizontal hypoactivity (*Fig. 2A*). Significantly decreased local motility was detected in the low-dose group (*Fig. 2B*). Changes in vertical activity were variable, the low dose of the toxin increased and the high dose decreased the rats' rearing activity (*Fig. 2C*). The time spent in immobility tended to increase in both treated groups (*Fig. 2D*).

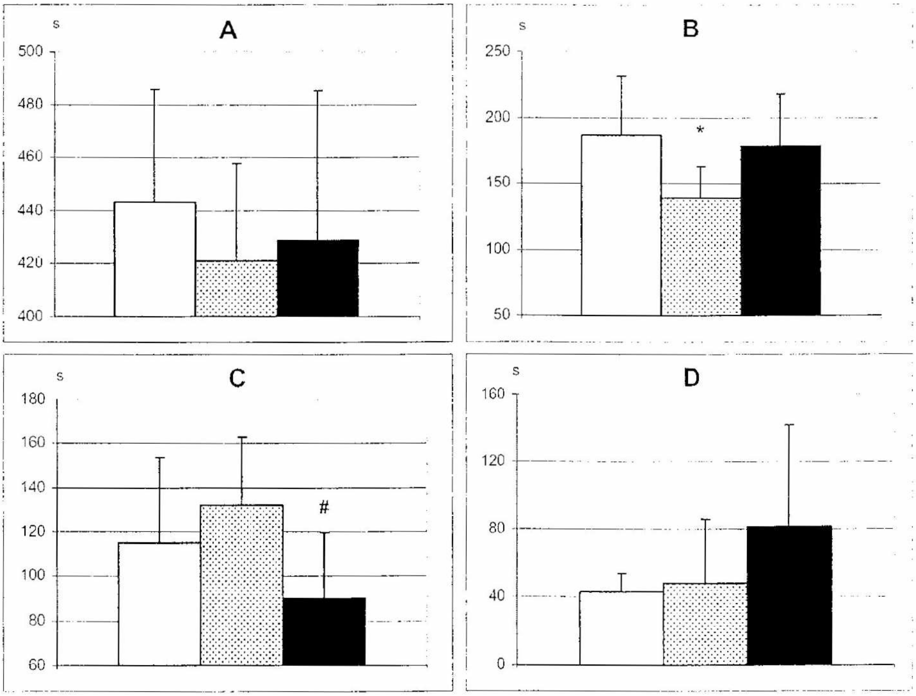

*Fig 2. Horizontal (A), local (B), vertical (C) open-field activity, and time spent in immobility (D) after subacute 3-NP treatment. Ordinate: time (s) spent in the given forms of activity, mean+SD. * p<0.05 vs. control, # p<0.05 vs. low dose. □ – control, ⬚ – low dose, ■ – high dose*

In the acute treatment, the number of ASR-positive reactions seemed to decrease in both treated groups, but this effect remained below the level of significance. In the control group, PPI was present (that is, the number of "noise-positive responses" decreased following a pre-pulse stimulus indicating an intact sensorimotor gating mechanism) in contrast to the treated groups where the number of positive reactions was not altered at all by the pre-pulse (*Fig. 3*). In the subacute treatment, dose-dependently increasing number of "noise-positive responses" was observed in the treated groups. Here, PPI did not significantly differ in the control and treated groups (*Fig. 4*).

Neither acute nor subacute 3-NP had any significant effect on the time spent on the rod.

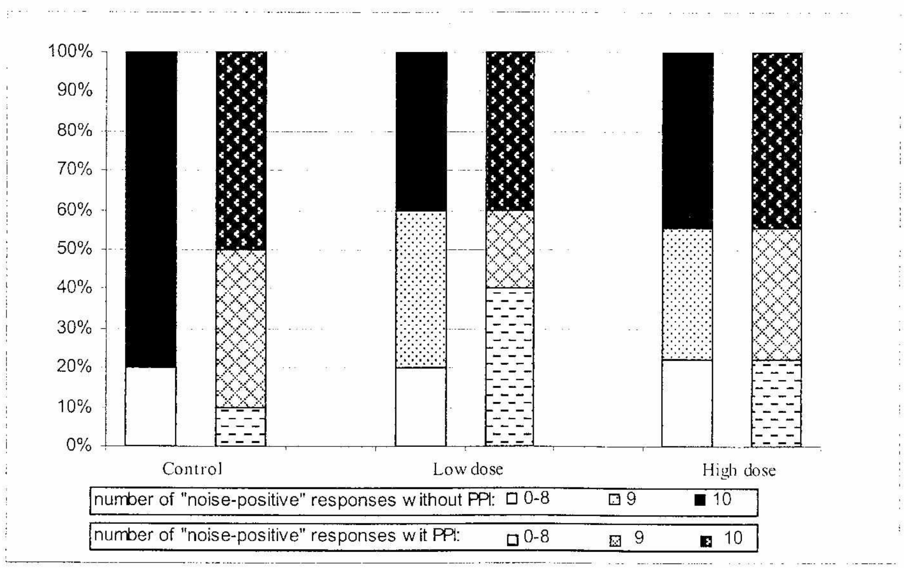

Fig 3. Number of "noise-positive responses" 30 minutes after acute 3-NP treatment. In each group, the left column represents the distribution of responses without pre-pulse and the right column that with pre-pulse stimulus (the difference of the two columns indicates the extent of pre-pulse inhibition). Ordinate: percentage of the animals giving as many noise-positive responses as indicated in the insert

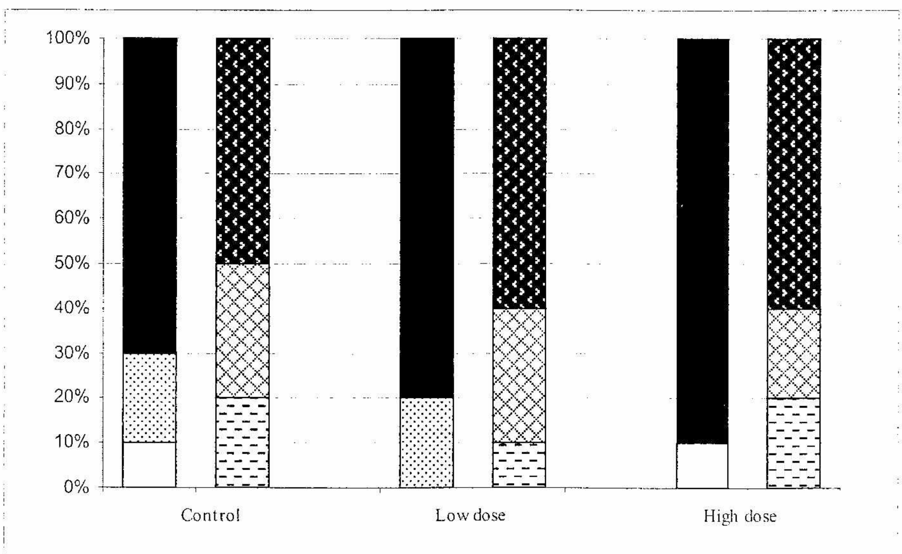

Fig 4. Number of "noise-positive responses" following subacute 3-NP treatment. The same display as in Fig. 3

DISCUSSION

In the rats treated with 3-NP, the horizontal and vertical components of the spontaneous motor activity were mostly reduced while the changes of local activity were dissimilar in acute vs. subacute application.

In the control of nocturnal spontaneous locomotor activity, the dorsal striatum may play a significant role in rats (Borlongan et al., 1995). According to the literature, 3-NP neurotoxicity is due to a neuronal loss in the striatum (Sato et al., 1997). Decreased horizontal activity is induced by changes in the dopaminergic system, which indicates that the effect of 3-NP observed in our study might have been a result of a damage in the striatal dopaminergic system. The alterations in the spontaneous locomotor activity were significant after the low, but did not reach the level of significance after the high dose of 3-NP, which may be explained by the dose-dependence of 3-NP-induced lesions and concomitant motor symptoms (Nishino et al., 1997). Kodsi and Swerdlow (1997) described that 10 or 15 mg/kg 3-NP caused no striatal lesions in the treated rats; whereas striatal lesions were observed in some of the rats treated with 20 mg/kg 3-NP, but in others, there was no histological damage at all. Variable sensitivity of rats seems thus to be reflected in the central nervous effects of 3-NP. Besides, at the 20 mg/kg dose, altered mitochondrial function and abnormal energy status may cover the effects resulting from NMDA excitotoxicity induced by 3-NP.

In the ASR test, decreasing tendency was seen in the rats' responsiveness following acute 3-NP treatment, but increasing in subacute application, whereas PPI of ASR was decreased by 3-NP in the acute, but not in the subacute experiment. PPI is known to be influenced by the sensitivity of dopamine autoreceptors in the nucleus accumbens (Yamada et al., 1998) and by the activity of the mesolimbic dopamine system (Koch and Schnitzler, 1997). This, together with the particular sensitivity of dopaminergic neurons to 3-NP (Pei and Ebendal, 1995), suggests that 3-NP altered PPI via acting on the dopaminergic system 30 minutes after its application. In the caudal pontine reticular nucleus, the main sensomotor interface of the PPI circuit (Koch, 1999), glutamatergic transmission has an important role. In the mechanism of 3-NP neurotoxicity – beyond the blockade of ATP synthesis – a secondary excitotoxicity is implicated. 3-NP inhibits glutamate uptake into rat brain synaptic vesicles (Tavares et al., 2001), leading to increased concentration of this excitatory transmitter which may contribute to excitotoxicity. Thus, an interaction between glutamate- and dopaminergic systems may underlie the mechanism of 3-NP toxicity.

In the present study, there were more significant changes immediately after a single dose of 3-NP than following multiple doses. According to the literature, in rats the plasma concentration of 3-NP fell to 10% of the peak value within 40 minutes (Schulz et al., 1996). Also, 3-NP decreased the activity of succinate dehydrogenase in a time- and dose-dependent way (Ray, 1999). The effect of 3-NP on behaviour seemed to be non-additive, on one hand, because massive inhibition of succinate dehydrogenase did not follow the earlier weak inhibition, and, on the other hand, because the 12 days of the subacute experiment were too short for any histological brain damage to develop.

The results of the present study, together with the recent ones (Lukács et al., 2005; Lukács et al., 2006) indicate that behavioural investigations may be suitable for the monitoring of the central nervous effects of 3-NP, which may be important in modelling of chronic, neurodegenerative diseases and may prove useful in evaluating potential neuroprotective therapies.

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