Stimulation of the respiratory and phosphorylating activities in rat brain mitochondria by idebenone (CV-2619), a new agent improving cerebral metabolism

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The effects of 6-(10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (CV-2619) on the respiration and non-respiratory oxygen consumption induced by ascorbate and Fe^{2+} in rat brain mitochondria were studied. When CV-2619 (100 and 300 mg/kg) was orally administered to rats for 3 days, it increased the state 3 respiration stimulated by ADP, slightly decreased the state 4 respiration after the consumption of ADP and resulted in a significant increase of the respiratory control index (RCI) by 14–19% for glutamate oxidation ($p < 0.01$) and 10–17% for succinate oxidation ($p < 0.05$), respectively. The RCI increasing effect of CV-2619 was dose dependent, but the compound had no effect on the ADP/O ratio. CV-2619 significantly suppressed by about 10% the non-respiratory oxygen consumption ($p < 0.02$), which closely associated with non-enzymatic reactions such as lipid peroxidation, membrane lysis and swelling of mitochondria. Thus, CV-2619 may contribute to stimulate the net ATP formation by the well-coupling of electron and energy transfer, and by the reduction of non-respiratory oxygen consumption in cerebral metabolism.

Idebenone (CV-2619) Brain mitochondria State 3 respiration Respiratory control index Non-respiratory oxygen consumption Cerebral metabolism

1. INTRODUCTION

CV-2619 is a novel compound that has protective and ameliorating effects on biochemical and neurological changes due to cerebral vascular lesions especially with ischemia or hypoxia [1–3]. It has also been reported that CV-2619 suppressed the reduction of ATP level in the rat ischemic brain [3].

From these findings, it is suggested that CV-2619 may influence or protect the respiration and energy metabolism in brain mitochondria. Here, we have studied the effects on the respiratory and phosphorylating activities of brain mitochondria from rats treated with CV-2619. We have also examined the non-respiratory oxygen consumption induced by ascorbate and Fe^{2+}, which is closely associated with non-enzymatic reactions such as lipid peroxidation, membrane lysis and swelling of mitochondria [6–8].

2. MATERIALS AND METHODS

2.1. *Materials*

6-(10-Hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, CV-2619) was synthesized in the Chemistry Laboratories of this Central Research Division [12]. Calcium hopantenate (hopate, Tanabe Seiyaku Co.) and rotenone (Professor H. Fukami, Kyoto University) were kindly provided. Antimycin A was purchased from ICN Pharmaceutical Inc. Other chemicals were obtained from commercial products of highest available purity. Male Wistar rats (9–11 weeks old) were used. They had free access to water and a laboratory chow, CE-2 (CLEA, Japan) until death. CV-2619 (100 and 300 mg/kg) and calcium hopantenate (500 mg/kg) suspended with gum arabic solution (5%) were orally administered to rats (n = 3–5) for 3 days (at 9 a.m.).

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2.2. *Isolation of mitochondria*

Brain mitochondria were isolated according to the method of Ozawa et al. [4] from the pooled brains of rats 1 h after the final administration of drugs. The isolation procedures are briefly described below. Following decapitation of the rats by a guillotine, the brain was removed and put into an ice-cold medium containing 0.3 M mannitol and 0.1 mM EDTA (pH 7.4) precisely 20 s after the decapitation. After the cerebellum and brain stem were removed, the pooled cerebral hemispheres from rats were washed and homogenized in the medium with a loosely fitted teflon homogenizer. The homogenate was centrifuged at 600 × *g* for 8 min and the supernatant was subjected to further centrifugation at 10000 × *g* for 10 min. The resultant pellet was washed once with the medium by centrifugation as above and finally suspended with 1 ml of the medium. Isolated mitochondria were kept on ice and used within 4 h. Mitochondrial protein was determined by the method of Lowry et al. [5] with bovine serum albumin as a standard.

2.3. *Measurement of oxygen consumption*

The mitochondrial respiration was measured by the oxygen electrode operated at room temperature (23–24°C) using an Oxygraph Model 5/6 (Gilson Medical Electronics) in a reaction mixture containing 0.3 M mannitol, 10 mM KCl, 5 mM potassium phosphate, 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4), 1.14–1.47 mg mitochondrial protein and 8 mM glutamate, in a final volume of 1.7 ml. In succinate oxidation, 8 mM succinate and 4 μg rotenone were substituted for glutamate, and 0.57–0.74 mg protein of mitochondria were used. State 3 respiration was initiated by the addition of 250 μM ADP with glutamate as substrate or 147 μM ADP with succinate as substrate. State 4 respiration was the rate of oxygen consumption after the consumption of ADP. The respiratory control index (RCI) was expressed as the ratio of the rate of oxygen consumption in state 3 and state 4. ADP/O ratio was calculated as the ratio of added amount of ADP to the total oxygen consumption during state 3 respiration.

The rate of non-respiratory oxygen consumption induced by ascorbate and Fe^{2+} was also measured in the same reaction mixture as described above without substrate and ADP. After the addition of mitochondria (0.57–0.74 mg protein) and $FeSO_4$ (29 μM), the reaction was followed by the addition of ascorbate (590 μM) for the measurement of oxygen consumption.

2.4. *Statistical analysis*

Data are represented as mean ± SD and statistical analyses were performed by the Student's *t*-test.

3. RESULTS

CV-2619 (100 and 300 mg/kg) and calcium hopantenate (500 mg/kg) for reference was orally

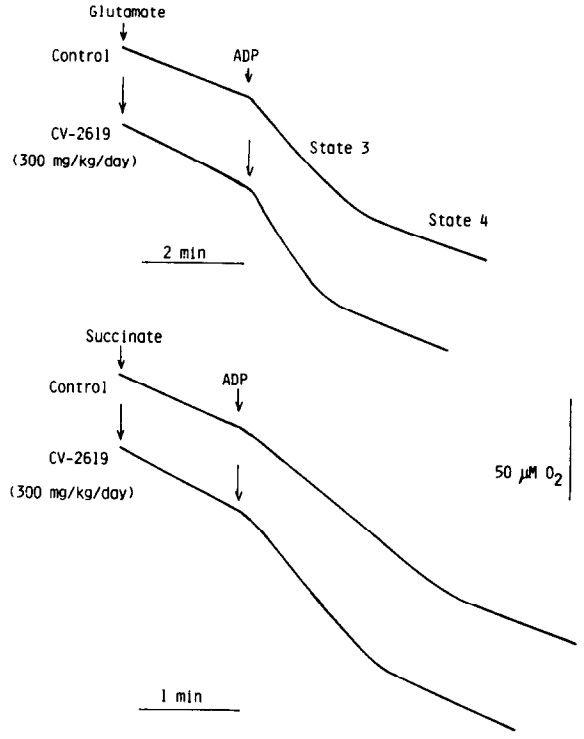

Fig.1. Oxygen electrode recording of brain mitochondria respiring in the presence of glutamate or succinate as substrate. CV-2619 (300 mg/kg) was orally administered to rats for 3 days. Brain mitochondria were isolated and respiration was measured in a reaction mixture containing 0.3 M mannitol, 10 mM KCl, 5 mM potassium phosphate, 0.2 mM EDTA, 10 mM Tris-HCl (pH 7.4) and 0.67 (control) or 0.83 (CV-2619) mg mitochondrial protein, in a final volume of 1.7 ml. When indicated, 4 mM of glutamate or succinate and 250 μM ADP were added. Other experimental details are described in section 2.

Table 1

Effects of CV-2619 and calcium hopantenate administration on the respiratory and phosphorylating activities of rat brain mitochondria

	Dose (mg/kg)	n	State 3	State 4	RCI	ADP/O
			(nmol O_2/min per mg protein)			
Glutamate						
Control	0	8	35.7 ± 1.7	8.72 ± 0.42	4.11 ± 0.33	2.46 ± 0.10
CV-2619	100	6	39.4 ± 2.2[b]	8.39 ± 0.46	4.71 ± 0.39[b]	2.54 ± 0.07
CV-2619	300	6	38.9 ± 1.4[b]	8.00 ± 0.60[a]	4.87 ± 0.37[b]	2.51 ± 0.07
hopantenate	500	6	39.3 ± 1.9[b]	8.58 ± 0.62	4.60 ± 0.44[a]	2.48 ± 0.07
Succinate						
Control	0	10	59.5 ± 4.1	29.6 ± 7.0	2.19 ± 0.23	1.46 ± 0.09
CV-2619	100	7	62.9 ± 5.1	27.3 ± 3.4	2.42 ± 0.18[a]	1.51 ± 0.04
CV-2619	300	7	61.9 ± 5.8	26.5 ± 8.8	2.56 ± 0.42[a]	1.53 ± 0.11
hopantenate	500	7	62.9 ± 4.1	28.7 ± 4.7	2.31 ± 0.25	1.48 ± 0.07

[a] $p < 0.05$, [b] $p < 0.01$ vs control

CV-2619 or calcium hopantenate was orally administered to rats for 3 days. Brain mitochondria were isolated and the respiration was measured as described in section 2. Values are the mean ± SD

administered to rats for 3 days. The respiration of the isolated brain mitochondria was measured. With glutamate as substrate, CV-2619 significantly increased the state 3 respiration stimulated by ADP ($p < 0.01$) and slightly decreased the state 4 respiration after the consumption of ADP (fig.1, table 1). Thus, the RCI was significantly increased by 14–19% in the mitochondria from rats treated with CV-2619 in a dose-dependent manner ($p < 0.01$). However, CV-2619 had no effect on the ADP/O ratio. With succinate as substrate, CV-2619 also increased the RCI in a dose-dependent fashion (11–16%, $p < 0.05$) accompanied by a slight enhancement of state 3 respiration and the suppression of state 4 respiration. On the other hand, calcium hopantenate had a similar increasing effect on the RCI, mainly by the enhancement of state 3 respiration in glutamate ($p < 0.05$) but not significant in succinate oxidation. Thus, the effect of calcium hopantenate was less potent than CV-2619. The ADP/O ratio was not affected by the treatment of calcium hopantenate.

Non-respiratory oxygen consumption induced by ascorbate and Fe^{2+} was also measured. Both CV-2619 and calcium hopantenate significantly decreased the activities of the oxygen consumption by 8–9% ($p < 0.02$, table 2).

Table 2

Effects of CV-2619 and calcium hopantenate administration on the non-respiratory oxygen consumption induced by ascorbate and Fe^{2+} in rat brain mitochondria

	Dose (mg/kg)	n	Oxygen consumption (nmol O/min per mg protein)
Control	0	12	49.9 ± 3.4
CV-2619	100	8	45.2 ± 4.4[a]
CV-2619	300	9	45.4 ± 2.6[b]
Hopantenate	500	9	44.6 ± 3.1[b]

[a] $p < 0.02$ and [b] $p < 0.01$ vs control

CV-2619 or calcium hopantenate was orally administered to rats for 3 days. Brain mitochondria were isolated and oxygen consumption was measured as described in section 2. Values are the mean ± SD

4. DISCUSSION

Here, we have prepared intact rat brain mitochondria with high respiratory and phosphorylating activities using the same tech-

nique as described by Ozawa et al. [4]. These values are in the same range as those reported by others [10]. By using this preparation, we have studied ex vivo effects of CV-2619 on mitochondrial functions such as the respiratory activity coupled with energy metabolism and the non-respiratory oxygen consumption.

It has been reported that CV-2619 improves or protects various abnormalities, such as cerebral vascular lesions and cell damage, caused by cerebral ischemia or hypoxia [1–3]. CV-2619 also has been demonstrated to be effective in improving cerebral metabolism by suppressing the decrease of ATP formation in the rat ischemic brain [3]. In the present study, CV-2619 showed the increasing effect of the RCI accompanied by stimulation of the state 3 respiration and the suppression of state 4 respiration, but the compound did not affect the ADP/O ratio. These results indicate that the brain mitochondria from the CV-2619-treated rat can efficiently perform ATP formation by the rigid coupling of electron and energy transfer.

CV-2619 reduced the non-respiratory oxygen consumption induced by ascorbate and Fe^{2+} in rat brain mitochondria. It has been reported that ascorbate and ferrous ion nonenzymatically induced the oxygen consumption, which closely associated with lipid peroxidation and swelling in mitochondria or microsomes [6–8]. In our experiments, the oxygen consumption induced by only ferrous ion was transient (not shown) but was continued by the following addition of ascorbate. Furthermore, in accordance with data reported by other investigators [6,7], the oxygen consumption was not inhibited by preincubating with electron transfer inhibitors such as rotenone, antimycin A and KCN (not shown). Therefore, the oxygen uptake induced by ascorbate and Fe^{2+} in rat brain mitochondria does not occur through the respiratory chain. These facts indicate that brain mitochondria from CV-2619-treated rats can save oxygen consumed by the non-respiratory system. This effect seems to be important to protect cell damage of the ischemic brain, because the supply of oxygen is limited in ischemic anoxia. Thus, the well-coupling of electron and energy transfer and the oxygen saving action by CV-2619 treatment may both contribute to protection of cerebral damage, possibly cell membranes, against ischemic anoxia through the increased production of ATP.

Although the mechanism of ex vivo action of CV-2619 remains unclear, studies on the direct action of CV-2619 on rat brain mitochondria are needed. Recently, Suno and Nagaoka [11,13] have reported that CV-2619 and its metabolites directly inhibited the lipid peroxidation by measuring the formation of malonedialdehyde (MDA) in rat brain homogenate [11] and mitochondria [13]. As it is well known that the radicals and lipid peroxides impair the mitochondrial function and construction [9,10], the inhibitory action of MDA formation by CV-2619 may contribute to the effects observed in this study.

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