Effects of Nitroglycerin on Stable Thromboxane A₂ Analogue-Induced, Nifedipine-Resistant Contraction in Canine Basilar Artery

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Accepted July 10, 1990

Abstract—The stable thromboxane A₂ analogue, STA₂, caused concentration-dependent contractions in the canine basilar artery. In Ca²⁺-free medium containing EGTA (0.1 mM) and nifedipine (10⁻⁶ M), the addition of Ca²⁺ (2.5 mM) in the presence of STA₂ (10⁻⁸ M) caused a tonic contraction (nifedipine-resistant Ca²⁺-induced contraction). In the basilar artery, nitroglycerin did not significantly affect such nifedipine-resistant Ca²⁺-induced contractions, but nearly abolished the contraction in the coronary artery. The present experiments suggest that the regulatory mechanism of mobilized Ca²⁺ for the nifedipine-resistant Ca²⁺-induced contraction produced by STA₂ in the basilar artery is different from that in the coronary artery.

In various vascular preparations, nifedipine-resistant Ca²⁺-induced contractions have been observed; for example, noradrenaline in rabbit aorta (1) and with 5-hydroxytryptamine (5-HT) in rabbit aorta, iliac and renal arteries (2). There is substantial evidence to suggest that receptor-controlled influx of Ca²⁺ through receptor-operated Ca²⁺ channels (ROCs) is additive to that induced by depolarization and is less sensitive to blockade by calcium channel antagonists than Ca²⁺ influx through voltage-operated calcium channels (VOCs) (3, 4). Thromboxane A₂ (TXA₂) is known as a potent vasoconstrictor. We have proposed that the endothelium-derived contracting factor (EDCF) in canine basilar artery might be TXA₂ (5–8) and have shown that canine cerebral endothelium produces TXA₂ (7). Nifedipine and nitroglycerin have been widely used as spasmolytic agents (9–11). The present experiments were undertaken to examine the effect of nitroglycerin on nifedipine-resistant Ca²⁺-induced contractions in the presence of the TXA₂ analogue STA₂ in canine basilar artery.

Mongrel dogs (10–20 kg) of either sex were anesthetized with sodium pentobarbital (25 mg/kg, i.p.) and bled to death from the common carotid arteries. The basilar arteries and coronary arteries were isolated and cut into helical strips. The strips were rubbed on the intimal surface to eliminate endothelial cells. Elimination of endothelial cells by the rubbing procedure has been shown by electron microscopy in previous studies (7, 12). The strips were fixed vertically between hooks in an organ bath containing a nutrient solution, maintained at 37±0.5°C and bubbled with a mixture of 95% O₂ and 5% CO₂. The composition of the solution was as follows: 120 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 25 mM NaHCO₃ and 10 mM glucose. The pH of the solution was 7.4. The end of the
strip was attached to the lever of a force-displacement transducer (NEC San-Ei Instrument Co., Tokyo, Japan) connected to an ink-writing oscillograph (NEC San-Ei Instrument Co., Tokyo, Japan) on which isometric tension changes were recorded. STA2 and KCl were dissolved in distilled water. Nifedipine was dissolved in dimethylsulphoxide and added 10 min prior to the administration of STA2 or KCl. In the next set of experiments, to obtain nifedipine-resistant Ca\(^{2+}\) contraction, the solution of the organ bath was changed to a Ca\(^{2+}\)-free solution containing EGTA (0.1 mM) and nifedipine (10\(^{-6}\) M). Then, after 10 min, STA2 (10\(^{-8}\) M) was applied; and after a further 15 min, Ca\(^{2+}\) (2.5 mM) was readded. Nitroglycerin was dissolved in ethanol and added 10 min after the administration of STA2. The final concentrations of dimethylsulphoxide and ethanol in the organ bath were less than 0.1% (v/v).

Nifedipine (Sigma Chemical Co., St. Louis, MO) and EGTA (ethylene glycol bis (\(\beta\)-aminoethyl-ether)-N,N,N',N'-tetraacetic acid) (Nacalai Tesque, Kyoto, Japan) were purchased. 9,11-Epithio-11,12-methano-TXA\(_2\) (STA2) (Ono Pharmaceutical Co., Osaka, Japan) and nitroglycerin (Nippon Kayaku Co., Tokyo, Japan) were kindly provided by the indicated companies.

All data are expressed as means±S.E., and statistical significance was analyzed using the two-tailed Student's t-test for paired and unpaired data. The criterion of statistical significance was a P value of less than 0.05.

| Table 1. Effects of nitroglycerin on nifedipine-resistant Ca\(^{2+}\)-contractions in the presence of STA2 in basilar and coronary arteries |
|----------------------------------|----------------------------------|----------------------------------|------------------|
|                                  | Control                          | Nitroglycerin                   |                   |
|                                  | 10\(^{-8}\) M                    | 10\(^{-7}\) M                   | 10\(^{-6}\) M     |
| Basilar                          | 1.31±0.15 (33)                   | 0.90±0.16 (9)                   | 1.08±0.25 (10)    |
| Coronary                         | 1.28±0.17 (20)                   | 0.37±0.09* (9)                  | 0.04±0.03* (5)    |

The nifedipine-resistant Ca\(^{2+}\)-contractions (Control) were obtained by the addition of Ca\(^{2+}\) (2.5 mM) into Ca\(^{2+}\)-free medium containing EGTA (0.1 mM), nifedipine (10\(^{-6}\) M) and STA2 (10\(^{-8}\) M). Nitroglycerin was added 10 min after the addition of STA2. Each value represents the mean±S.E. The number of experiments is shown in parentheses. *P<0.01: significantly different from the control value.
in the presence of possible EDCF (TXA₂ analogue, STA₂) in canine basilar artery was nitroglycerin-resistant. In contrast to this, in the coronary artery, nitroglycerin attenuated the nifedipine-resistant Ca²⁺-induced contraction in the presence of STA₂. These further confirm that there are two types of Ca²⁺-induced contractions mediated by ROCs in different arteries, in terms of nitroglycerin-susceptibility, as suggested by Akimoto et al. (4). Nitrovasodilators, including nitroglycerin, nitroprusside and nitric oxide, activate cytosolic guanylate cyclase in vascular preparations, increase cGMP production, decrease intracellular Ca²⁺-mobilization and cause vasodilation (13, 14). In rat aorta, nitroprusside activates cytosolic guanylate cyclase and inhibits norepinephrine-induced contraction and phosphatidylinositol hydrolysis (15). Indeed, the result that nitroglycerin attenuated the nifedipine-resistant Ca²⁺-induced contraction in coronary artery clearly indicates that the Ca²⁺-mobilization in coronary artery is regulated by guanylate cyclase. On the other hand, the result shows that such a guanylate cyclase-linked regulatory mechanism may be lacking in the basilar artery.

The present experiments provided pharmacological evidence that the regulatory mechanism of mobilized Ca²⁺ for the contraction (mediated by ROCs) produced by possible EDCF (TXA₂) in the basilar artery is different from that in the coronary artery. Drugs that effectively inhibit nifedipine-resistant, in addition to nitroglycerin-resistant, Ca²⁺-induced contractions in cerebral arteries may be useful for protection against cerebral vasospasms following subarachnoid hemorrhage.

Acknowledgment: This study was supported in part by a grant from the Smoking Research Foundation, Japan.

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