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SHORT COMMUNICATION

The Isomers of Thioctic Acid Alter ^{14}C-Deoxyglucose Incorporation in Rat Basal Ganglia

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ABSTRACT. Nigral cell death in Parkinson's disease is associated with decreased reduced glutathione (GSH) levels, impaired complex I activity and inhibition of α-ketoglutarate dehydrogenase (α-KGDH) in substantia nigra. Thioctic acid exerts antioxidant activity through a thiol-disulphide redox couple and is an essential cofactor for α-KGDH. However, it is not known whether or not thioctic acid enters basal ganglia or exerts beneficial effects in Parkinson's disease. As a global measure of altered cerebral function, the effect of R- and S-thioctic acid on ^{14}C-2-deoxyglucose (^{14}C-2DG) incorporation was investigated in rats. Rats were treated with either R- or S-thioctic acid (50 mg/kg IP) or 0.9% saline acutely or for 5 days and ^{14}C-2DG incorporation in basal ganglia was assessed. Following acute administration, R- but not S-thioctic acid caused an overall increase in ^{14}C-2DG incorporation that was significant in both substantia nigra zona compacta and zona reticulata. R-thioctic acid also increased the incorporation of ^{14}C-2DG in the medial forebrain bundle, thalamus, and red nucleus. S-thioctic acid decreased ^{14}C-2DG incorporation in the subthalamic nucleus, but increased it in the red nucleus. Following repeated administration, R-thioctic acid no longer increased ^{14}C-2DG incorporation in either zona compacta or zona reticulata of substantia nigra. However, both R- and S-thioctic acid now decreased ^{14}C-2DG incorporation in the subthalamic nucleus. The data suggest that thioctic acid does enter the brain and can alter neuronal activity in areas of the basal ganglia intimately associated with the motor deficits exhibited in Parkinson's disease. BIOCHEM PHARMACOL 51;7:983–986, 1996.

KEY WORDS. thioctic acid; R- and S-isomers; glucose utilisation; basal ganglia; rat

Parkinson's disease is characterised by degeneration of the dopamine-containing cells in the substantia nigra zona compacta [1, 2] and severe loss of dopamine in the basal ganglia [3], but the cause is still unknown. Postmortem studies on substantia nigra from patients with Parkinson's disease support the concept of oxidative stress as a cause of nigral cell death. Key components of the biochemical changes uncovered include inhibition of mitochondrial complex I [4], decreased α-KGDH activity [5], and decreased levels of reduced glutathione (GSH) [6–8].

The evidence for oxidative stress from postmortem studies provides the first opportunity to devise therapeutic strategies to prevent the progression of nigral cell death. A logical approach would be to increase cellular energy metabolism to prevent inhibition of complex I and α-KGDH and to reverse the decrease in GSH levels, because these appear to occur early in the disease [9]. However, to date no agents have been available for this purpose. Thioctic acid (α-lipoic acid) is an endogenous antioxidant which, with its reduced form dihydrolipoic acid, forms a thiol-disulphide redox system [10–13]. Thioctic acid may also replenish intracellular GSH levels [14–16] and is an essential cofactor for the multienzyme complexes, α-KGDH and pyruvate dehydrogenase. However, it is not known whether or not thioctic acid enters the brain or accumulates within the basal ganglia. A previous study by Gal and Razenska [17] showed a small fraction of ^{35}S-R,S-thioctic acid to be present in rat brain following acute intraperitoneal administration. However, these authors did not study specific brain regions and they did not perfuse the animals to remove the 50-fold higher levels of radioactivity present in brain capillaries. So, there is doubt over the validity of these findings. There is presently no highly labelled form of thioctic acid available to utilise for autoradiographic determination of its location in brain. Also, no sufficiently sensitive assay for the detection of thioctic acid in brain is available, so we have measured the potential actions of the isomers of thioctic acid to alter cerebral glucose utilisation as measured by ^{14}C-2-deoxyglucose (^{14}C-2DG) incorporation. Alterations in ^{14}C-2DG incorporation reflect changes in neuronal activity and have been routinely employed to determine sites of drug action in the brain. This approach is based on the previous demonstration that the R- and S-isomers of thioctic acid alter glucose uptake and glucose transporters in muscle cells [18].

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METHODS AND MATERIALS

Male Wistar rats (300 g; Tucks, U.K.) were employed. Animals were allowed food and water *ad lib*, housed 4 or 5 to a cage and kept under a 12-hr light/dark cycle at 20 ± 1°C and approximately 50% humidity.

Drug Treatment

R- and S-thioctic acid (Asta Medica, Germany) were dissolved (25 mg/mL) in sodium hydroxide (1 M) and neutralised to pH 7 with hydrochloric acid (1 M). In the acute investigation, animals were treated with R- or S-thioctic acid (50 mg/kg IP) 2 hr prior to the administration of ^{14}C-2DG. The time corresponds to the time of peak activity of thioctic acid (personal communication, Asta Medica). In the subchronic study, animals were treated with R- or S-thioctic acid (50 mg/kg IP) for 5 consecutive days prior to the administration of ^{14}C-2DG, the last dose being given 2 hr prior to the injection of ^{14}C-2DG.

Administration of ^{14}C-2-deoxyglucose

Under anaesthesia (Sagatal; sodium pentobarbitone 60 mg/kg IP), the tail vein and artery were cannulated with polythene tubing (gauges; ID 0.58 × OD 0.96 and ID 0.4 × OD 0.8 mm for the vein and artery, respectively; Portex, France). The animals were placed in a snug-fitting Perspex restrainer and allowed to recover from anesthesia before the intravenous administration of ^{14}C-2DG (25 μCi; specific activity 293 mCi/mmol; Amersham International, U.K.). Arterial blood samples were collected at 0, 15, 30, 45 sec, 1, 2, 3, 5, 7.5, 10, 15, 25, 35, and 45 min, and centrifuged immediately in a benchtop microcentrifuge at 1300 rpm for 1 min to obtain plasma. At 45 min, the animal was decapitated and the brain removed and rapidly frozen in precooled isopentane at approximately −60°C, before it was stored at −70°C.

Total Glucose Analysis

Plasma (50 μL) from each time point was added to 33% perchloric acid (500 μL) and centrifuged for 1 min in a benchtop microcentrifuge at 1300 rpm, to precipitate blood proteins. Total glucose analysis on the perchlorate supernatants (200 μL) was performed using a Test Combination Glucose kit (Boehringer Mannheim GmbH Diagnostica, U.K.).

^{14}C-Deoxyglucose Analysis

The perchlorate supernatants (200 μL) were added to scintillation vials, to which distilled water (0.5 mL) and Optisafe "Hisafe" II (LKB) scintillation fluid (5 mL) were added. The counting efficiency of the Minimax Tricarb 4000C scintillation counter (Packard, U.K.), in which the radioactivity of the samples was determined, was assessed using known ^{14}C standards and ranged from 40–93%.

Quantification of ^{14}C-Deoxyglucose Incorporation

Coronal sections of rat brain (20 μM) were cut (−7.64 to +3.7 mm from bregma [19]) using a Bright cryostat at −20°C and thaw-mounted onto coverslips before being apposed to β-max hyperfilm with autoradiographic ^{14}C-microscale standards (Amersham International, U.K.) for 1 week. The film was developed using Kodak D-19 developer and fixed with Kodak Unifix. ^{14}C-Deoxyglucose incorporation into specific brain areas was determined by comparing point measurements (μmol/100 g/min) of the optical densities to those from the standards on the same film, using computer-assisted densitometry (Imaging Research, Canada). At least 6 optical density readings were taken from each structure. Local cerebral glucose utilisation rates were calculated from the concentrations of radioactivity in the brain and from plasma glucose content, according to the method of Sokoloff and colleagues [20].

Statistics

The alterations in ^{14}C-deoxyglucose incorporation in response to the isomers of thioctic acid were analysed using the Mann Whitney U-Test.

RESULTS

Acute Administration of R- and S-thioctic Acid

The acute administration of R-thioctic acid (50 mg/kg IP) caused a general increase in ^{14}C-2DG incorporation in the areas of the basal ganglia studied. There were significant increases in ^{14}C-2DG incorporation in both zona compacta and zona reticulata of the substantia nigra (Table 1). In addition, ^{14}C-2DG incorporation was increased in the thalamus, red nucleus, and medial forebrain bundle (Table 1).

In contrast, the acute administration of S-thioctic acid had little overall effect on ^{14}C-2DG incorporation in basal ganglia. It increased ^{14}C-2DG incorporation in the red nucleus, but decreased it in the subthalamic nucleus (Table 1).

Repeated Administration of R- and S-Thioctic Acid

Following repeated administration of R-thioctic acid (50 mg/kg IP) for 5 days, ^{14}C-2DG incorporation in the substantia nigra zona compacta and zona reticulata was unchanged. The incorporation of ^{14}C-2DG was decreased in the globus pallidus and subthalamic nucleus, compared to control animals (Table 2).

Similarly, repeated administration of S-thioctic acid (50 mg/kg IP) for 5 days decreased ^{14}C-2DG incorporation in the globus pallidus, subthalamic nucleus, and thalamus (Table 2).

TABLE 1. Effects of acute administration of R- and S-thioctic acid (50 mg/kg IP) on ^{14}C-2DG incorporation in rat basal ganglia

Brain structure	Control	R-thioctic acid	S-thioctic acid
Striatum	195 ± 17	236 ± 18	206 ± 4
Globus pallidus	153 ± 5	170 ± 16	134 ± 10
Medial forebrain bundle	180 ± 15	223 ± 7*	204 ± 11
Substantia nigra			
zona compacta	152 ± 19	207 ± 5*	169 ± 16
zona reticulata	135 ± 7	199 ± 5*	142 ± 17
Subthalamic nucleus	183 ± 7	194 ± 8	116 ± 20†
Thalamus	190 ± 10	236 ± 18*	183 ± 9
Red nucleus	137 ± 28	241 ± 4†	207 ± 9*

The data expressed as μmol/100 g/min, are the means ± SEM of 5–6 animals per group and are compared to controls according to the Mann Whitney U-test. *$P < 0.05$ and †$P < 0.01$.

DISCUSSION

The object of this study was to determine whether thioctic acid enters the brain and alters glucose utilisation in the basal ganglia. From the experiments undertaken, R- and S-thioctic acid were found to alter ^{14}C-2DG incorporation in a manner that varied between acute and repeated treatment and between the R- and S-isomers of thioctic acid, but which suggests a direct action on brain. These findings validate the previous studies showing ^{35}S-R,S-thioctic acid in brain, extend the findings to specific brain regions, and demonstrate the isomeric nature of the effects observed.

The acute administration of R-thioctic acid shows altered ^{14}C-2DG incorporation in several basal ganglia structures, notably substantia nigra, but S-thioctic acid had, in general, much less effect. Although both isomers altered ^{14}C-2DG incorporation, the naturally occurring R-enantiomer was more effective than S-thioctic acid. R-thioctic acid is the naturally occurring enantiomer that is reduced by lipoamide dehydrogenase/diaphorase [21] to dihydrolipoic acid, which is a more potent antioxidant than thioctic acid itself. Thus, its antioxidant activity may be more marked than the S-enantiomer, which is not a good substrate for this enzyme. However, a recent study has shown that the isomers exert opposite stereoselectivity as a substrate for glutathione reductase, which preferentially reduces S-thioctic acid to dihydrolipoic acid [22]. Alterations in ^{14}C-2DG incorporation reflect altered neuronal activity and, indeed, in other studies we have shown thioctic acid to increase both dopamine and 5-HT turnover in the striatum [23]. Alternatively, alterations in ^{14}C-2DG incorporation may simply reflect the presence of thioctic acid in the brain, allowing it to exert its antioxidant activity and to influence GSH levels and α-KGDH activity. The precise mechanism by which the isomers of thioctic acid alter ^{14}C-2DG incorporation in the brain is not clear. However, the most likely explanation is an action on the glucose transporter.

Previous data has suggested an enantiomer-selective action of thioctic acid on glucose utilisation. Thus, while R,S-thioctic acid enhances glucose transport into skeletal muscle *in vivo* [24, 25], the effect is more apparent with the R-isomer than with S-thioctic acid. Similarly, R-thioctic acid stimulates glucose transport in isolated muscle cells to a greater extent than the S-isomer [18]. The R-isomer has an additive effect on insulin-stimulated glucose transport, but S-thioctic acid inhibits insulin action on glucose transport. In addition, R-thioctic acid promotes the translocation of GLUT 1 and GLUT 4 to the plasma membrane, whereas the S-isomer does not. Thioctic acid may exert a similar effect on brain, but at which level remains unknown. Thioctic acid may act at the neuronal level but it could, alternatively, cause changes in basal ganglia ^{14}C-2DG uptake through the blood-brain barrier.

To determine whether or not the effects of thioctic acid

TABLE 2. Effects of subchronic administration of R- and S-thioctic acid (50 mg/kg IP) for 5 days on ^{14}C-2DG incorporation in rat basal ganglia

Brain structure	Control	R-Thioctic acid	S-Thioctic acid
Striatum	176 ± 9	158 ± 12	168 ± 6
Globus pallidus	144 ± 5	117 ± 7*	122 ± 9*
Medial forebrain bundle	161 ± 7	152 ± 13	161 ± 8
Substantia nigra			
zona compacta	147 ± 13	144 ± 7	141 ± 8
zona reticulata	134 ± 13	132 ± 6	133 ± 8
Subthalamic nucleus	183 ± 7	147 ± 6*	151 ± 6†
Thalamus	183 ± 11	162 ± 10	156 ± 5*
Red nucleus	174 ± 19	173 ± 11	160 ± 9

The data expressed as μmol/100 g/min, are the means ± SEM of 5–6 animals per group and are compared to controls according to the Mann Whitney U-test. *$P < 0.05$ and †$P < 0.01$.

are enhanced by loading over several days and/or are maintained on repeated administration, we studied the effects of subchronic treatment. Repeated administration of R-thioctic acid no longer resulted in an increase in ^{14}C-2DG incorporation in the substantia nigra zona compacta and zona reticulata. As on acute administration, S-thioctic acid also had no effect on nigral ^{14}C-2DG incorporation. These results are in contrast to the finding that repeated administration of R,S- or R-thioctic acid for 10 days continues to stimulate glucose uptake into rat skeletal muscle [24, 25]. There were differences between the effects of acute administration of the R-thioctic acid on ^{14}C-2DG incorporation in rat brain and the results obtained on repeated treatment. Most interestingly, the subchronic administration of R-thioctic acid caused decreases in ^{14}C-2DG incorporation into both the globus pallidus and subthalamic nucleus. These are key areas of the basal ganglia outflow from the striatum, where neuronal activity is known to be altered in Parkinson's disease. Indeed, in MPTP-treated primates, ^{14}C-2DG incorporation is increased in the globus pallidus and decreased in the subthalamic nucleus [26].

In conclusion, thioctic acid alters glucose utilisation in rat basal ganglia. This may indicate its presence in the brain in sufficient amounts to prevent the progression of nigral cell death. Thioctic acid has not yet been utilised in the treatment of Parkinson's disease, but the results of this study would seem to warrant its examination.

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