ELSEVIER

European Journal of Pharmacology 330 (1997) 139–142

ejp

Short communication

The effect of 3-acetylpyridine on inferior olivary neuron degeneration in Lurcher mutant and wild-type mice

Keith W.T. Caddy [a,*], František Vožeh [b]

[a] *Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK*
[b] *Department of Pathophysiology, Medical Faculty, Charles University Plzeò, 301 66 Plzeò, Lidická 1, Czech Republic*

Received 22 May 1997; accepted 23 May 1997

Abstract

Lurcher mutant and wild-type mice were given intraperitoneal injections of 3-acetylpyridine to look at the toxic effects of this drug on the inferior olivary neurons. Intraperitoneal administration of 3-acetylpyridine is characterized by the different sensitivity of inferior olivary neurons in Lurcher mutant and wild-type mice. Lurcher mutants suffered a destruction of these neurons while wild-type mice were unaffected. The results show that there is a different effect of 3-acetylpyridine between genetic mutations and wild-type mice on the same inbred strain of mice. The different affinity of 3-acetylpyridine for the inferior olivary neurons of this mutant is briefly discussed. © 1997 Elsevier Science B.V.

Keywords: 3-Acetylpyridine; Inferior olivary neuron; Lurcher mutant mouse; NMDA (*N*-methyl-D-aspartate), inhibition; Niacinamide

1. Introduction

3-Acetylpyridine is a neurotoxin and a niacinamide receptor antagonist. It is known to be an effective agent for inducing degeneration in the inferior olivary nucleus and some other brain structures in rodents (Llinás et al., 1975; Balaban, 1985; Heikkila, 1985; Jones et al., 1994; Schulz et al., 1994). The neurotoxicity of 3-acetylpyridine is antagonized by a preceding, concurrent or sequential administration of niacinamide in vivo (Llinás et al., 1975; Balaban, 1985) or in vitro (Weller et al., 1992). These findings suggest that 3-acetylpyridine toxicity is caused by competition of 3-acetylpyridine with niacinamide in the formation of niacinamide dinucleotides (NAD) and NAD phosphate (NADP). The functional depletion of niacinamide leads to a disturbance of hydride ion transfer between enzymes and substrates using NADH and NADPH as cofactors. This particularly affects many dehydrogenases (e.g., malate dehydrogenase, lactate dehydrogenase, α-ketoglutarate dehydrogenase, glucose-6-phosphate dehydrogenase and glutamate dehydrogenase) and a depletion of substrate for the electron transport chain (Schulz et al., 1994).

The 3-acetylpyridine induces degenerative lesions after single intraperitoneal injections and because the inferior olivary neurons are the site of origin of the climbing fibres which innervate Purkinje cells in the cerebellum, this neurotoxin has been used extensively to study cerebellar pathophysiology. Other target structures of 3-acetylpyridine toxicity include the hippocampal formation, the substantia nigra, the nucleus ambiguus, the hypoglossal nucleus, the dorsal motor nucleus of the vagus nerve, the interpeduncular nucleus, the supraopticus and the paraventricularis nuclei, and the horizontal limb of the nucleus of the diagonal band (as shown by experiments performed mainly in rats) (Balaban, 1985; Schulz et al., 1994).

Our study focuses on the Lurcher mutant and wild-type mice. The Lurcher is characterized by genetically determined spontaneous degeneration of cerebellar Purkinje cells, granule cells and inferior olivary neurons. Heterozygote individuals (+/Lc) of this mutant suffer a rapid and complete loss of cerebellar Purkinje cells during postnatal development. By postnatal day 26 (P26) only 10% of Purkinje cells present in the wild type (+/+) remain in the +/Lc, and this figure probably falls to zero by P90. Granule cells and inferior olivary neurons are also reduced to 10% and 25% of the +/+ respectively (Caddy and Biscoe, 1979).

* Corresponding author. Tel.: (44-171) 419-3210; Fax: (44-171) 383-7005; e-mail: k.caddy@ucl.ac.uk

PII S0014-2999(97)01030-3

2. Materials and methods

In our experiments a total of 37 animals of both sexes were used. Infant mice were 8–19 days postnatal (P8–P19) and young adults were 62 days old (P62) when the animals received their injections. All the mice used in these experiments were on the same (C3H) background and all animals for each age group were littermates. The mice were maintained at a temperature of 23 ± 2°C and humidity of 55 ± 15% under a 12 h light/12 h dark cycle with free access to food and water. The Lurcher mutant and wild-type animals were treated with 3-acetylpyridine, harmaline and niacinamide, using modifications to the protocol of Llinás et al. (1975). The mice were given intraperitoneal (i.p.) injections of 200 mg/kg body weight of 3-acetylpyridine followed 15 min later by i.p. injection of 15 mg/kg body weight harmaline. The last i.p. injection of 300 mg/kg body weight niacinamide was given 3 h after the initial 3-acetylpyridine injection. Control animals ($n = 6$) were injected with physiological saline in place of the 3-acetylpyridine followed by the administration of harmaline and niacinamide as in the experimental animals. After 2 weeks the animals were given an overdose of pentobarbital sodium and perfused transcardially with a solution of 4% paraformaldehyde in phosphate buffer. The brains were removed and the dissected parts containing cerebellum, brain stem and medulla oblongata were placed into the same fixative solution for 1 h at room temperature. Afterwards the brains were immersed in a mixture of 10% polyvinylpyrrolidone and 6% sucrose in distilled water for 6 days at 4°C for cryoprotection. Frozen serial transverse sections, 40 μm thick, from both experimental and control animals were stained with cresyl violet and examined under the light microscope. Preliminary quantitative analysis of the number of neurons in the inferior olive was performed by counting the number of neurons in random areas and expressing the results as number of cells per unit area. This permits direct comparison between Lurcher control and injected mice.

3. Results

Histological examination of the inferior olivary nucleus revealed a destruction of the neurons in both young and adult Lurcher mutant animals when compared with wild-type and control animals. Compare the micrograph of the inferior olive from a Lurcher mouse treated with 3-acetylpyridine (Fig. 1A) with a Lurcher injected with saline (Fig. 1B) and a wild-type mouse injected with 3-acetylpyridine (Fig. 1C). There was a significantly lower number of the inferior olivary neurons in the Lurcher mutant animals, and the remaining neurons appeared shrunken and hyperchromatic with irregular nuclei (filled arrow in Fig. 1A). The density of inferior olivary neurons was estimated from the cresyl violet stained sections. For the wild-type and Lurcher control mice the density of olivary neurons was calculated to be 17.0 ± 4.1 ($\pm$S.D.) cells/0.01 mm^2 and 18.9 ± 3.1 ($\pm$S.D.) cells/0.01 mm^2. The density of olivary neurons in the 3-acetylpyridine treated wild-type animals was not

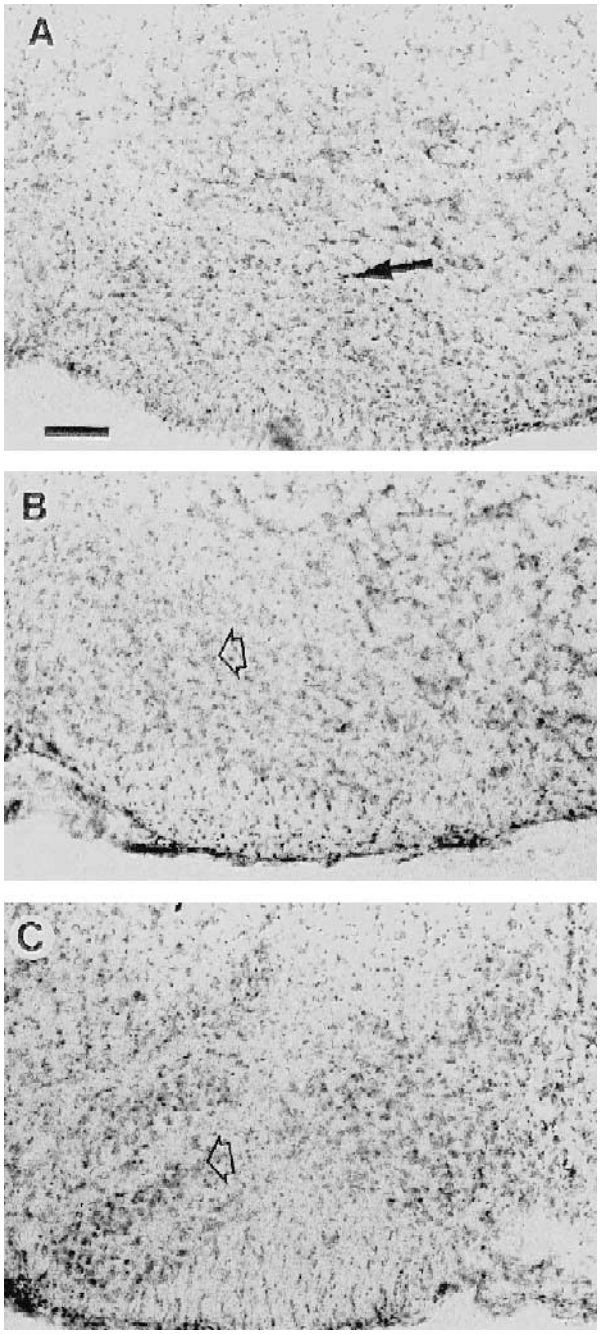

Fig. 1. These photomicrographs are taken from 40 μm thick frozen sections stained with cresyl violet. All these mice were injected on P14 and perfused on P28. Magnification bar = 100 μm. (A) Inferior olive from a Lurcher mouse injected with 3-acetylpyridine. Filled arrow indicates a group of hyperchromatic neurons. (B) Inferior olive from a Lurcher mouse injected with saline. Open arrow indicates a typical neuron which are infrequently seen in mice injected with 3-acetylpyridine. (C) Inferior olive from a wild-type mouse injected with 3-acetylpyridine. The neurons (open arrow) look normal and are no different from those found in the control wild-type mice (not illustrated).

different from the control values but the 3-acetylpyridine injected Lurcher animals had a reduced density of 10.6 ± 5.8 (± S.D.) cells/mm^2. These values indicated that there is a reduction in the Lurcher inferior olivary neuron density in mice treated with 3-acetylpyridine but not in the wild-type mouse. It appears that as a consequence of this loss of neurons there has been a proliferation of glial cells (see Fig. 1A). No significant differences were observed between the morphology of the inferior olive in the 3-acetylpyridine treated wild-type mice and controls of both age groups. Also no differences were found between male and female mice in both experimental and control animals.

The present results show a more destructive effect of 3-acetylpyridine on the inferior olivary neurons in the Lurcher mutant compared with wild-type mice (compare Fig. 1A and C).

4. Discussion

The results indicate that there is no difference in the inferior olivary neurons's sensitivity to 3-acetylpyridine in the immature Lurcher compared to the young adult Lurcher. These findings are unlike those found in rats by Anderson and Flumerfelt (1984). These authors showed that the inferior olivary neurons in young weanling animals were less sensitive to the toxic effects of 3-acetylpyridine than adult rats. In mice, there have been very few experiments studying the effect of 3-acetylpyridine on the inferior olivary neurons, especially in immature animals, but there have been many experiments using rats. Nevertheless Ozaki et al. (1983) described a partial degeneration of inferior olive complex and pyramidal cells in the CA3 hippocampal area in young adult mice of the ddN-F26 strain treated with 3-acetylpyridine. Heikkila (1985) has found a different toxic effect of another pyridine, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in agreement with variable neostriatal dopamine levels in young adult Swiss-Webster mice from different sources. Similarly Zimmer and Geneser (1987) have shown a higher brain monoamine oxidase B activity in the basal ganglia and the substantia nigra of C57 mice compared to adult albino NHRI mice at the same age. Because monoamine oxidase B is the key enzyme in the conversion of MPTP to the *N*-methyl-4-phenyl-pyridine ion (MPP$^+$) which causes degeneration of dopaminergic nigral neurons, the higher activity of this substance may explain differences in the susceptibility to MPTP. This conclusion was based on the demonstration of MPTP induced decrements of dopamine and its metabolites, which are a reflection of the neurotoxic process (Zimmer and Geneser, 1987). However, Deutch et al. (1989) have shown that the above mentioned neurotoxic influence of MPTP on dopaminergic nigral neuron degeneration in mice and other animals does not occur in rats. On the other hand the inferior olivary neuron degeneration caused by i.p. administration of 3-acetylpyridine in rats is accompanied by a degeneration of the nigrostriatal dopaminergic system resulting in a significant decrease in striatal dopamine concentration. This striatal 3-acetylpyridine toxicity and dopamine depletion does not depend on the production of a toxic metabolite generated by the action of monoamine oxidase B, but it is conceivable that a toxic 3-acetylpyridine metabolite is formed through another mechanism (Deutch et al., 1989).

The data from our investigation show different effects of 3-acetylpyridine between the Lurcher mutant and wild-type mice. At the same time the study has given us new information about the effects of site-specific pyridine neurotoxins in these two mice genotypes. We have shown that whereas the neurotoxin, 3-acetylpyridine, has no effect on the inferior olivary neurons in the wild-type mouse (C3H strain), in the Lurcher heterozygote on the same genetic background the inferior olivary neurons are susceptible. It appears therefore that in the Lurcher mutant although the inferior olivary neurons are not affected directly by the mutant gene their susceptibility to the neurotoxin is increased. Since this occurs in both young and mature Lurcher mice it would seem that the degeneration of Purkinje cells is not the cause of this increased sensitivity since we have injected the toxin at times when the Purkinje cells are present and after they have begun to degenerate. Since there appear to be some differences between the inferior olivary neurons in the Lurcher and wild-type mice we are beginning an in vitro study growing slices of Lurcher cerebellar cortex alongside slices of Lurcher and wild-type inferior olive. These cultures will show us what effect the innervation from these two different sources of afferents has on the growth of Purkinje cells. The study of the mechanism of 3-acetylpyridine degeneration in the mouse inferior olivary neurons helps us to understand the pathogenesis of the olivopontocerebellar atrophies in man (Deutch et al., 1989). Also the Lurcher mutant is a good model of olivopontocerebellar atrophy, and further investigation of the different affinity of 3-acetylpyridine for the inferior olivary neurons of this mutant in comparison with wild-type animals could be very useful in helping to clarify the mechanism involved in this disease.

References

Anderson, W.A., Flumerfelt, A., 1984. Sensitivity of rat inferior olivary neurons to 3-acetylpyridine. Dev. Brain Res. 12, 285–291.

Balaban, C.D., 1985. Central neurotoxic effects of intraperitoneally administered 3-acetylpyridine, harmaline and niacinamide in Sprague-Dawley and Long-Evans rats: a critical review of central 3-acetylpyridine neurotoxicity. Brain Res. Rev. 9, 21–42.

Caddy, K.W.T., Biscoe, T.J., 1979. Structural and quantitative studies on the normal C3H and Lurcher mutant mouse. Phil. Trans. R. Soc. London (Biol.) 287, 167–201.

Deutch, A.Y., Rosin, D.L., Goldstein, M., Roth, R.H., 1989. 3-Acetylpyridine-induced degeneration of the nigrostriatal dopamine system: an animal model of olivopontocerebellar atrophy-associated parkinsonism. Exp. Neurol. 105, 1–9.

Heikkila, R.E., 1985. Differential neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine (MPTP) in Swiss-Webster mice from different sources. Eur. J. Pharmacol. 117, 131–133.

Jones, N., Le Marec, N., Stelz, T., Caston, J., 1994. Effect of administration of 3-acetylpyridine followed by niacinamide injection on survival, extent of the inferior olivary complex lesion, and response to harmaline in the young rat. Brain Res. 656, 257–262.

Llinás, R., Kerry, W., Hillman, D.E., 1975. Inferior olive: its role in motor learning. Science 190, 1230–1231.

Ozaki, H.S., Murakami, T.H., Shimada, M., 1983. Learning deficits on avoidance task and hippocampal lesions in area CA3 following intraperitoneal administration of 3-acetylpyridine. J. Neurosci. Res. 10, 425–435.

Weller, M., Marini, A.M., Paul, S.M., 1992. Niacinamide blocks 3-acetylpyridine toxicity of cerebellar granule cells in vitro. Brain Res. 594, 161–164.

Schulz, J.B., Henshaw, D.R., Jenkins, G.J., Ferrante, R.J., Kowall, N.W., Rosen, B.R., Beal, M.F., 1994. 3-Acetylpyridine produces age dependent excitotoxic lesions in rat striatum. J. Cereb. Blood Flow Metab. 14, 1024–1029.

Zimmer, J., Geneser, F.A., 1987. Difference in monoamine oxidase B activity between C57 black and albino NMRI mouse strains may explain differential effects of the neurotoxin MPTP. Neurosci. Lett. 78, 253–258.