Inhibition of Lipid Peroxidation by some Dihydropyridine Derivatives

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The efficacy of dihydropyridine derivatives in inhibiting lipid peroxidation was studied using modified Buege and Aust's method. The method first involves keeping a decapitated rat head at 37°C for 30 min in order to induce global ischemia. Then, the cortex is removed and homogenized, and the homogenate is subsequently exposed to air for 30 min for reoxygenation. Finally, the amount of thiobarbituric acid-reactive substances (TBAR) is measured. With this method, nisoldipine, nimodipine, nitrendipine, nifedipine and nicardipine were all shown to have an antioxidant activity that correlated with their lipophilicity, which was determined by their octanol/water partition coefficients.
(Key words: brain ischemia, lipid peroxidation, antioxidant activity, dihydropyridine, lipophilicity)

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Among several 1, 4-dihydropyridines (DHPs) with calcium channel antagonistic activity, nimodipine is known to have cerebrovasodilatory and neuronal protective effects with little effect on peripheral circulation[1,2]. Nimodipine was suggested to have therapeutic value in the treatment of subarachnoid hemorrhage[3], brain ischemia[4–7] and epilepsy[8,9]. Although it has been assumed that the drug inhibits the calcium entry of single neurons by inactivating calcium channels (L-type), the precise mechanism of protection has not been established. DHPs were also shown to have some protective effects in several shock models. It has been reported that nimodipine has beneficial effects on traumatic shock in rats[10] and nitrendipine in hemorrhagic shocked cats[11]. Moreover, nisoldipine has been shown to prevent the sudden death of rabbits after arachidonate administration[12].

Recently, it has suggested that nifedipine has antioxidant activity[13]. Since it is generally accepted that free radicals may be the triggering factor of membrane damage in ischemia-reperfusion injury[14] and that antioxidants may play an important role in protecting the brain against injury[15–17], we undertook this study to measure the antioxidant activity of several DHPs. We have recently developed a simple *in vitro* assay of measuring the antioxidant activity of compounds using the rat brain[18]. Therefore, we applied this method to measure the antioxidant activity of five different DHPs.

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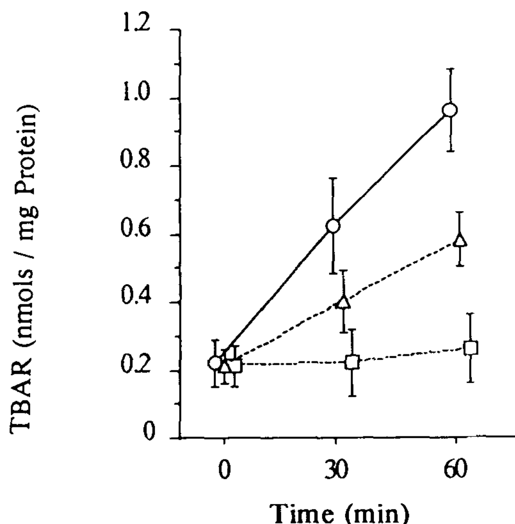

Fig. 1. The formation of TBAR during oxygenation of cortex homogenates. (○) The cortex was removed from the ischemic brain (kept at 37°C for 30 min). (△) The cortex was removed from the ischemic brain, but 50 $\mu g \cdot ml^{-1}$ of nimodipine was added before the start of oxygenation. (□) The cortex was removed from the brain which was not exposed to ischemia. Data are expressed as mean ± SD, n=5, each.

Methods

Chemicals

Nisoldipine, nitrendipine and nimodipine were supplied from Miles Lab. Inc. (Brooklyn, NY). Nifedipine was supplied from Pfizer Inc. (Brooklyn, NY). Nicardipine-HCI was supplied from Yamanouchi Pharmaceutical Inc. (Nicardipine was supplied in hydrochloric acid form). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO)

Method to measure the activity of antioxidants

Sprague-Dawley male rats were used in this study. The rat was decapitated and the head was kept in an ischemic condition for 30 min at 37°C. Then, the cortex was removed and was homogenized in a buffer solution containing 120 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.3 mM $MgSO_4$ and 10 mM Na_2HPO_4 (pH 7.4). The homogenate was exposed to air for 30 to 60 min using an apparatus described previously[19]. Then, the production of TBAR was measured by the method of Buege and Aust[20] with some modification. To 0.15 ml of tissue homogenates, 0.5 ml of ice-cold 0.8 N HCl containing 12.5% trichloroacetic acid was added to stop the reaction. Then, 0.4 ml of H_2O containing 50 μM desferrioxamine. Thiobarbituric acid (final concentration 0.67%) was finally added, and the sample was boiled in the presence of 0.05% butylated hydroxytoluene (BHT) for 20 min. The sample was then cooled, centrifuged at 1500 × g for 15 min, and the absorbance of the supernatant was read at 532 nm. Quantitation was based upon a molar extinction coefficient of 1.56×10^5. For the measurement of antioxidant activity of the drugs, one of DHPs was added before reoxygenation of the homogenate.

Measurement of lipophilicity

The octanol/water partition coefficient was determined as follows: 4 ml water-saturated octanol and 4 ml octanol-saturated water was pipetted into a glass tube. DHP (5–10 mg) was dissolved to the octanol layer and the tube was shaken vigorously for 2 min, then, it was centrifuged 2000g × 10 min. The absorption of each layer was measured at 350 nm. From the ratio of DHP concentrations in the organic phase and in the water phase (Co/Cw), the partition coefficient P was determined as P=log (Co/Cw).

Results

As indicated by open circles in figure 1, the amount of TBAR increased with time. If 50 $\mu g \cdot ml^{-1}$ nimodipine was added to the homogenate, the

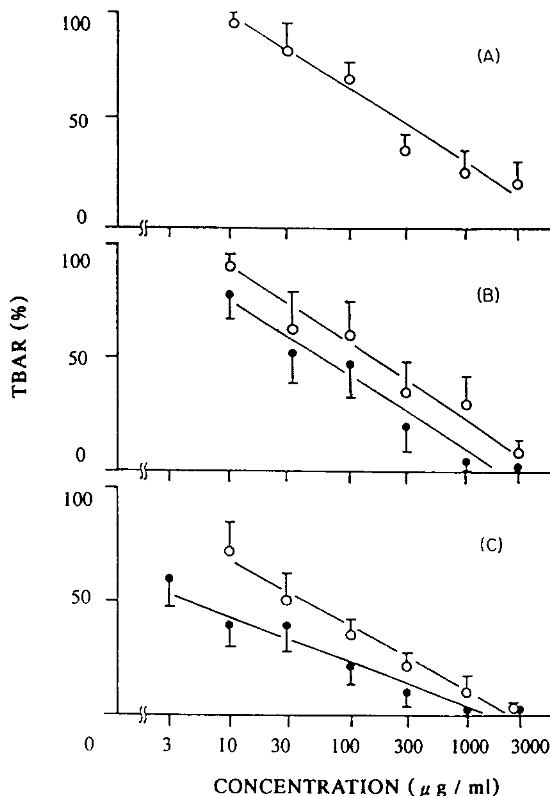

Fig. 2. Antioxidant activity of dihydropyridine compounds as measured by the inhibition of TBAR formation. (A) Nicardipine-HCl, (B) Open circles: nifedipine; Closed circles: nimodipine. (C) Open circles: nitrendipine; Closed circles: nisoldipine. Data are expressed as mean ± SD, n=3, each.

production of TBAR was inhibited by 50% (open triangles). The production of TBAR was related to ischemia/reoxygenation of the brain tissue. If the brain was not stored at 37°C for 30 min, very little TBAR was formed during the subsequent exposure to the air after homogenization, as indicated by open squares.

From the values of TBAR at 30 min of reoxygenation time, the dose-response relationship of different DHPs were measured. As shown in figure 2, all these DHPs had inhibited lipid peroxidation as measured by TBAR formation. Around concentrations of 1–3 mg·ml^{-1}, they completely inhibited lipid peroxidation. The concentration at which TBAR production was inhibited by 50% (IC_{50}) were 3, 20, 50, 200 and 300 μg·ml^{-1} for nisoldipine, nitrendipine, nimodipine, nifedipine and nicardipine-HCI, respectively.

The values of the partition coefficients P were 3.80, 3.40, 3.23, 2.78 and 1.02 for nisoldipine, nitrendipine, nimodipine, nifedipine and nicardipine-HCl, respectively. The antioxidant activity of DHP was related to their lipophilicity as indicated by the octanol/water partition coefficients. Figure 3 shows that there is a linear correlation between log (Co/Cw) and log (IC_{50})(nicardipine-HCl was exclude, because of its water soluble HCl form).

Discussion

When cell membranes are exposed to ischemic or hypoxic insults, lipid peroxidation takes place. By measuring TBAR formation in brain homogenates, we were able to screen the antioxidant activity of various DHPs. Our results suggest that the antioxi-

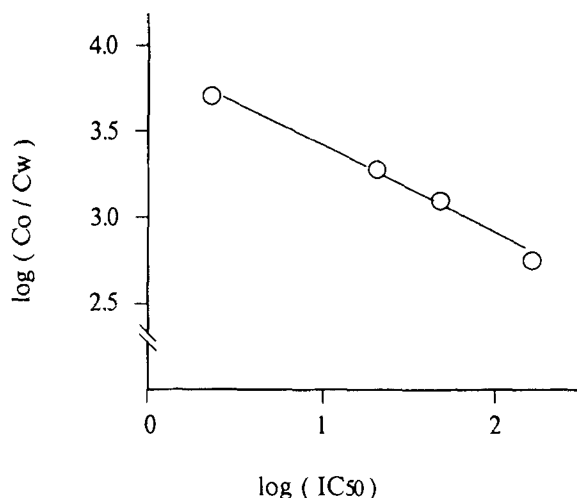

Fig. 3. A linear relationship between lipophilicity indicated by log (Co/Cw) and antioxidant activity indicated by log (IC_{50}). From left to right, nisoldipine, nitrendipine, nimodipine and nifedipine.

dant activity of DHP was related to their lipophilicity. It seems to be important that the ability of lipophilic compounds to bind or permeate cellular membranes is higher than that of the hydrophobic compounds when we use antioxidants during ischemia-reperfusion injury in *in vivo* situation.

Since nimodipine may have potential applications in treating central nervous system disorders[3–5,7–9,21], it is interesting to estimate whether or not a blood concentration of the order of 50 $\mu g \cdot ml^{-1}$ (which causes 50% inhibition in lipid peroxidation) could be attainable *in vivo*. In global ishemia in dogs[7], nimodipine was administered at 20 $\mu g \cdot kg^{-1}$ before and at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for two hours during and after ischemia and was found to reduce neurological deficit[6]. In pig-tailed monkeys, 10 $\mu g \cdot kg^{-1}$ i.v. nimodipine, administered five minutes after ischemia, followed by infusion at 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for ten hours, was also found to reduce neurological deficit. If we assume that the blood weight of an animal is approximately 1/10 of its body weight, and that the full dosage of the drugs administered are absorbed into the blood stream, the concentration attainable with 20 $\mu g \cdot kg^{-1}$ is approximately 0.2 $\mu g \cdot ml^{-1}$. This concentration is two orders of magnitude smaller than the concentration used in our experiment. It seems to be difficult to compare to changes in *in vivo* situation, however, in view of our results, the antioxidant effect of nimodipine may not be related to its beneficial effect in CNS disorders. If we could synthesize a compound with an anti-oxidant activity of two orders of magnitude greater than that of calcium blockers, then such a compound may have potential therapeutic value in treating CNS disorders[17].

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