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Endothelium-dependent relaxation induced by sodium fluoride in the rabbit ear artery

H. Tsuru

Department of Pharmacology, Hiroshima University School of Medicine, Kasumi, Minami-ku, Hiroshima 734 (Japan)
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Summary. Sodium fluoride (NaF) produced concentration-dependent relaxation of isolated rabbit ear artery precontracted with norepinephrine. In contrast, an arterial preparation with the endothelium rubbed off did not relax, but contracted in response to NaF. NaF-induced relaxation was not influenced by indomethacin but was inhibited by methylene blue or N^G-monomethyl-L-arginine. The results indicate that NaF relaxes the artery by releasing a so-called EDRF.
Key words. Sodium fluoride; endothelium-dependent vasorelaxation; rabbit ear artery.

It is well known that a number of endogenous as well as exogenous substances produce relaxation of blood vessels through the mediation of an increase in the level of intracellular cyclic AMP or cyclic GMP[1]. For instance, agonists for β-adrenoceptors, stimulators of adenylate cyclase such as forskolin, and inhibitors of phosphodiesterase including methylxanthines, are known to relax smooth muscles, including those of blood vessels, by increasing the intracellular cyclic AMP level[2,3]. So-called endothelium-derived relaxing factor (EDRF)[4,5], and a number of nitrogen oxide-containing substances such as nitroprusside and nitroglycerin, relax vascular smooth muscle cells by the mediation of cyclic GMP[6].

Sodium fluoride (NaF) has long been known to influence the activity of the adenylate cyclase system and to produce an increase in cyclic AMP[7]. NaF has therefore been frequently used for analysis of the functioning of the β-adrenoceptor-adenylate cyclase system in in vitro experiments, in both homogenized cell-free preparations and isolated tissue preparations.

It has recently been demonstrated in vascular tissues that NaF produces contraction[8] and that NaF stimulates synthesis of prostaglandin (PG) I_2, a potent vasodilator, in both vascular smooth muscles[9] and endothelial cells[10]. Very recently, Cushing et al.[11] reported that NaF produces endothelium-dependent relaxation by releasing both EDRF and prostanoid in the coronary artery in a number of mammals.

It was observed in the present study that NaF produces relaxation of the rabbit ear artery, one of the most popular preparations for pharmacological and physiological studies[12], primarily by releasing EDRF.

Methods

Male Japanese White rabbits, weighing 2 kg, were anesthetized with pentobarbital sodium at 30 mg/kg i.v. and exsanguinated from the common carotid artery. The ear artery was dissected out and placed in chilled Krebs' bicarbonate solution. The composition of the solution (in mM) was: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. The solution was previously aerated with a gas mixture of 95% O_2 and 5% CO_2.

Isolated arterial segments were cleaned of connective tissues under a dissecting microscope and made into ring preparations 4 mm in length. In some preparations, the endothelium was removed by gentle rubbing with a thin wooden stick. The absence of endothelium was confirmed by the absence of relaxation in response to acetylcholine- or calcium ionophore A23 187[13]. Each preparation was suspended in a tissue bath of 10 ml which contained Krebs' solution aerated with the gas mixture and maintained at 37 °C as described previously[3,13]. Preparations were given an optimum load of 2 g and equilibrated for 1.5 h before starting the experiments. Isometric tension was recorded on an ink-writing oscillograph (Nihon Kohden Kogyo, Tokyo, Japan, model WI-641G) via force-displacement transducers (Nihon Kohden Kogyo, Tokyo, Japan, model TB-611T).

Relaxation responses of blood vessels were examined after moderate contraction with 0.3 μM, which was about EC_{50}, of norepinephrine. The maximum relaxation of each preparation was obtained by adding 0.1 mM papaverine at the end of the experiment.

NaF was of special grade (Wako Pure Chemical Industries, Osaka, Japan). Norepinephrine bitartrate, indomethacin, atropine sulphate, methylene blue, N^G-monomethyl-L-arginine (L-NMMA) and calcium ionophore A23187 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetylcholine chloride (Ovisot, ACh) and papaverine hydrochloride were obtained from Daiichi Seiyaku (Tokyo, Japan) and Tokyo Kasei (Tokyo, Japan), respectively.

Results shown in the text and figures are expressed as mean ± SEM obtained from five to six experiments. Significance of difference between two means was determined by Student's t-test. Differences with P values less than 0.05 were considered to be statistically significant.

Under the conditions in which the artery was not precontracted, NaF produced only contraction. NaF-induced contraction was beyond the scope of the present study, but the contraction might be related to the opening of the calcium channel in the plasma membrane and perhaps a subsequent entry of extracellular Ca^{2+} into vascular smooth muscle cells[8].

As shown in figure 1, when the arterial preparation with intact endothelium was precontracted with 0.3 μM norepinephrine, the artery relaxed in response to ACh in a concentration-dependent way, and 0.1 μM atropine almost completely antagonized the relaxant effect of ACh. Subsequent addition of 10 mM NaF relaxed the artery again. In contrast, the arterial preparation without endothelium did not relax in response to ACh and NaF, but contracted in response to NaF, as seen in figure 1. These results indicate that NaF relaxes the artery by releasing some relaxing factor from the endothelium.

Nitroprusside similarly relaxed preparations both with and without endothelium. It is assumed that nitroprusside relaxes the blood vessel by increasing the intracellular level of cyclic GMP, in a similar way to EDRF[6]. Thus, the results in figure 1 indicate that both preparations have the ability to produce cyclic GMP, and to relax in response to cyclic GMP.

The relaxant response to NaF of the artery with intact endothelium was concentration-dependent, as shown in figure 2. NaF 10 mM induced more than 90% of the maximum relaxation induced by 0.1 mM papaverine, and concentrations of NaF higher than 10 mM produced more relaxation. Concentrations higher than 10 mM NaF, however, caused precipitation in the Krebs' bicarbonate solution aerated with O_2 and CO_2. Therefore, the concentration range of 1–10 mM was used in the present study. It should be emphasized that the phenomenon was observed in the range of concentrations used in biochemical experiments[2,7].

NaF-induced vasorelaxation may be mediated by prostanoids, since NaF-induced endothelium-dependent relaxation of the coronary artery was inhibited by indomethacin[11]. When the effect of indomethacin was tested in the present study, 10 μM indomethacin was added to the tissue bath 15 min before precontraction with norepinephrine. No significant effect of indomethacin was observed on the NaF concentration-response curve, as shown in figure 2. Thus, there was no significant difference in NaF pD_2 values, negative logarithms of EC_{50} values, between the control and the indomethacin-treated arteries. Mean pD_2 values ± SEM (N = 6) were 2.48 ± 0.06 in the control and 2.41 ± 0.05 in the in-

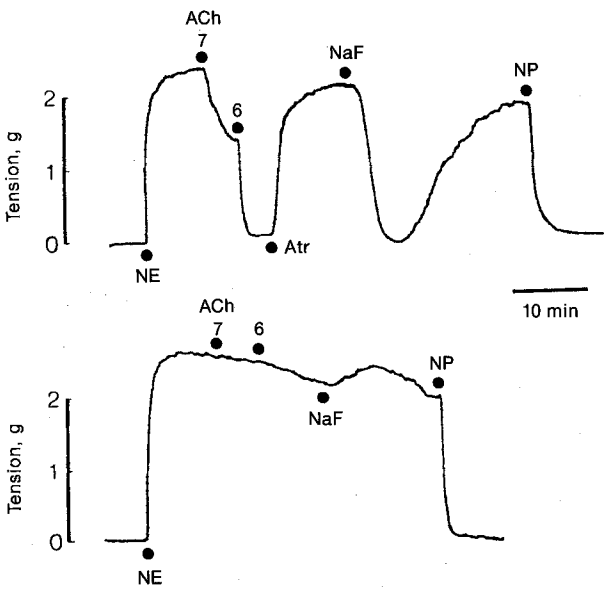

Figure 1. Typical recordings of the effects of 10 mM NaF on the rabbit ear artery precontracted with norepinephrine. Upper: endothelium intact and lower: endothelium rubbed off. NE, 0.3 μM norepinephrine; ACh, 0.1 and 1 μM acetylcholine; Atr, 0.1 μM atropine and NP, 30 μM nitroprusside. Note that relaxation responses to both acetylcholine and NaF are dependent on the endothelium.

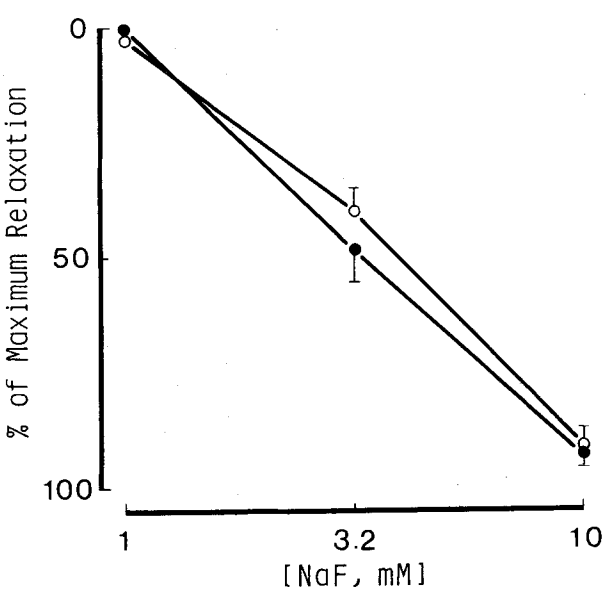

Figure 2. Effect of indomethacin on NaF concentration-response curves of the rabbit ear artery. ●—●: control artery, ○—○: 10 μM indomethacin-treated artery. Each point and vertical bar represents mean ± SEM, n = 6. Indomethacin had no significant effect on the NaF concentration-response curve. The maximum relaxation was obtained with 0.1 mM papaverine.

domethacin-treated artery. In other words, EC_{50} values were 3.3 mM and 3.9 mM, respectively. The results indicate that prostanoids are not involved in NaF-induced relaxation of the rabbit ear artery.

EDRF has been shown to behave in the same way as nitric oxide (NO), and methylene blue, L-NMMA and other agents have been used to inhibit the synthesis of EDRF (NO). Indeed, in the present experiment 10 μM methylene blue abolished the NaF-induced relaxation of the rabbit ear artery. L-NMMA also significantly depressed the NaF-induced relaxation, i.e., relaxation as a percentage of the maximum relaxation induced by 0.1 mM papaverine $90.4 \pm 5.2\%$ in the absence and $23.6 \pm 5.4\%$ in the presence of 0.1 mM L-NMMA ($N = 5$).

Data to date show that NaF affects not only stimulatory and inhibitory GTP-binding regulatory proteins (G-protein)[2] but also various other G-proteins, i.e., a G-protein coupling receptor activation to the breakdown of phosphatidylinositol 4,5-bisphosphate by a phosphodiesterase[14] and transducin[15]. Thus, Cushing et al.[11] have suggested that NaF-induced relaxation and contraction in the coronary artery may be G-protein-mediated, based on sensitivity to G-protein modulators, i.e., NaF can interact with a G-protein(s) and cause an increase in intracellular calcium concentration which leads to release of EDRFs. The suggestion is in accordance with the observation that the production of NO in the rat cerebellum is calcium-calmodulin-dependent[16]. Furthermore, Flavahan and Vanhoutte[17] have shown that NaF-induced endothelium-dependent relaxation of the canine coronary artery is mediated in part by activation of a pertussis toxin-sensitive G-protein.

It is concluded that NaF produces endothelium-dependent relaxation of the rabbit ear artery by releasing some relaxant substance, possibly an EDRF, from the endothelium. The process appears to be different from that in the coronary artery[11], and may not involve prostanoid.

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Effects of methoprene and juvenile hormone on the oxidative metabolism of isolated mitochondria from flight muscle of *Locusta migratoria* L.

G. Cotton* and J. H. Anstee

Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE (England)
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Summary. In vitro applications of juvenile hormone III and a juvenile hormone analogue, methoprene, were made to mitochondria isolated from dorsal longitudinal flight muscles of adult *Locusta migratoria* L. Both compounds completely inhibited oxygen consumption at the highest concentrations used. At lower concentrations, state 3 respiration and respiratory control were reduced but the ADP/O ratio was largely unaffected.
Key words. Locusta migratoria; methoprene; juvenile hormone; juvenile hormone analogue; mitochondria; oxidative metabolism.

In recent years, there has been considerable interest shown in the use of juvenile hormone analogues (JHAs) as novel agents for pest insect control. However, the majority of studies have investigated the morphological and pesticidal effects of the JHAs, so that relatively little is known about the effects of JHAs at the subcellular