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Research Report

The critical threshold of 3-nitropropionic acid-induced ischemic tolerance in the rat

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Abstract

3-nitropropionic acid (3-NPA) is a suicide inactivator of succinate dehydrogenase (SDH), commonly used as a pharmacological model of Huntington's disease in rodents. Several studies have shown that a single administration of 3-NPA given systemically provides subsequent ischemic tolerance. The present study has tested the hypothesis that 3-NPA is capable of inducing tolerance in a model of permanent focal cerebral ischemia and whether 3-NPA can be truly applicable as a tolerance-inducer to ischemia. Rats given 3-NPA intraperitoneally revealed that the mortality of 3-NPA of 15, 20, and 25 mg/kg groups was 20.5, 38.8, and 83.3%, respectively. All rats survived without behavioral sequelae at smaller doses. Three days after 3-NPA preconditioning, the rats showing no behavioral changes underwent the permanent middle cerebral artery occlusion. The groups treated with 10 and 15 mg/kg of 3-NPA showed significantly reduced neurological deficits and infarction volumes in comparison with the control group, whereas the groups treated with 5 and 20 mg/kg of 3-NPA revealed no tolerance effects. When the regional SDH activity (% of control) was photometrically semi-quantified, it was observed that the activity was reduced to 90.8, 76.1, 67.8, and 64.3% in the outer layers of the cerebral cortex, and to 79.4, 67.5, 63.2, and 62.9% in the striatum 1 h after 3-NPA application (5, 10, 15, 20 mg/kg), respectively. In conclusion, although the preconditioning with 3-NPA is clearly shown in the setting of permanent ischemia, the preconditioning with this mitochondrial toxin demonstrated a rather narrow safety margin (critical threshold).

Theme: Disorders of the nervous system
Topic: Ischemia

Keywords: 3-nitropropionic acid; Succinate dehydrogenase; Ischemic tolerance; Critical threshold; Neurotoxicity

1. Introduction

Ischemic tolerance is a phenomenon in which sublethal stress can induce resistance to subsequent lethal ischemia [7,9,15–17]. There are diverse types of sublethal stress, such as brief ischemia, hyperthermia, cortical spreading depression, anoxia/hypoxia, and inhibition of oxidative phosphorylation [15].

Several chemicals, e.g., acetylsalicylic acid [27], the antibiotics erythromycin and kanamycin [13], 2-deoxyglucose [32], and 3-nitropropionic acid (3-NPA) [25], all of which, applied in subtoxic doses, interfere with cellular energy metabolism, may also provide protection against subsequent lethal insults [23]. In clinical practice, if ischemic tolerance can be reinforced by some pharmacological treatment, a reasonable strategy would be to start pharmacological intervention in the face of transient ischemic attack or minor stroke, in order to potentiate the endogenous protection against a more profound ischemic event. This is applicable, for instance, to those scheduled for carotid endarterectomy [23].

3-NPA is a suicide inactivator of succinate dehydrogenase (SDH) in the tricarboxylic-acid cycle (inhibition of mitochondrial complex II), and exerts a nucleophilic effect on N-5 of the covalently bound flavin component of the enzyme [6,8]. It is commonly used as a pharmacological

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model for Huntington's disease in rodents, because systemic administration of 3-NPA results in selective striatal necrotic lesions, which are associated with slowly progressive abnormal motor behavior [3,5,20,22].

On the other hand, several studies have shown that a single administration of 3-NPA given systemically provides cerebral ischemic tolerance [10,12,18,21,28,31]. It has been reported that a burst of reactive oxygen species critically contributes to tolerance induction by 3-NPA [31], but effects further downstream remain unclear. Interestingly, the effectiveness of tolerance induction by 3-NPA was not dose-dependent, and it is possible that more profound pre-inhibition of SDH activity does not lead to an increase in neuronal tolerance in models of transient global ischemia [28].

The principal aim of this paper is to evaluate whether 3-NPA is truly applicable as a tolerance-inducer to ischemia. This paper describes the capability of 3-NPA to induce tolerance at various doses in permanent focal ischemic models. This study also refers to the "critical threshold" of metabolic impairment brought on by 3-NPA, if the preconditioning with 3-NPA has a wide therapeutic safety window.

2. Materials and methods

2.1. Animals and preliminary dosing of 3-NPA

Male Sprague–Dawley (SD) rats, 8–10 weeks old, weighing 250–350 g were used in all experiments and were allowed free access to food and water during the experiment. The experimental procedures were approved and followed the animal care guidelines of our institute.

According to previous reports, the dose of 3-NPA, at which ischemic tolerance is induced, was assumed to be 20 mg/kg in the rat [4,12,21,24,26,31,33]. However, because 38.8% of the rats had died with this dose (n = 54), as shown in Table 1, we examined the mortality rate for each dose of 3-NPA (Sigma; 5, 10, 15, 20, and 25 mg/kg, diluted in normal saline to a concentration of 1 mg/ml, adjusted to pH 7.4 with NaOH, i.p.). Also, among the rats that survived in each group, some presented with behavioral changes, such as uncoordinated gait with stereotypical paddling movements and ventral or lateral recumbent state [11]. These rats were excluded from subsequent surgical intervention.

Table 1
Morality rate after preconditioning with 3-NPA

3-NPA dose (n)	5 mg/kg (19)	10 mg/kg (25)	15 mg/kg (44)	20 mg/kg (54)	25 mg/kg (12)
Mortality rate (%)	0	0	20.5	38.8	83.3

2.2. Permanent focal cerebral ischemia

The rats were anesthetized with 2% halothane, and the left middle cerebral artery (MCA) was permanently occluded by a microsurgical technique [30]. During the operation, the ambient temperature was constantly maintained at a constant level, and the rectal temperature was kept between 36.5 °C and 37.5 °C with a heating pad. Before and after the operation, blood gas and blood sugar were measured directly from the tail artery, and systolic blood pressure was monitored with a tail cuff.

The animals were preconditioned with 3-NPA (5, 10, 15, 20 mg/kg) at an interval of 72 h before MCAO. The control group was injected only with physiological saline i.p. A neurological examination of each animal was performed 24 h after MCAO. All of the animals were sacrificed 4 days after MCAO for the measurement of the infarction volume.

2.3. Neurological deficits

Twenty-four hours after MCAO, neurological examinations were performed with modified Barone scales [2]; the forelimb scores were 0 (no observed deficit), 2 (contralateral forelimb flexion when suspended by the tail), and 4 (reduced resistance to lateral push toward the paretic, contralateral side). The hindlimb placement test consisted of pulling the contralateral hindlimb away from the rat over the edge of a table; the hindlimb scores were 0 (immediate placement of the limb back onto the table), 2 (slow placement of the limb back onto the table), and 4 (no limb placement/movement).

2.4. Measurement of the infarction volume

The rat brain was quickly removed and sliced into coronal sections at 2-mm intervals. Each slice was immersed in a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Tokyo Kasei) for 30 min at 37 °C and then fixed in 10% buffered formaldehyde solution. The stained brain slices were digitally photographed and the extent of infarction in each brain slice was determined on an image analysis system (Win ROOF, ver. 5.0, Mitani Corp., Japan) according to the indirect method proposed by Swanson and others [29]. The total infarction volume was calculated by summation of the infarcted area on 6 brain slices (2 to 14 mm frontal pole) and integration by the thickness (2 mm).

2.5. Succinate dehydrogenase activity measurements

One hour after the 3-NPA injection (5, 10, 15, 20 mg/kg), the rats were decapitated in deep anesthesia in order to detect SDH activity in freshly frozen brain sections. SDH histochemistry was performed as reported elsewhere [1,14,19]. In short, the brain was rapidly removed, frozen by immersion into isopentane in liquid nitrogen, and stored at −80 °C. Coronal sections at the level of the