Cardiovascular Effects of an Ionophorous Antibiotic, Lonomycin A, in Anesthetized Dogs

Katsuharu TSUCHIDA, Katsuyoshi KANEKO, Hironaka AIHARA and Shigetoshi CHIBA*

Research Laboratories, Taisho Pharmaceutical Co., Ltd., Ohmiya, Saitama 330, Japan
*Department of Pharmacology, Shinshu University School of Medicine, Matsumoto 390, Japan

Accepted February 22, 1985

Abstract—An intracoronary administration of lonomycin A, a K-selective ionophore, produced coronary vasodilatation in the presence of pindolol in anesthetized dogs. Coronary vasodilatation induced by lonomycin A, KCl and nifedipine was inhibited by pretreatment with an intracoronary injection of ouabain. This result suggests that lonomycin A-induced coronary vasodilatation may be partly due to a stimulation of Na⁺, K⁺-ATPase as well as KCl and/or a decrease in Ca²⁺ influx as well as nifedipine.

Most ionophorous antibiotics have recently been shown to produce hemodynamic and cardiotonic effects. Monensin (Na-selective ionophore) (1, 2), A23187 (Ca-selective) (3) and grisorixin (K-selective) (4) all reportedly produce coronary vasodilatation, in vivo or in isolated perfused hearts. The mechanism of relaxation of an isolated coronary artery by X-537A (nonselective) has been examined in vitro (5, 6), but X-537A has been shown not to produce any decrease in coronary vascular resistance in anesthetized dogs (7, 8). The actions of ionophores on arterioles seem to be different from those on large arteries, and there is no evidence concerning the mechanism of relaxation of the coronary artery by ionophores in vivo. Since, however, we have found that lonomycin A (LA), a K-selective ionophore (K⁺:Na⁺=6:1) (9), produces coronary vasodilatation in anesthetized dogs, its mechanism of action was studied.

Mongrel dogs of both sexes (9–15 kg) were anesthetized with sodium pentobarbital (30 mg/kg, i.v., supplemented with an additional dose as necessary) and ventilated artificially through an endotracheal tube with room air provided by a Shinano respirator (SN-408-3). Femoral blood pressure was measured with a pressure transducer (Nihon Kohden MPU-0.5) connected to a rigid polyethylene tube inserted into the femoral artery. The heart rate was obtained using a heart rate counter (Nihon Kohden AT-600G) driven by pressure waves. A left thoracotomy was performed in the fifth intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery, approximately 1–2 cm from its origin, was dissected free from surrounding connective tissues and then cannulated with a polyethylene cannula and perfused with blood from the right carotid artery. Coronary blood flow was measured with a flow probe inserted into the extracorporeal path between the carotid and coronary arteries. The flow probe was connected to an electromagnetic flowmeter (Nihon Kohden MF-26, MFV-1200).

The following drugs were used: LA Na (Taisho), ouabain (Takeda), pindolol (Sankyo), I-isoproterenol hydrochloride (Nikken Chemicals), nifedipine (Bayer), papaverine hydrochloride (Wako). LA, ouabain, papaverine and KCl were dissolved in distilled water to give concentrations of 1, 0.5, 5 and 50 mg/ml, respectively. Nifedipine was dissolved in 50% ethanol solution to give concentrations of 0.05 and 0.2 mg/ml.

Intra-arterial LA dose-dependently increased coronary blood flow. Percentage increases were 33±4% (n=8), 64±10%
(n=18) and 93%±8% (n=37) at doses of 10, 30 and 100 µg, respectively. Since it was possible that ouabain might increase the release of norepinephrine following inhibition of Na+, K+-ATPase in adrenergic nerve endings, the experiment was performed after pindolol administration. In the presence of 50 µg/kg intravenously pretreated pindolol, which completely abolished the coronary vasodilatation induced by 0.1 µg intracoronary isoproterenol, without affecting the LA-induced coronary vasodilatation throughout the course of the experiment, the inhibitory effect of ouabain on LA-, KCI-, nifedipine- and papaverine-induced coronary vasodilatation was studied. All four coronary vasoactive agents were administered intracoronarily within 1.5-3.0 min after intracoronary administration of ouabain. KCl (1 mg, i.a.) caused coronary vasodilatation without affecting any other hemodynamic parameters, and this was significantly inhibited by pretreatment with a 30 µg intrarterial dose of ouabain. This inhibition was observed to about the same extent approximately from 1 to 10 min after ouabain administration. More than 1 mg of KCl affected hemodynamics. With regard to LA (100 µg, i.a.)-induced coronary vasodilatation, the increased coronary flow was significantly inhibited by pretreatment with ouabain (30 µg, i.a.). Nifedipine (0.1-1 µg, i.a.)-induced coronary vasodilatation was also inhibited by pretreatment with ouabain (30 µg, i.a.). On the other hand, papaverine (100 µg, i.a.)-induced coronary vasodilatation was not significantly inhibited by pretreatment with ouabain (30 µg, i.a.). Prior treatment with LA (100 µg, i.a.) did not potentiate the coronary vasodilatatory action of KCl (1 mg, i.a.). One of the typical experiments is shown in Fig. 1. and summarized results are presented in Table 1. The increase in coronary blood flow as a result of intracoronary administration of LA was not blocked by several well-known antagonists such as pindolol, tripelennamine, atropine, aminophylline and indomethacin (data not shown). Therefore, it is suggested that LA-induced coronary vasodilatation was not attributable to a local release of norepinephrine, histamine, acetylcholine (ACh), adenosine (Ad) or prostaglandins. Furthermore, intracoronary administration of 100 µg LA increased not only coronary blood flow but also coronary sinus blood O2 tension (data not shown). This suggests that LA-induced coronary vasodilatation is not attributable to an increase in oxygen demand.

Moins et al. (10) have shown that the monocarboxylic ionophores grisorixin and albiorixin induce a marked increase in plasma potassium in the coronary sinus. Potassium ions locally released from cardiac muscle concomitant with increased cardiac work are one of the many factors which regulate coronary blood flow (11). Murray and Sparks (12) have shown that K+-induced coronary vasodilatation is significantly attenuated by the administration of a combination of ouabain and lidocaine in anesthetized dogs, and they concluded that the mechanism of K+-induced vasodilatation is attributable to an activation of the electrogenic Na+-K+ transport system of coronary smooth muscle. The vasodilatation induced by Ad or ACh has been shown to be attenuated by ouabain in isolated large arteries (13, 14), but not in arterioles (15). This suggests that the Na+-K+ transport system participates in the Ad or ACh-induced relaxation of large arteries, but not arterioles. With regard to ionophores, monensin has been reported to produce a relaxation by stimulation of Na+, K+-ATPase in the coronary artery of the dog (16), but the vasodilatatory action and/or the mechanism of ionophores appears to be different between arterioles and large arteries and different among the sites of vessels, species and various ionophores.

Although LA-induced coronary vasodila-
Table 1. Effect of ouabain (30 μg, i.a.) on coronary vasodilatatory agent (CVA)-induced peak increase in coronary blood flow (CBF, ml/min) and the effect of LA (100 μg, i.a.) on KCl-induced peak increase in anesthetized dogs

|       | Before treatment with ouabain | After treatment with ouabain |       |       |
|-------|-------------------------------|-----------------------------|-------|-------|
|       | Base line values | After CVA | Increase in CBF | Base line values | After CVA | Increase in CBF |
| LA    | 100 μg | 8 | 9.4±1.7 | 16.7±2.6 | 7.3±1.4 | 8.1±1.8* | 12.4±2.4 | 4.1±0.9** |
| KCl   | 1 mg  | 5 | 8.6±1.6 | 11.7±1.5 | 3.1±0.6 | 7.0±1.6* | 8.1±1.6 | 1.1±0.4* |
| Nifedipine | 0.1 μg | 6 | 8.6±2.5 | 13.5±3.4 | 4.9±1.1 | 9.0±2.4 | 12.2±2.8 | 3.2±0.6* |
| Nifedipine | 0.3 μg | 6 | 8.6±2.5 | 17.9±4.8 | 9.3±2.5 | 8.3±2.1 | 15.2±3.9 | 6.9±1.8* |
| Nifedipine | 1 μg  | 7 | 8.7±2.1 | 20.3±4.7 | 11.6±2.9 | 8.6±2.2 | 17.9±4.4 | 9.2±2.4* |
| Papaverine | 100 μg | 5 | 10.4±2.7 | 18.1±4.7 | 7.7±2.0 | 10.4±2.5 | 17.1±4.1 | 6.7±1.6 |

|       | Before treatment with LA | After treatment with LA |       |       |
|-------|--------------------------|-------------------------|-------|-------|
|       | Base line values | After KCl | Increase in CBF | Base line values | After KCl | Increase in CBF |
| KCl   | 1 mg  | 4 | 8.2±3.1 | 11.4±3.7 | 4.0±0.2 | 15.3±3.2* | 18.8±3.9 | 3.5±1.0 |

Administration of nifedipine and papaverine were conducted in a similar manner to the case of LA shown in Fig. 1. Two doses of nifedipine or one dose of both nifedipine and papaverine were administered to the same dog, so the second injection of these drugs was performed at a somewhat higher level of blood flow than the first one, which explains the higher mean base line values (mixture of the first and second injections) after treatment with ouabain in the cases of nifedipine and papaverine than those in the cases of KCl and LA. Values are means±S.E.M. N represents the number of experiments. *P<0.05 in comparison with the base line values before treatment with ouabain. **P<0.01 in comparison with the values of the increase in CBF before treatment with ouabain. †P<0.01 in comparison with the base line values before treatment with LA. Each p was obtained by the paired t-test.
tation was reduced by ouabain, the vasodilatory action of KCl was not significantly augmented by pretreatment with LA, despite the fact that an increase in vasodilation of KCl is reportedly induced under conditions where Na+, K+-ATPase is activated (17). However, this result does not necessarily indicate that LA has no influence on KCl-induced coronary vasodilatation because the base-line value of coronary blood flow has been shown to be elevated by pretreatment with LA, and so it may be difficult for KCl to induce further coronary vasodilatation (12). These results suggest that effluent potassium from the myocardium and/or a direct activation of the electrogenic Na+-K+ transport system by LA might play a partial role in the coronary vasodilatatory action of LA. Furthermore, the attenuation of LA response by ouabain could result from an increased influx of extracellular Ca2+ (18, 19), which would oppose any decrease in Ca2+ influx caused by LA as well as the nifedipine responses shown in the present study.

References
1 Kabell, G., Saini, R.K., Somani, P. and Pressman, B.C.: Effects of the carboxylic ionophore monensin on regional blood flow in normal and ischemic myocardium in anesthetized dogs. J. Pharmacol. Exp. Ther. 211, 231–237 (1979)
2 Saini, R.K., Hester, R.K., Somani, P. and Pressman, B.C.: Characterization of the coronary vasodilator and hemodynamic actions of monensin, a carboxylic ionophore. J. Cardiovasc. Pharmacol. 1, 123–138 (1979)
3 Schaffer, S.W., Safer, B., Scarpa, A. and Williamson, J.R.: Mode of action of the calcium ionophores X-537A and A23187 on cardiac contractility. Biochem. Pharmacol. 23, 1609–1617 (1974)
4 Moins, N., Gachon, P., Maublant, J. and Duchene-Marullaz, P.: Effects of the monovalent ionophores grisorixin and alborixin on cardiovascular function and plasma cation concentrations in the anesthetized dog. J. Cardiovasc. Pharmacol. 1, 659–671 (1979)
5 Watson, E.L.: Effects of ionophores A23187 and X-537A on vascular smooth muscle activity. Eur. J. Pharmacol. 52, 171–178 (1978)
6 Berner, P.F., Disalvo, J. and Schwartz, A.: Differential inhibitory effects of the ionophore RO 2–2985 (X-537A) on contactile responses to potassium and histamine in coronary artery smooth muscle. J. Pharmacol. Exp. Ther. 213, 59–63 (1980)
7 Schwartz, A., Lewis, R.M., Hanley, H.G., Munson, R.G., Dial, F.D. and Ray M.V.: Hemodynamic and biochemical effects of a new positive inotropic agent. Circ. Res. 34, 102–111 (1974)
8 deGuzman, N.T. and Pressman, B.C.: The inotropic effects of the calcium ionophore X-537A in the anesthetized dog. Circulation 49, 1072–1077 (1974)
9 Mitani, M. and Otake, N.: Studies on the ionophorous antibiotics. XV. J. Antibiot. (Tokyo) 31, 750–755 (1978)
10 Moins, N., Gachon, P. and Duchene-Marullaz, P.: Effects of two monovalent ionophores, grisorixin and alborixin, on cardiac function and plasma cation concentrations in the anesthetized dog. J. Cardiovasc. Pharmacol. 1, 659–671 (1979)
11 Driscoll, T.E. and Berne, R.M.: Role of potassium in regulation of coronary blood flow. Proc. Soc. Exp. Biol. Med. 96, 505–508 (1958)
12 Murray, P.A. and Sparks, H.V.: The mechanism of K+-induced vasodilation of the coronary vascular bed of the dog. Circ. Res. 42, 35–42 (1978)
13 DeMay, J.G. and Vanhoutte, P.M.: Interaction between Na+, K+ exchanges and direct inhibitory effect of acetylcholine on canine femoral arteries. Circ. Res. 46, 826–836 (1980)
14 Foley, D.H.: Diminished arterial smooth muscle response to adenosine during Na-K pump inhibition. Pfluegers Arch. 400, 88–95 (1984)
15 Chen, W.T., Brace, R.A., Scott, J.B., Anderson, D.K. and Haddy, F.J.: The mechanism of the vasodilator action of potassium. Proc. Soc. Exp. Biol. Med. 140, 820–824 (1972)
16 Anderson, H.L., III, Winquist, R.J., Webb, R.C. and Bohr, D.F.: Mechanism of canine coronary artery relaxation by monensin. Circ. Res. 53, 168–175 (1983)
17 Webb, R.C. and Bohr, D.F.: Potassium relaxation of vascular smooth muscle from spontaneously hypertensive rats. Blood Vessels 16, 71–79 (1979)
18 Toda, N.: Mechanism of ouabain-induced arterial muscle contraction. Am. J. Physiol. 239, H199–H206 (1980)
19 Belardinelli, L., Harder, D., Sperelakis, N., Rubio, R. and Berne, R.M.: Cardiac glycoside stimulation of inward Ca2+ current in vascular smooth muscle of canine coronary artery. J. Pharmacol. Exp. Ther. 209, 62–66 (1979)