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Research Report

1,2,3,4-Tetrahydroisoquinoline protects terminals of dopaminergic neurons in the striatum against the malonate-induced neurotoxicity

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Abstract

Malonate, a reversible inhibitor of the mitochondrial enzyme succinate dehydrogenase, is frequently used as a model neurotoxin to produce lesion of the nigrostriatal dopaminergic system in animals due to particular sensitivity of dopamine neurons to mild energy impairment. This model of neurotoxicity was applied in our study to explore neuroprotective potential of 1,2,3,4-tetrahydroisoquinoline (TIQ), an endo- and exogenous substance whose function in the mammalian brain, despite extensive studies, has not been elucidated so far. Injection of malonate at a dose of 3 μmol unilaterally into the rat left medial forebrain bundle resulted in the 54% decrease in dopamine (DA) concentration in the ipsilateral striatum and, depending on the examined striatum regions, caused 24–44% reduction in [^{3}H]GBR12,935 binding to the dopamine transporter (DAT). TIQ (50 mg/kg i.p.) administered 4 h before malonate infusion and next once daily for successive 7 days prevented both these effects of malonate. Such TIQ treatment restored DA content and DAT binding almost to the control level. The results of the present study indicate that TIQ may act as a neuroprotective agent in the rat brain. An inhibition of the enzymatic activities of monoamine oxidase and γ-glutamyl transpeptidase as well as an increase in the striatal levels of glutathione and nitric oxide found after TIQ administration and reported in our earlier studies are considered to be potential factors that may be involved in the TIQ-mediated protection of dopamine terminals from malonate toxicity.

Theme: Disorders of the nervous system
Topic: Neurotoxicity

Keywords: Malonate toxicity; 1,2,3,4-tetrahydroisoquinoline; Neuroprotection from malonate toxicity

1. Introduction

Although tetrahydroisoquinolines (TIQs) have attracted a considerable attention of pharmacologists and neurochemists since many years, their role in the mammalian brain has not been established, as yet. The existence of TIQs in plants was described much earlier before they were found in humans and animals organisms [69,90]. Using the most suitable method, gas chromatography-mass spectrometry, 1,2,3,4-tetrahydroisoquinoline (TIQ), the simplest representative of the group of non-catecholic tetrahydroisoquinolines, was finally identified as endogenous compound in the brains of parkinsonian patients and normal human subjects [57,59,62]. It was also confirmed to be present in rodent and monkey brains [34,87]. In general, TIQs are formed non-enzymatically by so-called Pictet–Spengler condensation from biogenic amines with a basic structure of 2-phenylethylamine and aldehydes or α-keto acids [69,90], though some of them are also synthesized enzymatically [86]. The preservation of the enzymatic system generating TIQ in the mammalian brain in the course of evolution suggests that it may play an important physiological function. TIQ found in the brain may be also of dietary origin, as it has been detected in many food-stuffs rich in 2-phenylethylamine [42,60]. Moreover, this compound easily penetrates into the

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brain [33,58] as it is actively transported across the blood–brain barrier by organic cation transporter system (OCT) and is quickly eliminated from it by P-glycoprotein [39]. Due to structural similarity of some TIQs to parkinsonism-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), they have been also considered to be potential endogenous and/or exogenous neurotoxins that may be involved in the degeneration of the nigrostriatal dopaminergic neurons [49,56].

Both in the idiopathic Parkinson disease and in the MPTP-induced parkinsonism, the loss of dopamine neurons in the substantia nigra pars compacta (SNc) is directly linked to a dramatic reduction of dopamine transporter (DAT) binding sites and to a severe decrease in dopamine (DA) concentration in the caudate nucleus and putamen [5,16,27,53,84]. TIQ administered chronically evoked some decrease in the number of tyrosine hydroxylase (TH)-positive neurons in the SNc of mice [61] and rats [36], but loss of these cells as judged by cresyl violet staining was not found [61]. Moreover, TIQ did not alter the radioligand-DAT binding neither in the rat [38] nor in the mouse striatum [28]. As for the DA concentration, small reduction or lack of any changes was observed in the rat striatum after chronic TIQ administration [2,36,38,72]. As the key neurochemical markers of PD were weakly affected in the rat nigrostriatal dopaminergic system, it is difficult to believe that TIQ itself is really parkinsonism-inducing substance. On the other hand, TIQ markedly affected DA catabolism both in the rat striatum and substantia nigra [2,36,38]. Therefore, it is assumed that this compound may act either as a neuromodulator or even as a neuroprotectant.

It is well known that, during the N-oxidation of dopamine catalyzed by mitochondrial enzyme monoamine oxidase (MAO), apart from 3,4-dihydroxyphenylacetic acid (DOPAC), hydrogen peroxide is produced, whose decomposition in the presence of iron-II is a source of a potent cytotoxic hydroxyl radical [9]. TIQ strongly depressed the level of DOPAC and enhanced that of the extraneuronal DA metabolite, 3-metoxytyramine (3-MT) [2,36,38]. The inhibition of MAO-dependent pathway of DA catabolism and activation of the COMT-dependent O-methylation suggest that TIQ, through changing DA catabolism, may contribute to the protection of dopamine neurons against the effect of free radicals [52]. This conclusion is in agreement with the fact that inhibitors of MAO_B possess neuroprotective properties [41,79], and TIQ is a moderate inhibitor both of MAO_A and MAO_B [66]. There are also other data that speak in favor of neuroprotective properties of TIQ. Recently, Storch et al. [80] using cell lines transfected with DAT cDNA have demonstrated that TIQ prevents DAT-mediated toxicity of MPP+ and 2[N]-methylisoquinolinium ion. Moreover, TIQ administered systemically increases the concentration of glutathione (GSH) in the dopaminergic structures of the rat brain [37,40], in contrary to the model proparkinsonian neurotoxin MPTP, which causes depletion of that antioxidant in the nigrostriatal dopaminergic system [63,77,88]. All these data seem to suggest that TIQ may help the cellular protective mechanisms by different routes.

To assess the neuroprotective abilities of TIQ in vivo, we performed the present study using malonate model of neurotoxicity. Malonate is a reversible competitive inhibitor of succinate dehydrogenase, which after stereotaxic injection into the striatum or substantia nigra, produces an energy impairment through its action on the mitochondrial respiratory chain with a subsequent damage of the nigrostriatal dopaminergic system [22,55,75,91,92]. Employing this model substance, it was examined whether TIQ administered intraperitoneally is able to prevent the loss of DA content and a decrease in the binding of [^{3}H]GBR12,935 to DAT in the striatum of rats with a unilateral malonate lesion of the medial forebrain bundle (MFB).

2. Materials and methods

2.1. Chemicals

1,2,3,4-Tetrahydroisoquinoline (TIQ) was obtained from the Aldrich Chemical Company (Milwaukee, USA) while the succinate dehydrogenase inhibitor malonic acid disodium salt (malonate) from Sigma-Aldrich Company (Poznań, Poland).

2.2. Animals and surgery

The study was conducted on male Wistar rats of initial body weight between 280–320 g kept under standard laboratory conditions; 8 animals per a large cage, at room temperature (22 °C) under an artificial light/dark cycle (12/12 h), with free access to standard laboratory food and tape water. Experiments were carried out according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publication no. 85-23, revised 1985) and were approved by the internal Bioethics Commission.

Rats were lightly anesthetized with pentobarbital (Vetbutal, 30 mg/kg i.p. Biowet, Poland) and then were placed in stereotaxic apparatus. A stainless steel needle (0.28 mm o.d.) was inserted unilaterally through a small hole in the skull, and the needle tip was placed into the left medial forebrain bundle (MFB). The lesion coordinates according to the atlas of König and Klippel [35] were as follows: A = 4.9, L = 1.6, H = −2.3. The malonate dissolved in an artificial cerebrospinal fluid was injected at a dose of 3 μmol in a volume of 2 μl into the rat left medial forebrain bundle (MFB) at a flow rate of 0.5 μl/min using a syringe pump. Control rats received an artificial cerebrospinal fluid instead of malonate. The cannula was left in place for at least 4 min to allow for diffusion. Experiments were performed on 5 groups of rats. Two of them were treated systemically with TIQ at a dose of 50 mg/kg while three others with 0.9% solution of NaCl. Each rat from the TIQ-treated groups