Quebec Cooperative Study
of Friedreich's Ataxia

Influence of Nicotinamide on Neurobehavioral Effects of 3-Acetylpyridine

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ABSTRACT: *The purpose of the present study was to examine the ability of nicotinamide to prevent the appearance of neurobehavioral symptoms induced by 3-acetyl pyridine (3-AP) in rats. Nicotinamide in doses of 5,50 and 500 mg/kg was injected immediately after administration of 65 mg/kg 3-AP, and neurobehavioral measurements were made at 6, 12, 24, 48 and 72 hours after injections. The effects of 500 mg/kg nicotinamide injected at 3 and 6 hours after 3-AP treatment were also investigated. The results indicate that, starting at 50 mg/kg, nicotinamide can protect animals against most of the neurobehavioral effects of 3-AP. However, the muscular rigidity induced by 3-AP can only be reversed by 500 mg/kg nicotinamide, and the depressing influence of 3-AP on locomotor activity is not blocked by any of the doses of nicotinamide tested. In terms of time course, the protective action of 500 mg/kg is seen when injected 3, but not 6 hours after 3-AP.*

RÉSUMÉ: *Le but de la présente étude était d'examiner l'habileté de la nicotinamide à prévenir l'apparition de symptômes neurocomportementaux produits chez le rat par la 3-acetyl pyridine (3-AP). La nicotinamide, à des doses de 5,50 et 500 mg/kg fut injectée immédiatement après l'administration de 3-AP (65 mg/kg) et les mesures comportementales faites 6, 12, 24, 48 et 72 heures après les injections. Nous avons également étudié l'effet d'injections de 500 mg/kg de nicotinamide 3 et 6 heures après le traitement au 3-AP. Les résultats obtenus indiquent qu'à compter de la dose de 50 mg/kg, la nicotinamide peut protéger les animaux contre la plupart des effets neurocomportementaux du 3-AP. Cependant la rigidité musculaire ne peut être renversée qu'à la dose de 500 mg/kg alors que l'action dépressive du 3-AP sur l'activité motrice n'est bloquée par aucune des doses de nicotinamide utilisées. L'action protectrice de 500 mg/kg se voit 3 heures, mais non 6 heures, après l'injection de 3-AP.*

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INTRODUCTION

Administration of 3-acetyl pyridine (3-AP) produces, within 24 hours, lesions in several brain stem nuclei, particularly in the inferior olivary nucleus where complete degeneration of the cell bodies of climbing fibers to the cerebellum is observed (Desclin, 1974, Desclin and Escubi, 1974). Competition of 3-AP with nicotinamide for incorporation into NAD is thought to be responsible for the neurotoxicity of this substance (Kaplan et al, 1954). In accordance with this view, administration of nicotinamide has been shown to protect animals against the lethal effects of 3-AP and to block the inhibitory influence of 3-AP on harmaline induced tremors (Kaplan et al, 1954, Simantov et al, 1976).

Neurobehaviorally, administration of 3-AP to animals results in a variety of neurological signs, such as a marked ataxic gait, decreased locomotor activity, catalepsy, muscular rigidity and the loss of various reflexes (Jolicoeur et al, 1979). The purpose of the present study was to examine the ability of nicotinamide to prevent the appearance of these neurobehavioral symptoms in 3-AP treated animals.

In the present study we examined the antidotal properties of a relatively wide range of nicotinamide doses (5, 50 and 500 mg/kg) injected immediately after administration of 3-AP (65 mg/kg to rats. In order to determine the time course of any protective action of nicotinamide, the effects of 500 mg/kg injected at 3 and 6 hours after adminstration of 3-AP were also investigated.

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METHODS

Male hooded rats, 275-300 g in weight, were used. They were divided into seven groups of eight animals each.

On treatment day, six groups were injected first 65 mg/kg 3-AP (Sigma). Immediately afterwards, four of these groups received a second injection of either 0.9% NaCL, 5,50 or 500 mg/kg nicotinamide (Sigma). The two other groups of 3-AP treated animals were also administered 500 mg/kg nicotinamide, one group at 180, the other at 360 minutes after injections of 3-AP. A final group of animals received two consecutive injections of 0.9% NaCL and constituted the control group of the study. All injections were performed via the intraperitoneal route. Both 3-AP and nicotinamide were mixed in 0.9% NaCL to obtain injection volumes of 1 ml/kg.

At 6, 12, 24, 48 and 72 hours following 3-AP administration, animals were submitted to the following battery of neurobehavioral tests. The tests were performed in the order presented here. A more detailed description of testing procedures has been published previously. (Jolicoeur et al, 1979).

Locomotor activity

Spontaneous locomotor activity was measured for two minutes by means of a photocell activity apparatus (Lehigh Valley Electronics).

Muscular Tone

A rat was suspended by its front paws grasping a metal rod (0.5 cm diameter) which was held by the experimenter about 50 cm above a table top. The time the animal remained on the bar (maximum: 60 sec.) was recorded.

Catalepsy

If present, intensity of catalepsy was determined by placing an animal's front paws on a horizontal bar (1 cm in width) suspended 10 cm above a table top. Time spent in that position, up to a maximum of 60 seconds, was recorded.

Landing foot spread

After staining the hindfeet with ink, an animal was held horizontally 30 cm above a table covered with absorbent paper. The rat was dropped and the distance between the prints of each hind limb was measured.

Gait analysis

Hindfeet were again stained with ink and the animal was walked through an enclosed 90 cm long corridor with a paper covered floor. When two consecutive steps were obtained, the stride width, length and angle between consecutive steps on contralateral sides were calculated.

Reflexive responses

First the presence of a normal righting reflex was verified. Then the animal's ability to shift its weight contralaterally to a gravitational pull, and the position of its hindlimbs when held vertically were checked. Subsequently, the animal, placed on a table, was lifted by the tail and the presence of a normal extension of the hindlimbs was noted. Finally, in the traction test, the animal was held by the tail and pulled horizontally on a table; the presence of a spontaneous hunched posture during the pull was recorded.

RESULTS

Data obtained on activity, catalepsy, muscular tone, foot landing spread and the three gait components were analysed by individual multifactorial ANOVA's for repeated measures. (WINER, 1971). Factors included in each analysis were groups and test periods. When significant groups by test periods interactions were found, simple main effect analyses were carried out at each level of the test period factor to determine when groups differed from each other. When ap-

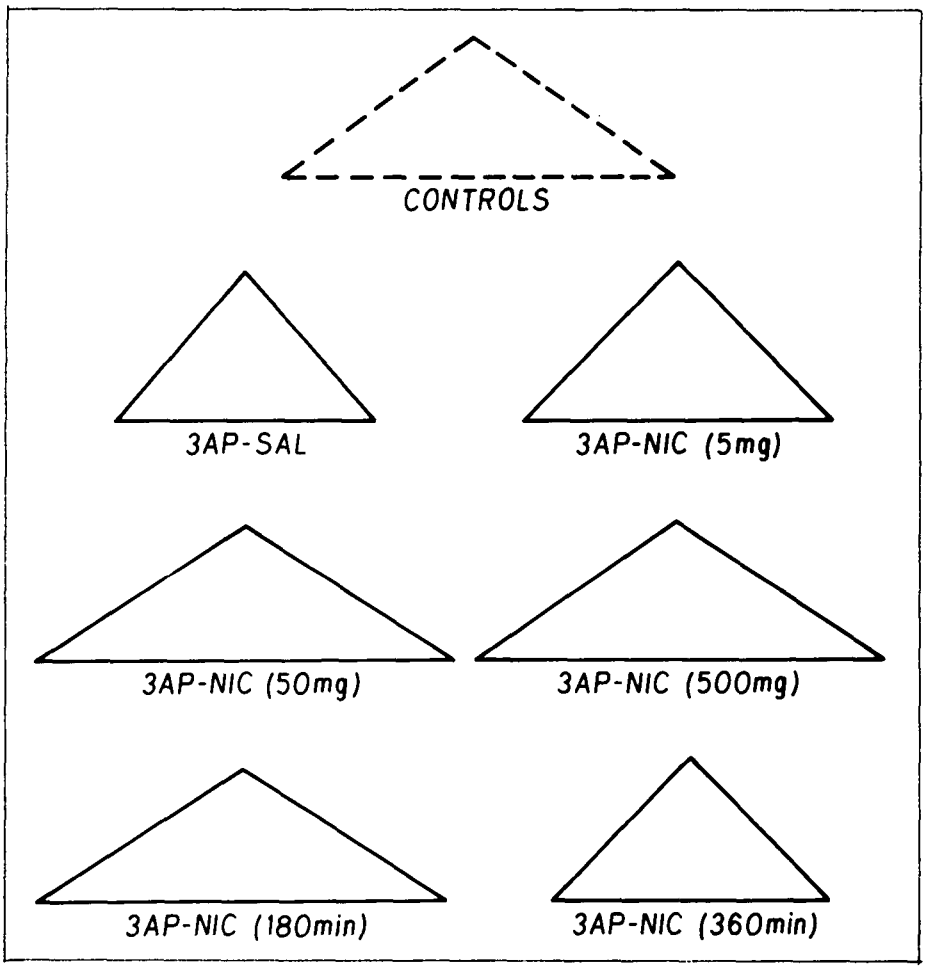

Figure 1 — Schematic representations of gait of control and experimental animals subjected to the various treatments of the present study. Patterns were obtained at the 48 hour post injections test period.

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TABLE 1

OCCURENCE OF NEUROLOGICAL SIGNS

	TREATMENTS					
	3-AP 0.9% NaCl (0 min.)*	3-AP-5mg/kg Nicotinamide (0 min.)*	3-AP-50 mg/kg Nicotinamide (0 min.)*	3-AP-500 mg/kg Nicotinamide (0 min.)*	3-AP-500 mg/kg Nicotinamide (180 min.)*	3-AP-500 mg/kg Nicotinamide (360 min.)*
Length of steps	•↓	•↓				•↓
Width of steps	•↑	•↑				•↑
Angle of steps	•↓	•↓				•↓
Activity	•↓	•↓	•↓	•↓	•↓	•↓
Muscular tone	•↑	•↑	•↑			•↑
Catalepsy	•↑	•↑				•↑
Landing foot spread	•↑	•↑				•↑
Traction	o (4)	o (2)				o (5)
Hindlimb position	o (6)	o (4)				o (6)
Hindlimb extension	o (6)	o (5)				o (5)
Weight shift	o (6)	o (5)				o (6)
Righting	o (5)	o (1)				o (4)

- Full circles indicate significant differences from control group as revealed by ANOVA ($p < 0.05$).
- Arrows indicate direction of change
- Open circles refer to the observed absence of a given reflex in a group. Number of animals affected is given in parentheses (n:8)
- * Time span between the two injections.

propriate, significant differences between individual 3-AP treated groups and the control animals were assessed by means of Dunnet tests. In the group injected with 3-AP and 0.9% NaCL, two animals died between the 48 and 72 hours test periods. Because no mortality occurred in the other groups during the course of the experiment, statistical analyses were carried out taking into account unequal cell frequencies (Winer 1971). For all statistical analyses a difference between groups was considered significant if it had a probability of random occurrence of less than 5 percent.

Results are summarized in *Table 1* where statistically significant differences between groups, as well as numbers of animals in each group displaying losses of various reflexes, are given. Schematic representations of animal's gait patterns obtained in the various groups at the 48 hour test period, are presented in *Figure 1*.

Results obtained with the groups injected with either 3-AP and 0.9% NaCL, 3-AP and 5 mg/kg nicotinamide, or 3-AP and, 360 minutes later, 500 mg/kg nicotinamide were similar. Compared to control animals, significant differences in the overall five test periods scores of activity, catalepsy, muscular tone, landing foot spread and in the length, width and angle of steps were found for the three groups (Table 1). For each of these groups, motor activity was significantly decreased and this effect was first seen at the 6 hour test period; also starting at 6 hour, landing foot spread was significantly increased; catalepsy and muscular tone scores were significantly elevated, initially at the 24 hour test period; by the 48 hour test period, angles and lengths of steps were significantly decreased and width significantly increased (Figure 1).

Results obtained in the various reflex tests were also similar in these three groups (Table 1). Starting at 12 hour, some animals of these groups displayed an abnormal hindlimb position characterized by the feet being retracted and held against the body. By the 24 hour test period, some animals in the three groups had lost the righting, weight shift and hindlimb extension reflexes, and were unable to maintain a normal hunched posture during the traction test. As can be seen in Table 1, although these disturbances were observed in all three groups, they were more frequent in the 3-AP treated animals administered 0.9% NaCL or 500 mg/kg nicotinamide 360 minutes later than in those given 5 mg/kg nicotinamide immediately after 3-AP.

For the group administered 3-AP and 50 mg/kg nicotinamide, the overall five test periods scores for activity and muscular tone were significantly decreased and increased respectively in comparison to control animals. Both effects were first detected in the 6 hour test period. No significant effect was seen in any of the other measures, and no animal in this group manifested disturbances in the various reflexive responses (Table 1). For the groups treated with 3-AP followed by 500 mg/kg nicotinamide immediately after or 180 minutes after, the only significant effect was a decrease in motor activity scores which occurred solely in the 6 hour test period. All other measures were not significantly dif-

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ferent from controls, and all animals presented normal reflexes.

In summary, the results of the present experiment demonstrate that, starting at 50 mg/kg, nicotinamide can protect animals against most of the deleterious neurobehavioral effects of 3-AP. However, the muscular rigidity induced by 3-AP can only be reversed by 500 mg/kg nicotinamide and the hypokinetic effect of 3-AP is not blocked by any of the doses of nicotinamide tested. In terms of time course, the protection action of 500 mg/kg nicotinamide is seen when injected 3 hours, but not 6 hours after 3-AP.

DISCUSSION

Administration of 65 mg/kg 3-AP induced a profile of neurobehavioral effects which is very similar to that previously observed (Jolicoeur et al, 1979). A decrease in locomotor activity, an increase in landing foot spread, the presence of catalepsy, the appearance of muscle rigidity, as well as distinctive disturbances in various reflexes, were again observed within 72 hours after 3-AP injection.

It is unlikely that such a diversity of neurological signs reflects a common mechanism and/or locus of action of 3-AP. In this respect, the results obtained with the various nicotinamide treatments point to a multifaceted neurotoxic property of 3-AP. Whereas most symptoms induced by 3-AP were blocked by 50 mg/kg nicotinamide, muscular rigidity was only abolished with 500 mg/kg, and the decrease in locomotor activity was not reversed with any of the doses tested. It should be mentioned that the decreases in locomotor activity were seen mostly in the first 6 hours test period. However it is difficult to determine if this represents the actual time course in the hypokinetic influence of 3-AP since, even though the activity scores of 3-AP treated animals remained generally low in the subsequent test periods, due to a habituation effect, activity of control animals gradually decreased to a minimum, so that statistically significant differences could no longer be obtained in the latter test periods.

The inability of nicotinamide to influence the hypokinetic effect of 3-AP is difficult to understand. One possibility is that this effect is mediated by a still unspecified action of 3-AP which is not related to the competition with nicotinamide for incorporation into NAD. It is also possible that some brain areas, involved in the central control of motor activity, may be very susceptible to the action of 3-AP. One such area could be the substantia nigra, a region definitely involved in the regulation of motor activity, and which has been shown to be histopathologically affected following 3-AP administration (Desclin, 1974).

The observed time course in the antidotal action of nicotinamide against most neurobehavioral disturbances produced by 3-AP parallels that of the reported protective influence of nicotinamide against 3-AP induced lethality and blockade of harmaline tremors (Simantov et al, 1976). The fact that the neurotoxic actions of 3-AP are irreversible after 6 hours, is concordant with the known time course in 3-AP induced neurohistopathological changes. (Desclin 1974, Desclin et al, 1974).

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