Effects of DN-9693, a Selective Inhibitor of Cyclic AMP Phosphodiesterase, in Isolated and Perfused Canine Large Coronary Arteries

Tokio NAKANE and Shigetoshi CHIBA
Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan
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Abstract—In isolated canine epicardial coronary arteries perfused with Krebs-Henseleit solution at 37°C, DN-9693, a newly synthesized selective cyclic AMP phosphodiesterase inhibitor, induced vasodilation in a dose-related manner. The threshold dose for inducing vasodilation to DN-9693 was very small, but the maximum vasodilation was not great. The order of potency for ED20 (20% dilatation) was DN-9693>IBMX>aminophylline, but that for ED70 was IBMX>aminophylline >DN-9693, suggesting that these three PDE inhibitors have different properties.

It has been well recognized that inhibitors of cyclic nucleotide phosphodiesterase (PDE) induce a vasodilation via an intracellular increase in cyclic AMP in the vasculature. In 1985, DN-9693 was developed as one of the water-soluble platelet aggregation inhibitors (1). Moreover, DN-9693 has been demonstrated to inhibit selectively cyclic AMP PDE (isozyme III) in platelets (2). It was also reported that DN-9693 might exert its cardio-protective action by preventing adhesion and aggregations of leukocytes and platelets (3-5). More recently, Hashimoto et al. (6) reported that DN-9693 and isobutylmethylxanthine (IBMX) increased blood flow in a similar dose range with intraarterial injection to the canine coronary, femoral, mesenteric and renal arterial beds. They observed vasodilating effects of DN-9693 in resistance vessels because they investigated the vascular responses of in situ arterial preparations (6). In the present study, we attempted to observe the effects of DN-9693, a selective cyclic AMP PDE inhibitor, comparing them with those of non-selective PDE inhibitors, IBMX and aminophylline, on epicardial large coronary arteries of dogs that were perfused with Krebs-Henseleit solution. The method was developed as the cannula inserting method by Hongo and Chiba (7).

Mongrel dogs (7-18 kg) of either sex were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). After i.v. injection of sodium heparin (200 units/kg), the dogs were killed by rapid bleeding. The left epicardial coronary artery was isolated from the heart and cleaned of loose adipose and connective tissues in cold Krebs-Henseleit solution (4-10°C). The artery was cut into segments (0.7-2.6 mm in outside diameter and 1.5 cm length). Side branches were tied with silk threads. The arterial segment was carefully cannulated with a stainless steel cannula (0.6-2.2 mm outer diameter) and was set up for perfusion vs. previously described (8). Krebs-Henseleit solution was perfused with a peristaltic pump (Harvard Apparatus 505-1210). The flow rate was kept constant through the experiments (approximately 2 ml/min). The basal perfusion pressure was between 60-200 mmHg. The pressure changes were measured with an electric manometer (Nihon Kohden AP621 G). The drug solution was administered into the rubber tube connecting the cannula in a volume of 10-30 µl for 4 sec by a micro-injector (Terumo Co.). Vasodilation, therefore, was recorded as a decrease in perfusion pressure. Krebs-Henseleit solution contained 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO4, 2.5 mM CaCl2, 1.2 mM KH2PO4, 25 mM NaHCO3 and 11 mM glucose; it was gassed
with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C (Haake EF2). The experiments were started when the arteries had equilibrated for about 1 hr, until perfusion pressure increased spontaneously and a stable base line appeared.

Drugs used were DN-9693 (7-pyperidinyl-1,2,3,5-tetrahydroimidazo [2,1-b]-2-one hydrochloride) (Daichi Seiyaku), IBMX (Aldrich Chemical) and aminophylline (Eisai Co., Ltd.). These drugs were dissolved in and diluted with 0.9% saline to the desired concentrations. Statistical significance between two data samples was tested by Student’s t-test. A probability level of P<0.05 was considered statistically significant.

When DN-9693 was bolusly injected into the cannulated, isolated dog coronary artery, a decrease in perfusion pressure was dose-dependently produced. IBMX and aminophylline also induced dose-related vasodilations. Typical response patterns to the 3 compounds are shown in Fig. 1. At small doses, DN-9693 readily induced a clear vasodilation, but IBMX and aminophylline did not produce any vasodilation in the threshold doses of DN-9693. At relatively large doses, IBMX and aminophylline produced a profound vasodilation, but the DN-9693-induced maximum dilation reached only approximately 70% of the maximum dilation by IBMX or aminophylline. The summarized data are shown in Fig. 2. The order of potency for ED₂₀ (20% dilatation) was DN-9693>IBMX>aminophylline, but that for ED₇₀ was IBMX>aminophylline>DN-9693. The reason why the DN-9693-induced maximum dilation was less than the IBMX- or aminophylline-induced dilatation is unknown. It may be one of the reasons why cyclic GMP may readily cause a very great vasodilation (9). Because IBMX and aminophylline, non-selective PDE inhibitors, induced marked vasodilations that were much greater than that by DN-9693, a selective cyclic AMP PDE inhibitor, with a high dose range, it will be necessary to use other selective PDE inhibitors to confirm maximal vasodilation in the future. It is still unclear why both cyclic AMP and cyclic GMP seem to be involved in mediating or modulating vascular smooth muscle relaxation. It is necessary to investigate each

Fig. 1. Vasodilatory effects of increasing doses of IBMX, aminophylline and DN-9693 on an isolated and perfused dog coronary artery.
physiological role of both cyclic nucleotides in vasodilation.

In the small dose range, DN-9693 readily induced clear vasodilatation in this study that used large coronary arteries. This may be a beneficial action for the therapy of ischemic heart disease, because it has been well recognized that the potent antianginal drug nitroglycerin does not appear to dilate the coronary arterial resistance bed, but does dilate large coronary epicardial vessels (10). Hashimoto et al. (6) demonstrated that DN-9693 induced vasodilator responses in situ dog coronary arteries to a lesser extent than IBMX in any dose range. On the other hand, in the present study by the use of isolated large epicardial coronary arteries, DN-9693 at small doses induced greater vasodilation than IBMX at the same dose, suggesting that conducting and resistance vascular beds may have different sensitivity to cyclic GMP and cyclic AMP.

![Fig. 2. Dose-response curves for increasing doses of IBMX, aminophylline (AMINO) and DN-9693. Results are shown as the mean±S.E.M. The number in parenthesis indicates the number of preparations perfused. The response to 6 μmol IBMX is considered as 100% of the response. * ○ is significantly greater than either ○ or Δ (P<0.01). ** Δ is significantly smaller than either ○ or Δ (P<0.01).](image)

### References


