Protective Effects of the Antianginal Agent Nicorandil on Arachidonate-Induced Sudden Death in Rats: Comparison with Several Antianginal Agents and Cyclooxygenase Inhibitors

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Abstract—In anesthetized rats, intra-carotid injections of arachidonate-Na (20 mg/kg) elicited a marked pressor response, producing death within 10 min in untreated rats. The antianginal agents (nicorandil, nitroglycerin and diltiazem) and cyclooxygenase inhibitors (indomethacin and aspirin), applied i.v. or p.o., effectively protected the rats from death. In the surviving rats, these drugs significantly prevented intravascular thrombosis in cerebral vessels and the marked pressor response to arachidonate-Na. The protective mechanism of the antianginal agents tested seems to be different from that of cyclooxygenase inhibitors.

In various organs, arachidonic acid is the precursor of a variety of bisenoic prostaglandins (PGs) including thromboxane A₂ (TxA₂), a powerful vasoconstrictor and activator of platelet aggregation (1). Several workers have shown that arachidonate-Na injected into the carotid artery of the rat (2) and the veins of the rabbit (3) causes sudden death, probably by vasoconstriction and platelet aggregation related to the production of TxA₂. Calcium channel blockers such as verapamil and nisoldipine partially protect rabbits from arachidonate-induced death (4). Further studies revealed that 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate (TMB-8), an intracellular calcium antagonist (5), attenuated the proaggregatory (6) and the vasoconstrictor effects of TxA₂ (7). Nicorandil is a newly developed, efficacious antianginal agent (8). Its action seems to be partly dependent on intracellular calcium pools (9, 10).

The present study was undertaken to examine the effects of nicorandil administered i.v. or p.o. on stroke in rats produced by the intra-carotid injection of arachidonate-Na and to compare the effects of nicorandil with those of the other antianginal agents, nitroglycerin and diltiazem, and with the cyclooxygenase inhibitors, indomethacin and aspirin.

Male Wistar-Imamichi rats (400–500 g), allowed free access to food and water overnight prior to the experiments, were anesthetized with pentobarbital-Na, 65 mg/kg, i.p. They were tracheotomized, and polyethylene catheters were introduced into the left femoral artery and vein to monitor systemic blood pressure (SBP) and to inject i.v. drugs, respectively. The SBP was measured with a Nihon Kohden pressure transducer (MPU-0.5) and recorded on a Yokogawa Pen Recorder (Type 3066). A four-series experimental protocol was utilized to study drug effects on arachidonate-induced sudden death: I. group not treated with any drugs; II. groups treated i.v. with nicorandil, nitroglycerin or diltiazem; III. groups treated p.o. with nicorandil or diltiazem; IV. groups treated p.o. with indomethacin or aspirin. In the I and II series of experiments, after completion of the surgical procedure, at least 15–20 min was allowed for stabilization of the preparation. Then, in the I series, arachidonate-Na (5–20 mg/kg) was administered into the left
common carotid artery (i.c.) without pretreatment with any drugs. In the II series, the tested drugs were administered i.v., and arachidonate-Na (20 mg/kg) was injected i.c. at 10 or 30 min after drug administration. In the III and IV series, just before pentobarbital injection, the animals were given the tested drugs orally and were then anesthetized and operated on. Arachidonate-Na (20 mg/kg) was administered i.c. about 30 min after drug application. The animals, which did not die within 1 hr following arachidonate administration were considered survivors.

Drugs used were nicorandil, N-(2-hydroxyethyl)nicotinamide nitrate (ester) (Chugai), diltiazem-HCl (Tanabe), nitroglycerin (ampoule, Nippon Kayaku), arachidonate-Na and indomethacin (free) (both from Sigma), and acetylsalicylic acid (aspirin, Iwaki). Nicorandil and diltiazem were dissolved in 0.9% saline solution, and nitroglycerin was diluted with 0.9% saline solution. Indomethacin and aspirin were suspended in 0.3% arabic gum solution. The drugs were given i.v. or p.o. during 10 sec in a volume of 1 ml/kg and flushed in with 0.9% saline solution. Only a single dose of each tested drug was administered for each preparation. The arachidonate-Na was dissolved in distilled water, just before the experiments, and the solution in a syringe was immersed in ice-cold water in order to preserve the pharmacological activity over long periods. The left common carotid artery was exposed at the midcervical region. A 3 cm 28 gauge needle with a polyethylene tube (PE 10) was inserted by piercing the vessel wall and fixed there with biotissue adhesive (Aron Alpha®, Sankyo). As quickly as possible (1—2 sec), 0.4 ml/kg of the arachidonate solution was administered through the tube into the carotid artery. Then, the artery was transiently occluded distally to the needle.

Values in the text represent means±S.E. Statistical significance of differences between unpaired mean values was analyzed by Student’s t-test and expressed as P values. The difference was regarded to be significant at P<0.05.

Arachidonate-Na in doses of 5 to 20 mg/kg was injected rapidly, i.c., and the lethal effects were examined. At a dose of 5 mg/kg, the arachidonate induced only a decrease in systemic blood pressure (SBP) in most of the preparations. Eighty percent of the animals in the group survived. At a dose of 10 mg/kg of the arachidonate, the SBP response varied: either just a decrease, mild increase preceded by a decrease, or a marked increase preceded by a decrease. Sixty percent of the animals in the group survived. A marked increase preceded by an initial decrease in SBP occurred in the animals challenged with 20 mg/kg of the arachidonate. All of the animals died within 10 min (5.4±0.5 min, n=15). The i.v. administration of 20 mg/kg of the arachidonate caused only a decrease in SBP and did not lead to death. Also, the injection of an equivalent volume of the vehicle, at the same speed into the carotid artery caused no death. Thus, for the present study, 20 mg/kg of arachidonate-Na was chosen as a dose adequate to cause stroke.

When nicorandil, nitroglycerin and diltiazem were administered i.v. in doses of 0.125 to 2 mg/kg, they all caused a decrease in the SBP. These drugs given at 10 or 30 min prior to the arachidonate were effective in protecting rats from death (Table 1). Significant inhibition of the pressor response to the arachidonate (20 mg/kg) injected i.c. was observed in the surviving animal group compared with the non-surviving one. However, the protective effect of these drugs against death was neither dose-dependent nor universal.

Significant protection against death was observed in oral doses of 0.25 to 2 mg/kg of nicorandil and diltiazem (Table 2). However, the highest survival rate produced by diltiazem was lower than that induced by nicorandil. Nicorandil increased the survival rate to 100% at doses of 1 and 2 mg/kg, while the survival effect of diltiazem was neither dose-dependent nor universal. There were no significant differences in the SBP between the groups pretreated with nicorandil and diltiazem, e.g., the mean SBP just before arachidonate-Na (20 mg/kg) injection was as follows: group treated with 1 mg/kg nicorandil, 92.0±1.5 mmHg (n=5); group treated with 1 mg/kg diltiazem, 94.3±4.8
mmHg (n=6); P>0.05. Indomethacin (100 mg/kg) and aspirin (200 mg/kg) pretreated p.o. also demonstrated a survival rate of 100%. In surviving rats, all of the drugs significantly inhibited remarkable pressor response to the arachidonate.

Our present results agree well with those reported previously (2-4), in view of the intravascular thrombosis in cerebral vessels that we found in the rats that died: light microscopic examination showed intense congestion of arteries and arterioles in both cerebral hemispheres resulting from the intracarotid injections of arachidonate-Na, although there were no detectable platelet thrombi in the lungs, hearts or livers (not shown). The dose of arachidonate used by us was much larger than that by Furlow and Bass (2), and the arachidonate injected i.c. was distributed not only to the left but also to the right cerebral hemisphere. I.v. injections of the arachidonate in the same dose and injection speed used in the artery injections caused no deaths. Although the cause of sudden death still remains unknown, it probably is due to the combination of intravascular thrombosis and arterial vasoconstriction in the cerebral vessels, mediated mainly by the synthesis of TxA2 from arachidonate-Na.

Nicorandil given i.v. or p.o. protected rats from arachidonate-induced death. Similar protective effects were obtained by pretreatment with nitroglycerin and diltiazem and pretreatment with indomethacin and aspirin. Recent studies indicate that nicorandil (11) and nitroglycerin (12) do not directly influence the synthesis of PGs and TxA2.

### Table 1. Influence of nicorandil, nitroglycerin and diltiazem administered i.v. on survival rate in arachidonate (AA)-induced sudden death

| Drugs (mg/kg) | Number of survivors | Number of fatalities | Percentage survival |
|--------------|---------------------|----------------------|---------------------|
| Control (without drugs) | 0                  | 15                   | 0                   |
| Nicorandil | a b                | a b                  | a b                 |
| 0.125       | 3 1                | 5 5                  | 37.5 16.7           |
| 0.25        | 3 2                | 5 4                  | 37.5 33.3           |
| 0.5         | 4 3                | 4 3                  | 50.0 50.0           |
| 1.0         | 4 3                | 4 3                  | 50.0 50.0           |
| 2.0         | 5 5                | 3 1                  | 62.5 83.3           |
| Nitroglycerin | 1 2                | 5 4                  | 16.7 33.3           |
| 0.25        | 3 3                | 3 3                  | 50.0 50.0           |
| 0.5         | 5 4                | 1 2                  | 83.3 66.7           |
| 1.0         | 4 5                | 2 1                  | 66.7 83.3           |
| 2.0         | 3 4                | 3 2                  | 50.0 66.7           |
| Diltiazem   | 0.125              | 3 3                  | 5 5                  | 50.0 37.5 |
| 0.25        | 3 3                | 3 5                  | 50.0 37.5           |
| 0.5         | 4 3                | 2 5                  | 66.7 37.5           |
| 1.0         | 4 4                | 2 4                  | 66.7 50.0           |
| 2.0         | 1 4                | 5 4                  | 16.7 50.0           |

Only a single dose of each drug was used for each preparation. At 10 or 30 min after drug administration, arachidonate-Na (20 mg/kg) was injected into the left common carotid artery. The animals that did not die within 1 hr following arachidonate administration were considered survivors. All animals (control) not pretreated with any drugs died at 5.4±0.5 min (n=15) after arachidonate (20 mg/kg) administration.
in organs and microsomal fractions of various animal species. However, in in vitro experiments, these agents inhibit platelet aggregation activated by naturally occurring substances such as collagen and arachidonic acid (13) (Prof. Katori, personal communication for nicorandil). In addition, nicorandil, nitroglycerin and diltiazem all possess vasospasmolytic effects (14) and powerfully dilate cerebral vessels (9, 15, 16) as well as other vessels (8) in anesthetized animals.

It is universally accepted that calcium ions are vital in many biologic processes, including a variety of vascular smooth muscle actions and hemostasis (17). Gorman (6) reported that in human platelets, the initial aggregatory response to TxA2 is a mobilization of intracellular calcium. Furthermore, Smith et al. (7) postulated that the vasoconstrictor response to TxA2 is dependent on both intracellular and extracellular calcium pools. Diltiazem is a potent inhibitor of the transmembrane Ca++ influx, but in high doses it also exerts intracellular effects in vascular smooth muscle (18). Also, the effects of nicorandil and nitroglycerin seem to be in part ascribable to inhibition of mobilization of intracellular calcium (9, 10). Altogether, taking these reports into consideration, it is not surprising that the three antianginal agents used in the present study were effective in preventing rats from arachidonate-induced death. The protective mechanism of these agents, however, seems to be different from that of cyclooxygenase inhibitors.

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### Table 2. Influence of nicorandil, diltiazem, indomethacin and aspirin administered p.o. on survival rate in arachidonate (AA)-induced sudden death

| Drugs (mg/kg) | Number of survivors | Number of fatalities | SBP (mean, mmHg) | Percentage survival |
|--------------|---------------------|---------------------|------------------|---------------------|
| Control (without drugs) | 0 | 15 | 172.3±4.3† (107.9±3.9) | 0 |
| Nicorandil | | | | |
| 0.25 | 1 | 4 | 106.6± 6.4*** (92.0± 1.9) | 20 |
| 0.5 | 1 | 4 | 102.7± 3.4*** (98.4± 3.4) | 20 |
| 1.0 | 5 | 0 | 103.4±10.0*** (93.2± 5.2) | 100 |
| 2.0 | 5 | 0 | 102.7± 3.4*** (98.4± 3.4) | 100 |
| Diltiazem | | | | |
| 0.25 | 3 | 3 | 102.7± 3.4*** (98.4± 3.4) | 50 |
| 0.5 | 3 | 3 | 102.7± 3.4*** (98.4± 3.4) | 50 |
| 1.0 | 3 | 3 | 102.7± 3.4*** (98.4± 3.4) | 50 |
| 2.0 | 4 | 2 | 102.7± 3.4*** (98.4± 3.4) | 66.7 |
| Indomethacin | | | | |
| 100 | 7 | 0 | 102.7± 3.4*** (98.4± 3.4) | 100 |
| Aspirin | | | | |
| 200 | 5 | 0 | 120.4±10.2*** (105.8± 6.3) | 100 |

At 30 min after drug administration, arachidonate-Na (20 mg/kg) was injected into the left common carotid artery. Peak systemic blood pressure (SBP) responses to the AA in the groups treated with nicorandil, indomethacin and aspirin, all of which survived the AA challenge, are also shown. Values presented are means±S.E. ***P<0.001 vs. † control. The values in parenthesis were measured just before AA application. Other explanations are as in Table 1.
References

1 Moncada, S. and Vane, J.R.: Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A₂, and prostacyclin. Pharmacol. Rev. 30, 293–331 (1979)

2 Furlow, T.W., Jr. and Bass, N.H.: Stroke in rats produced by carotid injection of sodium arachidonate. Science 1, 658–660 (1974)

3 Silver, M.J., Hoch, W., Kocsis, J.J., Ingerman, C.M. and Smith, B.: Arachidonic acid causes sudden death in rabbits. Science 183, 1085–1087 (1974)

4 Okamatsu, S., Peck, R.C. and Lefer, A.M.: Effects of calcium channel blockers on arachidonate-induced sudden death in rabbits. Proc. Soc. Exp. Biol. Med. 166, 551–555 (1981)

5 Chiou, C.Y. and Malagodi, M.H.: Studies on the mechanism of action of a new Ca²⁺ antagonist, 8-(N,N-diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride in smooth and skeletal muscle. Br. J. Pharmacol. 53, 279–285 (1975)

6 Gorman, R.R.: Modulation of human platelet function by prostacyclin and thromboxane A₂. Fed. Proc. 38, 83–88 (1979)

7 Smith, E.F., Lefer, A.M. and Nicolaou, K.C.: Mechanism of coronary vasoconstriction by carbocyclic thromboxane A₂. Am. J. Physiol. 240, H493–H497 (1981)

8 Sakai, K., Nakano, H., Nagano, H. and Uchida, Y.: Nicorandil. In New Drugs Annual: Cardiovascular Drugs. Edited by Scriabine, A., p. 227–242, Reven Press, New York (1983)

9 Nakagawa, Y., Takeda, K., Katano, Y., Tsukada, T., Kitagawa, T., Otorii, T. and Imai, S.: Effects of 2-nicotinamidoethyl nitrate on the cardiovascular system. Japan. Heart J. 20, 881–895 (1979)

10 Imai, S., Ushijima, T., Nakazawa, M., Nabata, H. and Sakai, K.: Mechanism of relaxant effects of nicorandil on the dog coronary artery. Arch. Int. Pharmacodyn. Ther. 265, 274–282 (1983)

11 Kadowitz, P.J., Armstead, W.M., Hyman, A.L., Gross, G. and Lippton, H.L.: Cyclooxygenase-independent vascular responses to nitroglycerin, nitroprusside and nicorandil. Fed. Proc. 42, 500 (1983)

12 Retkowskii, W., Pönicke, K., Block, H.U., Gießler, C.H., Dunemann, A., Zehl, U. and Förster, W.: Studies of the influence of nitroglycerin on the synthesis of prostaglandins and thromboxane A₂ and on platelet aggregation. Arzneimittelforsch. 32, 194–200 (1982)

13 Schafer, A.I., Alexander, R.W. and Handin, R.I.: Inhibition of platelet function by organic nitrate vasodilators. Blood 55, 649–654 (1980)

14 Nabata, H. and Sakai, K.: Effects of a new anti-anginal agent, nicorandil, on normoxic and anoxic contractions in isolated miniature pig coronary arteries exposed to 5-hydroxytryptamine and norepinephrine: comparison with nitroglycerin and diltiazem. Eur. J. Pharmacol. 96, 37–44 (1983)

15 Chin, W., Imai, S., Nakano, U., Takeda, K., Tamatsu, H. and Ushijima, T.: Effects of drugs on the cerebral circulation of the dog in relation to the cerebral oxygen consumption. Br. J. Pharmacol. 79, 897–906 (1983)

16 Murata, S., Nagano, T. and Nakajima, H.: Cerebral vasodilation and spasmolytic activity of diltiazem in anesthetized animals. Japan. J. Pharmacol. 32, 1033–1040 (1982)

17 Braunwald, E.: Mechanisms of action of calcium-channel-blocking agents. N. Engl. J. Med. 307, 1618–1626 (1982)

18 Saida, K. and van Breemen, C.: Mechanism of Ca** antagonist-induced vasodilation: intracellular actions. Circ. Res. 52, 137–142 (1983)