Protective Effect of DM-9384, a Novel Pyrrolidone Derivative, against Experimental Cerebral Anoxia

Takeo SAKURAI, Satoshi HATANAKA, Satoru TANAKA, Terukiyo YAMASAKI, Hiroshi KOJIMA and Akira AKASHI
Research Institute, Daiichi Pharmaceutical Co., Ltd., 16-13, Kita-Kasai 1-chome, Edogawa-ku, Tokyo 134, Japan
Accepted June 1, 1990

Abstract—The protective effects of DM-9384 \([N-(2,6\text{-dimethylphenyl})\cdot 2\cdot (2\text{-oxo-1-pyrrolidinyl})\text{ acetamide}]\) against cerebral anoxia were investigated using various animal models. Oral administration of DM-9384 resulted in a significant prolongation of survival time in mice and rats subjected to the normobaric hypoxia; its minimal effective doses were 30 and 10 mg/kg, respectively. A significant protection by this drug against hypobaric hypoxia, histotoxic anoxia and cerebral ischemia also occurred in mice at a dose of 100 mg/kg, p.o. Bifemelane (100–300 mg/kg, p.o.) was protective against these models except for hypobaric hypoxia, and the effects of piracetam, aniracetam and pramiracetam (1000 mg/kg, p.o.) were variable depending on the type of anoxia model used. DM-9384 (100 mg/kg and lower) attenuated the hypolocomotion and the disturbance of cerebral energy metabolism such as a decrease in ATP, an increase in lactate and lactate/pyruvate ratio induced by hypoxia in rats. The spontaneous motor activity, uptake and utilization of brain glucose in normal animals, however, were not influenced by this drug. Based on these results, DM-9384 is characterized as a broad spectrum anti-anoxic drug with negligible CNS depression, and the cerebral protective effect of this drug may be, at least in part, attributable to its ability to improve the cerebral energy metabolic disturbance.

It is well-known that a decrease in the oxygen content (hypoxia) depresses brain function in experimental animals and humans. The cerebral hypoxic and ischemic models are widely used in the preclinical evaluation of drugs for the treatment of cerebrovascular disorders (1–4). For example, bifemelane, which was recently developed as a new cerebroacting drug, was reported to prolong the duration of respiratory activity in normobaric hypoxic, KCN-induced anoxic and decapitation-induced cerebral ischemic animals (5).

DM-9384 is a new pyrrolidone derivative. It has been shown to improve electroconvulsive shock-, scopolamine- and GABA antagonist-induced amnesia, and to accelerate acquisition of learning both negatively and positively reinforced tasks in rodents (6, 7). Accordingly, DM-9384 is considered to be a cognition-enhancing agent like piracetam (8, 9), aniracetam (10) and pramiracetam (11).

In the present study, we examined the cerebral protective effect of DM-9384 using various anoxia models in comparison with those of piracetam, aniracetam, pramiracetam and bifemelane. Additionally, the effect of DM-9384 on the cerebral energy metabolism was also investigated to elucidate the mechanism(s) of the anti-anoxic effect of this drug.

Materials and Methods

Animals: Male ddY mice weighing 22–28 g, male Wistar rats weighing 170–290 g and male Sprague-Dawley (SD) rats weighing 310–420 g were purchased from Japan SLC, Inc. The animals were housed in rooms controlled at 23±2°C and 55±15% of relative
humidity on a 12-hr light-dark cycle (7:00–19:00).

**Drugs:** DM-9384 (Daiichi Pharmaceutical Co., Ltd.). Piracetam, aniracetam, pramiracetam sulfate and bifemelane hydrochloride used as reference drugs were synthesized at our Research Institute. DM-9384, piracetam, aniracetam and pramiracetam were suspended in 0.5% sodium carboxymethyl cellulose solution, and bifemelane was dissolved in distilled water. Doses of pramiracetam and bifemelane were expressed in terms of their salts. These drugs were administered orally. [14C]-2-Deoxy-D-glucose (14C-2-DG, specific activity: 2.04 MBq/mmol) was obtained from New England Nuclear Corp. (Boston, MA).

**Hypobaric hypoxia in mice:** Mice were put into a chamber (3-L desiccator), and the inside pressure of this chamber was lowered to 200 mmHg in approximately 25-26 sec with a vacuum pump. Survival time was defined as the time interval between the start of hypoxia and the cessation of respiratory movements, and the survival time of longer than 10 min was assigned the value of 10 min in the calculations (12). Drugs were administered 1 hr before the hypoxia.

**Normobaric hypoxia in mice:** Mice were put into a 500-ml glass container after 1 hr medication. Normobaric hypoxia was produced by introducing pure nitrogen gas into the container at the rate of 5 L/min. The time when the respiratory arrest occurred was recorded as the survival time.

**Histotoxic anoxia in mice:** Histotoxic anoxia was produced by i.v. injection of 4 mg/kg KCN. The survival time was measured with a limit of 180 sec (13). Drugs were administered 1 hr before the KCN injection.

**Complete ischemia in mice:** Cerebral ischemia was produced by intersection between the medulla and the spinal cord. The time between decapitation and the last gasp was recorded as gasping duration (13). Drugs were administered 1 hr before the decapitation.

**Normobaric hypoxia in rats:** Wistar rats were put into a 3.5-L plastic chamber after 1 hr medication. Hypoxia was produced by introducing pure nitrogen gas into the chamber at the rate of 20 L/min. The time when respiratory arrest occurred was recorded as the survival time (14).

**Locomotor activity in normal and hypoxic rats:** One hour after the drug administration, locomotor activity of Wistar rats was recorded every 10 min for 30 min with an Animex (Farad Electronics). Hypoxia was induced by lowering the concentration of oxygen in the chamber (22.5 x 30.0 x 18.5 cm) to about 3.6% (Pocket oxygen alarm, Yokogawa Hokushin Electric) for a 9-min period by blowing pure nitrogen at the flow rate of 10 L/min for 110 sec in the chamber, just before the locomotion test (15).

**Brain glucose uptake in mice:** The procedure used was that of Shibota et al. (16). The mice were injected with 14C-2-DG (185 KBq/kg) intravenously at 60 min after drug administration. Ten minutes later, the animals were decapitated and the brain was removed. The blood samples were centrifuged, and plasma levels of 14C-2-DG and glucose were determined by a liquid scintillation counter, and the glucose oxidase technique, respectively. Whole brain was combusted with an automatic sample combustion system (Aloka Co., Ltd.), and the 14CO2 formed by combustion was assayed by liquid scintillation counting. The brain glucose uptake (mg/g/10 min) was calculated using the following equation (16):

\[
\text{brain glucose uptake} = \text{brain }^{14}\text{C activity (dpm/g)} \\
\times \frac{\text{plasma glucose level (mg/dl)}}{\text{plasma }^{14}\text{C activity (dpm/ml)}} \\
\times \frac{1}{100}
\]

**Local cerebral glucose utilization (LCGU) in rats:** LCGU in the fully conscious rat was measured by the 14C-2-deoxyglucose method described by Sokoloff et al. (17) with some modification. SD rats, fasted for approximately 16 hr, were anesthetized with halothane, and polyethylene catheters (PE-50, Clay-Adams) were implanted into the femoral artery and vein for timed sampling of arterial blood and for the injection of 14C-2-DG, respectively. Halothane was discontinued, the animals were allowed to recover for at least 1 hr, and then the drug was administered. Sixty minutes later, 14C-2-DG (740 KBq/kg) was injected, and timed arterial blood samples were then withdrawn during the following 45 min (baseline, 15 and 30 sec, and 1, 2, 3, 5, 10, 15,
20, 30 and 45 min). At the end of the 45 min sampling period, the rats were sacrificed with an overdose of sodium pentobarbital (50 mg, i.v.), and the brain was removed as rapidly as possible and frozen. The plasma \(^{14}\text{C}\) or glucose level and \(^{14}\text{C}\) activity in each region of the brain were measured by the same method as described in the brain glucose uptake test. LCGU (R) was calculated by the following equation and the constant reported by Sokoloff et al. (17) using a computer (FACOM 6658K).

\[
R = \frac{C_i(\tau) - k_1 e^{-((k_2 + k_3)\tau)} \int_0^\tau C_p e^{(k_2 + k_3)\tau} d\tau}{L_c \left[ \int_0^\tau (C_p/C_p) dt - e^{-(k_2 + k_3)\tau} \int_0^\tau (C_p/C_p) e^{(k_2 + k_3)\tau} dt \right]}
\]

Where \(C_i(\tau)\) is the local cerebral tissue concentration of \(^{14}\text{C}\); \(C_p\) is the time courses of the plasma radioactivities; \(C_p\) is the time courses of the plasma glucose concentrations; \(k_1\), \(k_2\) and \(k_3\) are kinetic constants for phosphorylation of deoxyglucose; and \(L_c\) is the lumped constant (0.464).

**Cerebral energy metabolites in hypoxic rats:** Wistar rats were used; the procedures of drug administration and the load of hypoxia were the same as described in the locomotor test. Immediately after the hypoxia, the rats were sacrificed by focussed microwave irradiation (4.8 kW for 1.2 sec; Muromachi Kikai Co., Ltd.). The microwaved brains were immediately removed, weighed, and homogenized with 7 vol. of 0.6 N perchloric acid and then centrifuged (10000 rpm for 15 min, at 4°C). The protein-free acid extract was used as samples of energy metabolites assay. ATP, pyruvate and lactate levels were determined according to the enzymatic methods using reagent kits (Boehringer Mannheim Biochemicals).

**Statistical analysis:** The results are expressed as the mean±S.E.M. Statistical significance was assessed using one-way analysis of variance and multiple comparison by Dunnett.

**Results**

**Effect on hypobaric hypoxia in mice:** The protective effects of DM-9384 and reference drugs against hypobaric hypoxia are shown in

| Drug            | Dose (mg/kg, p.o.) | Survival time (min) |
|-----------------|--------------------|---------------------|
| DM-9384         | 0                  | 2.7±0.4             |
|                 | 30                 | 3.3±0.3             |
|                 | 100                | 6.5±0.6**           |
| Piracetam       | 0                  | 2.8±0.4             |
|                 | 300                | 2.7±0.5             |
|                 | 1000               | 4.4±0.9             |
| Aniracetam      | 0                  | 3.0±0.4             |
|                 | 300                | 2.7±0.3             |
|                 | 1000               | 4.9±0.7*            |
| Pramiracetam    | 0                  | 2.9±0.4             |
|                 | 300                | 2.8±0.3             |
|                 | 1000               | 2.8±0.4             |
| Bifemelane      | 0                  | 2.3±0.3             |
|                 | 30                 | 2.4±0.4             |
|                 | 100                | 3.5±0.8             |
|                 | 300                | 3.1±0.2             |

Drugs were administered 1 hr before the hypoxia (200 mmHg). The control group was treated with vehicle. Each value represents the mean±S.E.M. (n=10). *P<0.05, **P<0.01 vs. control (0 mg/kg).
Table 1. Oral treatment of the hypoxic mice with DM-9384 resulted in a significant prolongation of the survival time with a threshold dose of 100 mg/kg. After administration of aniracetam, there was a significant prolongation of the survival only at doses as high as 1000 mg/kg, p.o. Bifemelane at doses of 100 and 300 mg/kg, p.o. tended to increase the survival, but the difference did not reach statistical significance. Piracetam and pramiracetam were also without significant effect on the survival at doses up to 1000 mg/kg, p.o.

Effect on normobaric hypoxia in mice: As summarized in Table 2, DM-9384, like aniracetam and bifemelane, significantly increased the survival of hypoxic mice in a dose-dependent manner. The minimum effective oral doses were estimated to be 30 mg/kg for DM-9384, 1000 mg/kg for aniracetam and 100 mg/kg for bifemelane. As in the normobaric hypoxic model, the effects of piracetam and pramiracetam (100–1000 mg/kg, p.o.) were not significant.

Effect on KCN-induced anoxia in mice: The summarized data are illustrated in Table 3. DM-9384 at doses of 10–100 mg/kg, p.o. provided a dose-dependent protective effect, and the prolongation of survival of KCN-treated mice became more prominent and significant after the 100 mg/kg dose. Bifemelane was highly effective in this model, and the response to the 100 mg/kg dose was of similar magnitude as compared with the same dose of DM-9384. The effects of piracetam, aniracetam and pramiracetam were significant only at doses of 1000 mg/kg, p.o.

Effect on gasping duration in mice: The results obtained are presented in Table 4. DM-9384 at a dose of 100 mg/kg, p.o. produced a significant prolongation of the gasping duration. Bifemelane (300 mg/kg, p.o.) also significantly prolonged the gasping duration, an effect which was similar in magnitude to

Table 2. Effects of DM-9384 and reference drugs on the normobaric hypoxia in mice

| Drug       | Dose (mg/kg, p.o.) | Survival time (sec) |
|------------|--------------------|---------------------|
| DM-9384    | 0                  | 31.7±0.7            |
|            | 10                 | 32.4±0.9            |
|            | 30                 | 36.4±1.0*           |
|            | 100                | 41.3±1.1**          |
| Piracetam  | 0                  | 31.6±1.2            |
|            | 100                | 31.5±0.7            |
|            | 300                | 32.4±0.7            |
|            | 1000               | 33.8±0.6            |
| Aniracetam | 0                  | 31.4±0.7            |
|            | 100                | 32.0±0.5            |
|            | 300                | 32.8±0.7            |
|            | 1000               | 35.3±1.1**          |
| Pramiracetam| 0                  | 32.5±0.6            |
|            | 100                | 32.7±0.7            |
|            | 300                | 32.8±0.7            |
|            | 1000               | 32.2±1.1            |
| Bifemelane | 0                  | 33.7±0.7            |
|            | 30                 | 36.5±0.9            |
|            | 100                | 39.4±1.5**          |
|            | 300                | 44.8±1.5**          |

Drugs were administered 1 hr before the hypoxia (pure nitrogen gas). The control group was treated with vehicle. Each value represents the mean±S.E.M. (n=10). *P<0.05, **P<0.01 vs. control (0 mg/kg).
that seen after the 100 mg/kg dose of DM-9384. In contrast, piracetam, aniracetam and pramiracetam failed to have any protective effect even at doses of 1000 mg/kg, p.o.

Effect on normobaric hypoxia in rats: As shown in Table 5, DM-9384, at doses ranging from 10 to 100 mg/kg, p.o. produced a significant and dose-related prolongation of the survival time of hypoxic rats, although its 3 mg/kg dose was inactive (data not shown). Bifemelane (100 mg/kg, p.o.) was also effective, whereas piracetam, aniracetam and pramiracetam lacked the ability to improve the survival at doses up to 1000 mg/kg, p.o.

Effect on locomotor activity in normal and hypoxic rats: DM-9384 at 30 and 100 mg/kg, p.o. did not cause any significant changes in the locomotor activity of normal rats, but atenuated significantly and dose-dependently the hypolocomotion of hypoxia-induced rats at 10 min after the hypoxia (Fig. 1). At 10 mg/kg, p.o., however, there was no significant alteration during the 30-min observation period (data not shown). Bifemelane (100 mg/kg, p.o.) exerted no effect on locomotor activity in both normal and hypoxia rats. Aniracetam (1000 mg/kg, p.o.) also had almost no effect (Fig. 2).

Effect on brain glucose uptake in mice: DM-9384 had no effect on the glucose and $^{14}$C activity level of the plasma and no effect on the $^{14}$C activity level and glucose uptake of the brain at doses up to 100 mg/kg, p.o. (Table 6).

Effect on LCGU in rats: The LCGU was quantitated in 19 regions of the rat brain. The values of LCGU in control animals were similar to those reported previously for conscious male rats (17). DM-9384 at 10 and 30 mg/kg, p.o. decreased LCGU in the sensory motor cortex, the septal nucleus and the pons by 10–12% as compared with the control values, although these changes did not reach statistical significance (Fig. 3). The mean

| Drug       | Dose (mg/kg, p.o.) | Survival time (sec) |
|------------|--------------------|---------------------|
| DM-9384    | 0                  | 37.8±1.6            |
|            | 10                 | 40.8±1.6            |
|            | 30                 | 45.7±1.6            |
|            | 100                | 67.6±5.3**          |
| Piracetam  | 0                  | 36.4±1.7            |
|            | 100                | 40.7±2.4            |
|            | 300                | 39.5±1.4            |
|            | 1000               | 43.1±2.0*           |
| Aniracetam | 0                  | 37.9±1.8            |
|            | 100                | 43.5±2.3            |
|            | 300                | 45.3±3.7            |
|            | 1000               | 51.2±4.4*           |
| Pramiracetam| 0                 | 34.6±0.8            |
|             | 100                | 33.9±1.2            |
|             | 300                | 36.7±1.6            |
|             | 1000               | 43.2±2.4**          |
| Bifemelane | 0                  | 38.4±1.7            |
|            | 30                 | 43.5±2.2            |
|            | 100                | 68.1±12.7*          |
|            | 300                | 173.1±6.9**         |

Drugs were administered 1 hr before the KCN (4.0 mg/kg, i.v.) injection. The control group was treated with vehicle. Each value represents the mean±S.E.M. (n=10). *P<0.05, **P<0.01 vs. control (0 mg/kg).
values of the other regions were somewhat lower than those of the control, but did not achieve statistical significance.

Effect on the changes of cerebral energy metabolites in hypoxic rats: As shown in Table 7, the hypoxic animals treated with vehicle alone exhibited a pattern of metabolic rearrangement that was characterized by a moder-
Fig. 1. Effects of DM-9384 on locomotor activity in normal and hypoxic rats. DM-9384 was administered 1 hr before the activity test by Animex. The control group was treated with vehicle. Locomotor activity was measured immediately after the hypoxia (3.6% O₂, 9 min). ○: Control; ●: DM-9384 30 mg/kg, p.o.; ▲: DM-9384 100 mg/kg, p.o. Each point represents the mean±S.E.M. (n=8). *P<0.05, **P<0.01 vs. control.

Fig. 2. Effects of aniracetam and bifemelane on locomotor activity in normal and hypoxic rats. Drugs were administered 1 hr before the activity test by Animex. The control group was treated with vehicle. Locomotor activity was measured immediately after the hypoxia (3.6% O₂, 9 min). ○: Control; ●: Aniracetam 1000 mg/kg, p.o.; ▲: Bifemelane 100 mg/kg, p.o. Each point represents the mean±S.E.M. (n=8). *P<0.05 vs. control.

A slight decrease in ATP and an increase in lactate and lactate/pyruvate (L/P) ratio (these changes were significantly different from normal animals). A slight increase in cerebral pyruvate level occurred in the hypoxic rats, but this was not significantly different from that of the normal control. DM-9384 (10–100 mg/kg, p.o.) improved these changes of
Table 6. Effect of DM-9384 on brain glucose uptake in mice

| Drug    | Dose (mg/kg, p.o.) | Brain $^{14}$C (Kdpm/g) | Plasma $^{14}$C (Kdpm/ml) | Plasma G. (mg/dl) | Brain G. uptake (mg/g/10 min) |
|---------|-------------------|-------------------------|---------------------------|-------------------|-------------------------------|
| Control | 0                 | 33.3±1.9                | 22.8±1.3                  | 167±11            | 2.40±0.20                     |
| DM-9384 | 10                | 30.4±1.4                | 20.4±1.4                  | 175±7             | 2.66±0.15                     |
|         | 30                | 36.0±1.8                | 22.1±1.6                  | 162±5             | 2.61±0.15                     |
|         | 100               | 30.9±1.1                | 23.3±0.9                  | 179±4             | 2.40±0.11                     |

DM-9384 was administered 1 hr before the decapitation. The control group was treated with vehicle. Each value represents the mean±S.E.M. (n=10). G.: glucose.

Table 7. Effect of DM-9384 on the changes in cerebral energy metabolites in hypoxic rats

| Drug         | Dose (mg/kg, p.o.) | ATP     | Lactate (L) | Pyruvate (P) | L/P ratio |
|--------------|-------------------|---------|-------------|--------------|-----------|
| Control      | 0                 | 1.60±0.05 | 13.6±0.8    | 0.34±0.07    | 46.7±8.5 |
| DM-9384      | 10                | 1.63±0.03 | 13.5±1.2    | 0.35±0.07    | 43.8±6.7 |
|              | 30                | 1.67±0.04 | 12.3±0.5    | 0.42±0.08    | 34.0±5.6 |
|              | 100               | 1.74±0.02* | 10.3±0.6*   | 0.40±0.07    | 31.3±6.1 |
| Non hypoxia  | 0                 | 1.74±0.02* | 4.2±0.3**   | 0.25±0.05    | 20.9±4.6* |

DM-9384 was administered 1 hr before the microwave irradiation. The control group was treated with vehicle. Each value represents the mean±S.E.M. (n=6). *P<0.05. **P<0.01 vs. control.

Fig. 3. Effect of DM-9384 on local cerebral glucose utilization (LCGU) in conscious rats. The vehicle (open column) or DM-9384 (dotted column: 10 mg/kg, hatched column: 30 mg/kg) were administered orally 1 hr before the measurement of LCGU. C.: cortex. Vertical bars represent the mean±S.E.M. (n=5).
cerebral metabolism induced by hypoxia in a dose-dependent manner, as evidenced by a significant increase in the ATP content with a significant decrease in lactate content after a dose of 100 mg/kg.

Discussion

The causes of hypoxidosis in the brain can be classified into three types: hypoxic, histotoxic and ischemic anoxia. In the present study, therefore, hypobaric-, normobaric hypoxia (hypoxic), KCN-induced anoxia (histotoxic) and cerebral ischemia induced by decapitation (ischemic anoxia) were used for the evaluation of the anti-anoxic activity of DM-9384.

The present study demonstrates that DM-9384 exerted an anti-anoxic effect in all the anoxic models studied, as assessed by the protection against impaired respiratory activity. Furthermore, DM-9384 was found to improve the decrease in locomotor activity of rats subjected to hypoxia without affecting the locomotion in normal rats. These results suggest that the improving effect of DM-9384 on the hypolocomotion appears to be mediated through its protective action against cerebral anoxia. The anti-anoxic effect of bifemelane was also confirmed in our experiments; however, this drug failed to improve the hypolocomotion induced by hypoxia, suggesting that the mode of the anti-anoxic effect of bifemelane is different from that of DM-9384. Concerning this point, Tobe et al. (15) provided evidence that, when administered repeatedly, bifemelane is capable of producing an improvement in the hypoxia-induced hypolocomotion similar to that employed in the present experiment.

Although the anti-anoxic effect of piracetam has been often reported (2, 18), little information is available regarding the relative anti-anoxic potency of pyrrolidone derivatives used in the present study. Our data indicate that piracetam and pramiracetam were effective in prolonging the survival time only in the KCN-anoxia model, whereas aniracetam increased the survival to a similar extent in mice exposed to the hypobaric-, normobaric hypoxia and KCN-anoxia. On the other hand, as mentioned above, DM-9384 was found to be protective against all the anoxia models, and it was characterized by its ability to produce the most potent protection against the normobaric hypoxia. These observations suggest that pharmacological properties of these pyrrolidone drugs are not homogeneous in spite of their close structural similarity.

A number of investigators have reported apparent protective effects of barbiturate and other CNS depressants, e.g., anti-anxiety drugs, against cerebral anoxia (12–14). The mechanism of the cerebral protective effect of these drugs have been explained by cerebral metabolic depression and/or suppression of the energy demand (12, 19). Additionally, these CNS depressants were demonstrated to decrease LCGU in rats (17, 20). Thus, we examined whether DM-9384 affects the cerebral energy state in normal animals using the LCGU and the glucose brain uptake technique. In this study, DM-9384 neither caused significant changes in the LCGU in 19 regions of rats nor inhibited the brain glucose uptake in mice. Therefore, it was assumed that the pharmacological mode of action of DM-9384 in anoxia models is distinguishable from that of CNS depressants.

The relationship between hypoxia or cerebral ischemia and cerebral energy metabolism disturbance has already been well-recognized (19, 21). In our study on cerebral energy metabolism which employed a 3.6% oxygen concentration to produce the same hypoxic condition as the hypolocomotion model, a significant decrease in ATP level and a significant increase in lactate and L/P ratio were demonstrated. This finding indicates that anaerobic glycolysis was dominant in the energy production of the hypoxic state, a phenomenon which coincided with that reported by Gottesfeld and Miller. (22) who have demonstrated that a 4% oxygen concentration produced a decrease in cerebral ATP level.

DM-9384 dose-dependently improved the disturbance of cerebral energy metabolism under the hypoxic condition, which seemed likely to be correlated with its protective effect against hypolocomotion. Accordingly, the improvement by DM-9384 of cerebral energy metabolism may be responsible for its anti-hypolocomotion effect. Although the exact
mechanism(s) by which DM-9384 improves cerebral energy metabolism is still unclear, the effect of this drug can be considered to be regulated by the facilitation of aerobic glycolysis rather than that of the anaerobic one if you take into consideration the changes in ATP, lactate level and L/P ratio. On the basis of the electrophysiological studies in vitro (23), a decrease in ATP level during hypoxia was suggested to be relate to the disturbance of neurotransmission due to the functional inhibition of the Na-K pump. At present, electrophysiological studies of the effect of DM-9384 on hypoxia are in progress in our laboratory.

It is known that complete ischemia induced by decapitation is dependent on the glucose level in the brain and not correlated with the supply of oxygen from the cerebral circulation (24). Therefore, the anti-anoxic effect of DM-9384 on this model may not be explained by only stimulation of aerobic glycolysis. Gibson and Duffy (25) and Gibson et al. (26) reported that the impairment of ACh synthesis occurs in hypoxic brain. DM-9384 has been reported to produce an elevation of choline acetyltransferase activity and choline uptake in rat brain (27, 28). Thus, these neurochemical changes appear to be a possible mechanism contributing to the cerebral protective action of DM-9384.

In conclusion, DM-9384 produced protective effects against cerebral anoxia without inducing CNS depression, and it is likely that this effect may be, at least in part, mediated through the improvement of cerebral energy metabolic disturbance.

Acknowledgments: The authors would like to thank Mrs. T. Sakamoto for her excellent technical assistance.

References
1 Wauquier, A., Ashton, D., Clincke, G. and Fransen, J.: "Calcium entry blockers" as cerebral protecting agents: Comparative activity in tests of hypoxia and hyperexcitability. Japan. J. Pharmacol. 38, 1–7 (1985)
2 Kriegstein, V.J., and Heuer, H.: On the usefulness of a model of acute hypoxia for testing cerebroprotective drugs. Arzneimittelforschung 36, 1568–1571 (1986)
3 Masuda, Y., Ochi, Y., Ochi, Y., Karasawa, T., Hatano, N., Kadokawa, T. and Okegawa, T.: Protective effect of a new prostaoyclin analogue OP-2507 against cerebral anoxia and edema in experimental animals. Eur. J. Pharmacol. 123, 335–344 (1986)
4 Nikolov, R., Dikova, M., Nikolova, M., Voronina, T., Nerobkova, L. and Garibova, T.: Cerebroprotective effect of nicergoline and interference with the anti-hypoxic effect of prostaoyclin. Methods Find. Exp. Clin. Pharmacol. 9, 479–484 (1987)
5 Tobe, A., Egawa, M. and Hashimoto, N.: Anti-anoxic effect of 4-(o-benzylphenoxy)-N-methylbutylamine hydrochloride (MCI-2016). Folia Pharmacol. Japon. 81, 421–429 (1983) (Abs. in English)
6 Sakurai, T., Ojima, H., Yamasaki, T., Kojima, H. and Akashi, A.: Effects of N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide (DM-9384) on learning and memory in rats. Japan. J. Pharmacol. 50, 47–53 (1989)
7 Nabeshima, T., Noda, Y. and Kameyama, T.: Effect of DM-9384, a pyrrolidone derivative, on an amnesia model animal having GABA-ergic neuronal dysfunctions. Xth Int. Cong. Pharmacol. Aug 23–28, Sydney (Abstract), 301 (1987)
8 Giurgea, C., Lefevre, D., Lescrenier, C. and David-Remacle, M.: Pharmacological protection against hypoxia induced amnesia in rats. Psychopharmacologia (Berlin) 20, 160–168 (1971)
9 Sara, S.J. and David-Remacle, M.: Recovery from electroconvulsive shock-induced amnesia by exposure to the training environment: Pharmacological enhancement by piracetam. Psychopharmacologia (Berlin) 36, 59–66 (1974)
10 Cumin, R., Bande, E.F., Gamzu, E. and Haefely, W.E.: Effects of the novel compound aniracetam (Ro13-5057) upon impaired learning and memory in rodents. Psychopharmacology (Berlin) 78, 104–111 (1982)
11 Poschel, B.P.H., Marriott, J.G. and Gluckman, M.I.: Pharmacology of the cognition activator primiracetam (CI-879). Drugs Exp. Clin. Res. 9, 853–871 (1983)
12 Nakanishi, M., Yasuda, H. and Tsumagari, T.: Protective effect of anti-anxiety drugs against hypoxia. Life Sci. 13, 467–474 (1973)
13 Wauquier, A., Ashton, D., Clincke, G. and Niemeegers, C.J.E.: Anti-hypoxic effects of etomidate, thiopental and methohexitol. Arch. Int. Pharmacodyn. Ther. 249, 330–334 (1981)
14 Yasuda, H., Shuto, S., Tsumagari, T. and Nakajima, A.: Protective effect of a novel imidazole derivative against cerebral anoxia. Arch. Int. Pharmacodyn. Ther. 233, 136–144 (1978)
15 Tobe, A., Egawa, M., Saito, K. and Hashimoto, N.:
Effects of 4-(o-benzylphenoxy)-N-methylbutylamine hydrochloride (MCI-2016, bifemelane hydrochloride) on spontaneous motor activity under different experimental conditions. Folia Pharmacol. Japon. 86, 51–60 (1985) (Abs. in English)

16 Shibota, M., Kakihana, M. and Nagaoka, A.: The effect of vinpocetine on the brain glucose uptake in mice. Folia Pharmacol. Japon. 80, 221–224 (1982) (Abs. in English)

17 Sokoloff, L., Reivich, M., Kennedy, C., Des Rosiers, M.H., Patlak, C.S., Pettigrew, K.D., Sakurada, O. and Shinohara, M.: The [14C]-deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J. Neurochem. 28, 897–916 (1977)

18 Berga, P., Beckett, P.R., Roberts, D.J., Llenas, J. and Massingham, R.: Synergistic interactions between piracetam and dihydroergocristine in some animal models of cerebral hypoxia and ischemia. Arzneimittelforschung 36, 1314–1320 (1986)

19 Yasuda, H., Nakanishi, M., Tsumagari, T., Nakajima, A. and Nakanishi, M.: The mechanism of action of a novel cerebral protective drug against anoxia 1. The effect on cerebral energy demand. Arch. Int. Pharmacodyn. Ther. 242, 77–85 (1979)

20 Ableitner, A., Wüster, M. and Herz, A.: Specific changes in local cerebral glucose utilization in the rat brain induced by acute and chronic diazepam. Brain Res. 359, 49–56 (1985)

21 Duffy, T.E., Nelson, S.R. and Lowry, O.H.: Cerebral carbohydrate metabolism during acute hypoxia and recovery. J. Neurochem. 19, 959–977 (1972)

22 Gottesfeld, Z. and Miller, A.T., Jr.: Metabolic response of rat brain to acute hypoxia: Influence of polycythemia and hypercapnia. Am. J. Physiol. 216, 1374–1379 (1969)

23 Lipton, P. and Whittingham, T.S.: The effect of hypoxia on evoked potentials in the in vitro hippocampus. J. Physiol. (Lond.) 287, 427–436 (1979)

24 Holowach-Thurston, J., Hauhart, R.E. and Jones, E.M.: Anoxia in mice: Reduced glucose in brain with normal or elevated glucose in plasma and increased survival after glucose treatment. Pediatr. Res. 8, 238–243 (1974)

25 Gibson, G.E. and Duffy, T.E.: Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. J. Neurochem. 36, 28–33 (1981)

26 Gibson, G.E., Peterson, C. and Sansone, J.: Decreases in amino acid and acetylcholine metabolism during hypoxia. J. Neurochem. 37, 192–201 (1981)

27 Kawajiri, S., Sakurai, T., Ojima, H., Hatanaka, S., Yamasaki, T., Kojima, H. and Akashi, A.: Effect of DM-9384, a new pyrrolidone derivative, on learning behavior and cerebral choline acetyltransferase activity in rats. Psychopharmacology (Berlin). Supp. 96, 306 (1988)

28 Watabe, S., Yamaguchi, H. and Ashida, S.: Effects of DM-9384, a new cognition-enhancing agent, on GABA release and choline uptake in rat cortex. 19th Annual Meeting. Society for Neuroscience (abstract) 15, Part 1, 601 (1989)