Naunyn-Schmiedeberg's Arch Pharmacol (1983) 322:319–321

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Short Communication

Relationship Between Relaxation and Cyclic GMP Formation Caused by Nicorandil in Canine Mesenteric Artery

Masao Endoh and Norio Taira

Department of Pharmacology, Tohoku University School of Medicine, 980 Sendai, Japan

Summary. In the isolated canine mesenteric artery relaxation caused by nicorandil [N-(2-hydroxyethyl)nicotinamide nitrate ester; SG-75] in the presence of noradrenaline was accompanied by a concomitant elevation of cGMP level, whilst the cAMP level was not changed by the drug. The relaxation and cGMP level after the administration of nicorandil showed a good correlation. In this respect nicorandil is very similar to nitrogen oxide-containing vasodilators.

Key words: Cyclic GMP – Vasodilation – Nicorandil – Mesenteric artery – Dog

Introduction

Nicorandil [N-(2-hydroxyethyl)nicotinamide nitrate ester; SG-75] is a vasodilator developed recently as a potential antianginal drug (Uchida et al. 1978). Although the drug is a nitrate ester, the profile of its cardiovascular action differs from that of nitroglycerin in several respects. Unlike nitroglycerin, nicorandil increases venous return in open-chest anaesthetized dogs (Taira et al. 1979). The drug hyperpolarizes the resting membrane potential and reduces the action potential duration in canine left atrial muscle fibres, suggesting an increase in the membrane potassium conductance (Yanagisawa and Taira 1980). In porcine and guinea-pig coronary arterial smooth muscles the drug produces relaxation and hyperpolarization at the same concentration (Furukawa et al. 1981), whereas nitroglycerin causes hyperpolarization in concentrations much higher than those required for producing relaxation (Ito et al. 1980). Nevertheless, the nitrate site undoubtedly plays an important role in the vasodilator action of nicorandil (Taira et al. 1979; Sakai et al. 1980; Maruyama et al. 1982). On the other hand, recent studies have shown that cyclic guanosine 3′,5′-monophosphate (cGMP) formation is involved in relaxation of vascular and nonvascular smooth muscles elicited by nitrogen oxide-containing vasodilators such as nitroglycerin, sodium nitroprusside and sodium nitrite (Schultz et al. 1977; Kukovetz et al. 1979; Axelsson et al. 1981; Gruetter et al. 1981). Therefore, we investigated whether the relaxation of vascular smooth muscle produced by nicorandil is accompanied by changes in intracellular levels of cyclic nucleotides.

Send offprint requests to M. Endoh at the above address
Correspondence: M. Endoh, M.D. at the above address

Methods

Helical strips of the mesenteric artery were obtained from dogs anaesthetized with sodium pentobarbital (30 mg/kg i.v.), given sodium heparin (200 U/kg i.v.) and exsanguinated. Strips were mounted in 20 ml organ baths containing Krebs-Henseleit solution bubbled with 95% O_2 and 5% CO_2 at a temperature of 37° C. In order to minimize autoxidation of noradrenaline in the baths, ascorbic acid (57 μmol/l) and disodium EDTA (27 μmol/l) were added to the solution. Strips were stretched by a tension of about 10 mN. After an equilibration period of 30 min, noradrenaline (3 μmol/l) was administered repeatedly at 15-min intervals until successive responses became virtually identical. Then, (1) the concentration-response curve for noradrenaline, (2) the concentration-response curve for nicorandil in the presence of 3 μmol/l noradrenaline, and (3) the time course of relaxation and of changes in cGMP and cyclic adenosine 3′,5′-monophosphate (cAMP) levels in response to 100 μmol/l nicorandil in the presence of 3 μmol/l noradrenaline were determined. The maximal relaxation caused by 100 μmol/l papaverine was taken as 100% in the determination of the concentration-response curves for nicorandil. For the assay of cyclic nucleotides in the tissue, strips were quickly frozen in liquid nitrogen at various times after the administration of nicorandil. In this series of experiments nicorandil was added 5 min after 3 μmol/l noradrenaline, i.e., when the contraction elicited by noradrenaline reached a steady level. After homogenization by procedures described previously for the heart (Endoh et al. 1982), the cyclic nucleotide content was determined by a sensitive radioimmunoassay. Experimental values were presented as means ± S.E. Significant differences between mean values were estimated by the use of Student's *t*-test. A test for paired values was used when applicable. Drugs used were nicorandil (Chugai, Tokyo, Japan), (–)-noradrenaline base (Fluka, Buchs, Switzerland) and papaverine hydrochloride (Iwaki Seiyaku, Tokyo, Japan). For radioimmunoassay of cyclic nucleotides anti-cAMP and anti-cGMP antisera were used, and [^{125}I]succinyl cAMP and cGMP tyrosine methyl ester (Yamasa Shoyu Co., Choshi, Japan).

Results

The canine isolated mesenteric artery was contracted by noradrenaline in a concentration-dependent manner in concentrations of 0.1 μmol/l and higher. The response to each concentration of noradrenaline reached a steady level

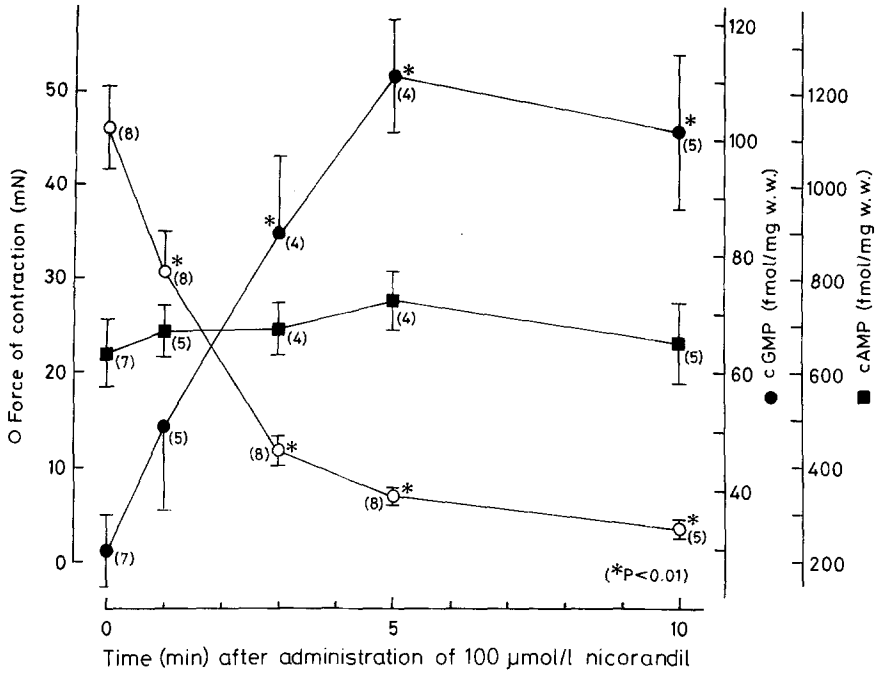

Fig. 1
The time course of the changes in cGMP and cAMP levels, and that of the changes in force in response to nicorandil in the presence of 3 µmol/l noradrenaline. Nicorandil was added 5 min after 3 µmol/l noradrenaline. *Vertical bars:* S.E. of the mean; *number in parentheses:* number of experiments; * significantly different from the corresponding control values prior to administration of nicorandil

Table 1. Relaxant effect of nicorandil on the canine isolated mesenteric artery in the presence of 3 µmol/l noradrenaline

Drug	Concentration (µmol/l)	Force (mN)	Relaxation (%)
Noradrenaline	3	32.1 ± 3.43	0
+ nicorandil	1	28.2 ± 3.65*	13 ± 2
	3	23.4 ± 3.68*	29 ± 4
	10	17.3 ± 3.14*	48 ± 5
	30	8.8 ± 2.73*	75 ± 6
	100	3.0 ± 1.74*	93 ± 5
	300	1.1 ± 0.96*	98 ± 3

Given are means ± S.E. of 6 preparations
* $P < 0.05$ (paired comparison) vs. the value with noradrenaline alone

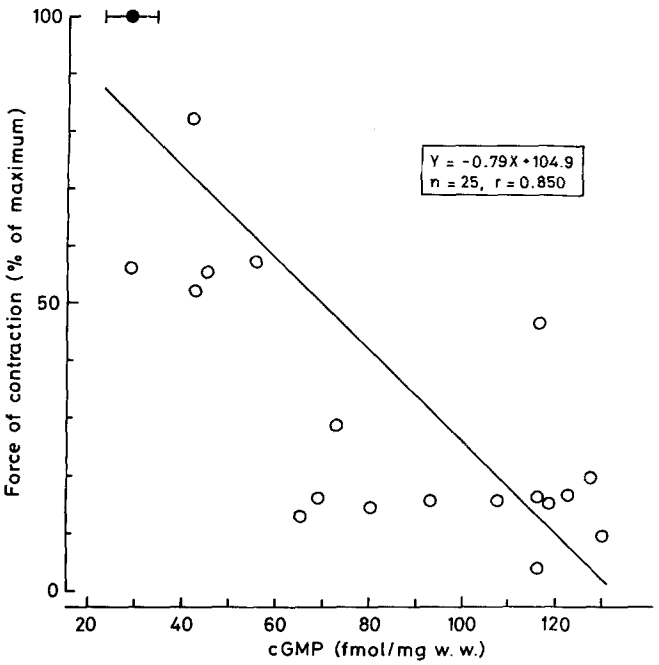

Fig. 2. Correlation between the force and the cGMP level after the administration of 100 µmol/l nicorandil in the presence of 3 µmol/l noradrenaline in the isolated canine mesenteric artery. The value prior to the administration of nicorandil, which is shown as a solid point with the horizontal bar, was also included in calculating the correlation coefficient

3–5 min after the administration and remained at the same level thereafter in most preparations. The pD_2-value for noradrenaline was 5.64 ± 0.14 ($n = 4$). Noradrenaline, in a concentration of 3 µmol/l, elicited a contractile response amounting to 55 ± 7% ($n = 4$) of the maximal response. In the presence of 3 µmol/l noradrenaline nicorandil (≥ 1 µmol/l) caused a concentration-dependent relaxation (Table 1). The contractile response to noradrenaline was abolished in the presence of 300 µmol/l nicorandil (2.0 ± 2.8% of the maximal response, $n = 6$). The pD_2-value for the relaxant effect of nicorandil in the presence of 3 µmol/l noradrenaline was 4.99 ± 0.09 ($n = 6$).

The time course of the relaxation and of the changes in cGMP and cAMP levels in response to 100 µmol/l nicorandil in the presence of 3 µmol/l noradrenaline is shown in Fig. 1. Five minutes after the addition of 3 µmol/l noradrenaline the contractile force amounted to 46.1 ± 4.6 mN ($n = 8$) above the resting level of 10 mN. The cGMP and cAMP levels were 29.9 ± 6.1 ($n = 7$) and 640 ± 71 ($n = 7$) fmol/mg wet weight, respectively. One minute after the addition of 100 µmol/l nicorandil relaxation reached a significant level. The elevation of cGMP level reached a significant level 3 min after the administration (Fig. 1). The elevated cGMP level was maintained until 10 min when the relaxation reached a maximal level. The cAMP level did not change significantly during the relaxation elicited by nicorandil.

A good correlation was found between the force and the cGMP level after the administration of 100 µmol/l nicorandil in the presence of 3 µmol/l noradrenaline (Fig. 2).

Discussion

In the presence of noradrenaline nicorandil relaxed the canine isolated mesenteric artery in a concentration-dependent manner. The threshold concentration and the concentration range to elicit relaxation are consistent with those reported to cause electrophysiological and mechanical responses in the guinea-pig and porcine coronary (Furukawa et al. 1981) and porcine mesenteric arteries (Itoh et al. 1981).

In the present study it was shown that the relaxation elicited by nicorandil was accompanied by the simultaneous elevation of intracellular cGMP level. The time course of changes in force and that of changes in cGMP level were very similar to each other, and a good correlation was found between both parameters. In this respect nicorandil is very similar to nitrogen oxide-containing vasodilators. Pieces of evidence to support the involvement of cGMP in the smooth muscle relaxation are, however, circumstantial and controversial at present (Diamond and Janis 1978; Janis and Diamond 1979). The detailed mechanism through which the intracellular cGMP causes relaxation of smooth muscle has to be established before we can conclude that cGMP mediates the relaxant action of nitrogen oxide-containing vasodilators.

Acknowledgement. We are grateful to Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, for nicorandil and to Yamasa Shoyu Co., Ltd., Choshi, Japan, for the anti-cGMP and -cAMP antisera, [^{125}I]-succinyl cGMP and cAMP tyrosine methyl ester.

References

Axelsson KL, Andersson RGG, Wikberg JES (1981) Correlation between vascular smooth muscle relaxation and increase in cyclic GMP induced by some organic nitro esters. Acta Pharmacol Toxicol 49:270–276

Diamond J, Janis RA (1978) Increases in cyclic GMP levels may not mediate relaxant effects of sodium nitroprusside, verapamil and hydralazine in rat vas deferens. Nature (Lond) 271:472–473

Endoh M, Yamashita S, Taira N (1982) Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J Pharmacol Exp Ther 221:775–783

Furukawa K, Itoh T, Kajiwara M, Kitamura K, Suzuki H, Ito Y, Kuriyama H (1981) Vasodilating actions of 2-nicotinamidoethyl nitrate on porcine and guinea-pig coronary arteries. J Pharmacol Exp Ther 218:248–259

Gruetter CA, Gruetter DY, Lyon JE, Kadowitz PJ, Ignarro LJ (1981) Relationship between cyclic guanosine 3′:5′-monophosphate formation and relaxation of coronary arterial smooth muscle by glyceryl trinitrate, nitroprusside, nitrite and nitric oxide: Effects of methylene blue and methemoglobin. J Pharmacol Exp Ther 219:181–186

Ito Y, Kitamura K, Kuriyama H (1980) Actions of nitroglycerine on the membrane and mechanical properties of smooth muscles of the coronary artery of the pig. Br J Pharmacol 70:197–204

Itoh T, Furukawa K, Kajiwara M, Kitamura K, Suzuki H, Ito Y, Kuriyama H (1981) Effects of 2-nicotinamidoethyl nitrate on smooth muscle cells and on adrenergic transmission in the guinea-pig and porcine mesenteric arteries. J Pharmacol Exp Ther 218:260–270

Janis RA, Diamond J (1979) Relationship between cyclic nucleotide levels and drug-induced relaxation of smooth muscle. J Pharmacol Exp Ther 211:480–484

Kukovetz WR, Holzmann S, Wurm A, Pöch G (1979) Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. Naunyn-Schmiedeberg's Arch Pharmacol 310:129–138

Maruyama M, Satoh K, Taira N (1982) Effects of nicorandil and its congeners on musculature and vasculature on the dog trachea in situ. Arch Int Pharmacodyn Ther 258:260–266

Sakai K, Ohba Y, Akima M, Kamiyama H, Hinohara Y, Nakano H (1980) Pharmacodynamic and metabolism studies on a new coronary vasodilator, N-(2-hydroxyethyl)nicotinamide nitrate (SG-75). Jpn J Pharmacol 30:881–890

Schultz KD, Schultz K, Schultz G (1977) Sodium nitroprusside and other smooth muscle-relaxants increase cyclic GMP levels in rat ductus deferens. Nature 265:750–751

Taira N, Satoh K, Yanagisawa T, Imai Y, Hiwatari M (1979) Pharmacological profile of a new coronary vasodilator drug, 2-nicotinamidoethyl nitrate (SG-75). Clin Exp Pharmacol Physiol 6:301–316

Uchida Y, Yoshimoto N, Murao S (1978) Effect of 2-nicotinamidethyl nitrate (SG-75) on coronary circulation. Jpn Heart J 19:112–124

Yanagisawa T, Naira N (1980) Effect of 2-nicotinamidoethyl nitrate (SG-75) on the membrane potential of left atrial muscle fibres of the dog: Increase in potassium conductance. Naunyn-Schmiedeberg's Arch Pharmacol 312:69–76

Received January 20, 1983/Accepted February 22, 1983