Fluoride Produces Endothelium-Dependent Relaxation and Endothelium-Independent Contraction in Coronary Artery[1]

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ABSTRACT

NaF produced endothelium-dependent relaxation and endothelium-independent contraction in porcine, bovine, canine and human coronary artery rings precontracted with either KCl or prostaglandin $F_{2\alpha}$. For practical reasons the porcine coronary artery was selected to investigate the mechanisms responsible for these responses. Methylene blue, indomethacin, N-ethylmaleimide, pertussis toxin and cholera toxin all significantly attenuated the endothelium-dependent relaxation caused by fluoride. Pretreatment with deferoxamine had no effect on relaxation and superoxide dismutase/catalase potentiated the relaxation produced by fluoride. Fluoride also contracted vessels with or without the endothelium to equal tension levels and had no apparent relaxing effect on basal tone. The contraction produced by fluoride was significantly attenuated by pertussis toxin and cholera toxin; however, none of the other agents examined significantly altered contraction. Bradykinin also caused endothelium-dependent relaxation and this response was significantly attenuated by methylene blue but not indomethacin. Therefore, fluoride appears to relax the arteries by releasing an endothelium-derived relaxing factor similar to that released by bradykinin (methylene blue sensitive) and one or more prostanoid type endothelium-derived relaxing factor(s) (indomethacin sensitive). Furthermore, fluoride relaxation and contraction may be guanine nucleotide-binding regulatory protein-mediated based on sensitivity to the guanine nucleotide-binding regulatory protein modulators.

Various agents stimulate endothelial cells to produce and release factor(s) (EDRFs) which cause relaxation of vascular smooth muscle (Furchgott 1984). Some of the known examples of these agents are acetylcholine, BK, histamine and A23187. In cultured porcine aortic endothelial cells, BK and A23187 have been shown to release nitric oxide and another relaxing factor (Boulanger *et al.*, 1989). The mechanism by which some of these agents cause the production and release of EDRFs is not well understood but may involve interaction with membrane associated G proteins (Weinheimer and Osswald 1989).

EDRF has been suggested to be nitric oxide based on many biochemical, chemical and pharmacological similarities between EDRF and nitric oxide (Ignarro 1989). However, nitric oxide is not the only EDRF that has been proposed. Boulanger *et al.* (1989) using cultured endothelial cells concluded that more than one EDRF was being released from these cells. One of these EDRF's was nitric oxide and the other was a cyclooxygenase product.

Recent experimentation in our laboratory investigating the

G protein coupling to the coronary adenosine receptor (Sabouni *et al.*, 1989) led to the observation that fluoride caused endothelium-dependent relaxation and endotyhelium-independent contraction in coronary artery. Fluoride has been shown previously to contract various types of smooth muscle and is known to have a positive inotropic effect on the heart (Zeng *et al.*, 1989; Nguyen-Duong, 1985; Casteels *et al.*, 1981; Berman, 1966; Loewi, 1955). The purpose of this study was to investigate the nature of the endothelial-dependent relaxing and endothelial-independent contracting effects of fluoride in coronary artery. Specifically, this study was designed to determine pharmacologically whether fluoride was acting through a G protein and if fluoride was releasing relaxing and contracting factors from the coronary endothelium.

Materials and Methods

Tissue acquisition and preparation. Porcine and bovine hearts were obtained (with no regard to the sex of the animal) from three different local abattoirs within 30 min of slaughter. Canine hearts were obtained from the animal resource center at this university within 15 min of death. Human hearts were obtained with the aid of the Virginia Tissue Bank. Human hearts were transported in cold buffer within 24 to 36 hr of removal from donors and only those hearts with a warm

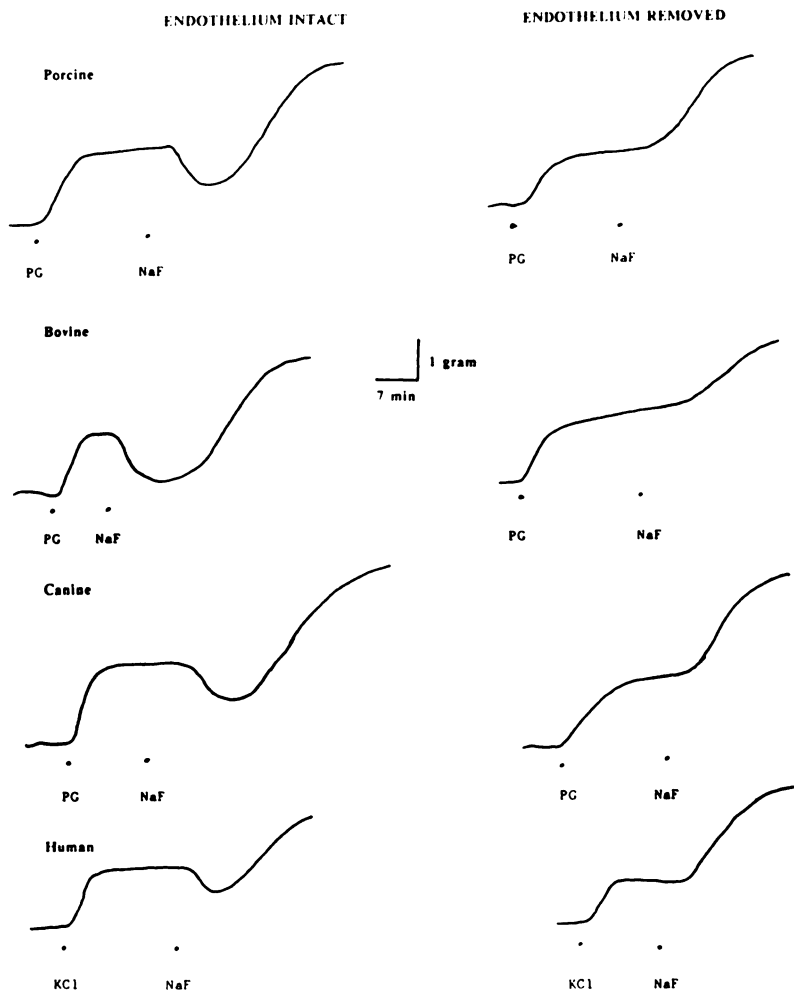ENDOTHELIUM INTACT   ENDOTHELIUM REMOVED

Fig. 1. Representative tracings from porcine, bovine, canine and human coronary artery in response to NaF (10 mM) addition in rings precontracted with PGF$_{2\alpha}$ (10 μM) (porcine, bovine or canine) or KCl (human). The left panel contains rings with the endothelium intact and the right panel contains rings with the endothelium removed.

TABLE 1

Contraction produced by NaF (10 mM) on basal tone in porcine coronary rings with and without an intact endothelium

Data are the mean ± S.E. from 32 rings taken from eight hearts. There was no significant difference between vessels with an intact endothelium and denuded vessels (P > .05; Student's t test).

Treatment	Response
	g tension
Endothelium intact	2.60 ± 0.22
Endothelium removed	2.83 ± 0.27

ischemia time of less than 2 hr were used. The left anterior descending coronary artery was dissected from each heart (of all species), cleaned of fat and adhering tissue and cut into rings approximately 4 mm in length and 2 mm outer diameter. When porcine or bovine hearts were used, four to eight hearts were obtained each day and the rings from all hearts were pooled and selected randomly for experimentation. Only one heart was obtained per day for human and canine experiments.

Isolated muscle bath preparation. Coronary rings were mounted horizontally between two stainless-steel hooks in 10-ml organ chambers filled with Krebs-Henseleit solution and oxygenated with 95% O$_2$-5% CO$_2$ (pH 7.4; 37°C). The composition of Krebs-Henseleit was (millimolar): NaCl, 118; KCl 4.8; MgSO$_4$, 1.2; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 25; CaCl$_2$, 2.5; and glucose, 11. Changes in isometric tension were measured with force transducers (Grass FT03c) connected to Sensormedics dynographs (R611). Rings were equilibrated for 1 hr under an initial tension of 2 g and the buffer was changed every 15 min. After equilibration rings were challenged with KCl (50 mM) until constant and reproducible contractions were achieved. The integrity of the endothe-

lium was evaluated with 100 nM BK (porcine, bovine and human) or acetylcholine (canine). It should be noted that BK (100 nM) produces endothelium-dependent relaxation in canine rings and the reason for testing the canine rings in this study with acetylcholine was merely one of convenience. In some experiments the endothelium was removed mechanically by gently rubbing the intimal surface of the rings with a pair of forceps.

Relaxation and contraction responses to fluoride were performed on porcine, bovine and canine rings precontracted with PGF$_{2\alpha}$ (10 μM). The relaxation and contraction responses to fluoride were performed on human coronary rings precontracted with KCl (35 mM) as PGF$_{2\alpha}$ causes phasic-type contractions in human vessels (Sabouni and Mustafa, 1989). Because the relaxation and contraction responses to fluoride appeared identical in all species tested (fig. 1), the porcine coronary was chosen as the model to investigate the mechanism(s) mediating fluoride responses. The primary basis for this decision was tissue availability.

Chemicals. Indomethacin, DEF, PGF$_{2\alpha}$, PT, CT and NEM were purchased from Sigma Chemical Co. (St. Louis, MO). SOD, MB and CAT were a gift from Dr. Dean Loven. Fluoride was purchased from Fisher Chemical Co. (Atlanta, GA).

Statistical analysis. The data are the mean ± S.E. from several determinations where "n" represents the number of porcine coronary rings examined. It should be noted that the relaxation response to 10 mM NaF in porcine coronary rings contracted with PGF$_{2\alpha}$ was not significantly different between animals on different experimental days (P > .05; analysis of variance). Each determination was generated from no fewer than eight porcine hearts. Single determinations between experimental and control responses were measured with a t test and
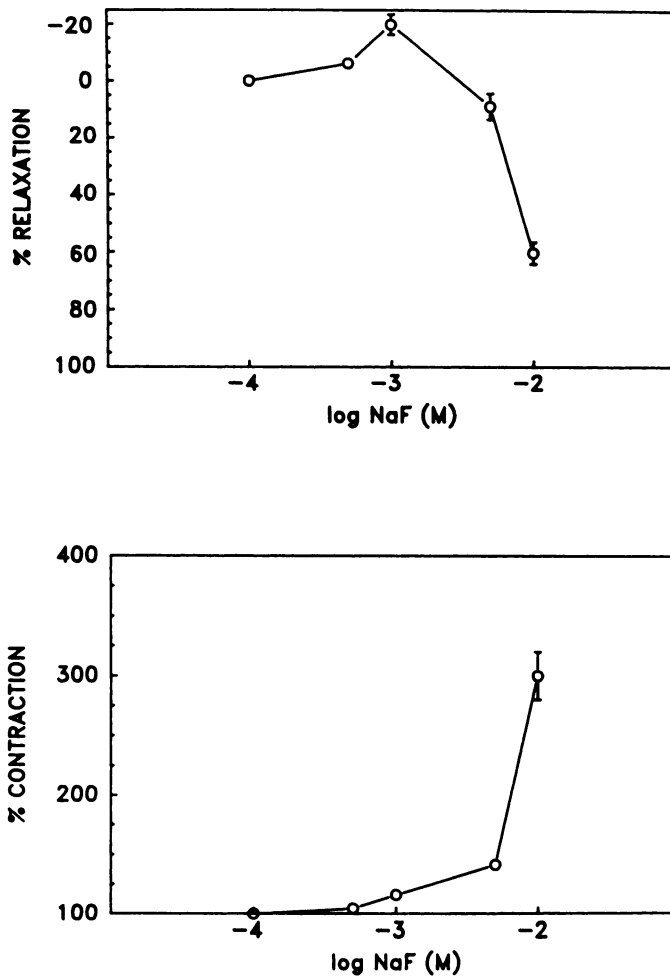
Fig. 2. Concentration-response curve for NaF in $PGF_{2\alpha}$ contracted porcine coronary rings, with an intact endothelium (upper panel). Concentration-response curve for NaF in $PGF_{2\alpha}$ contracted porcine coronary rings without the endothelium (lower panel). Data are the mean ± S.E. from 16 rings taken from eight hearts.
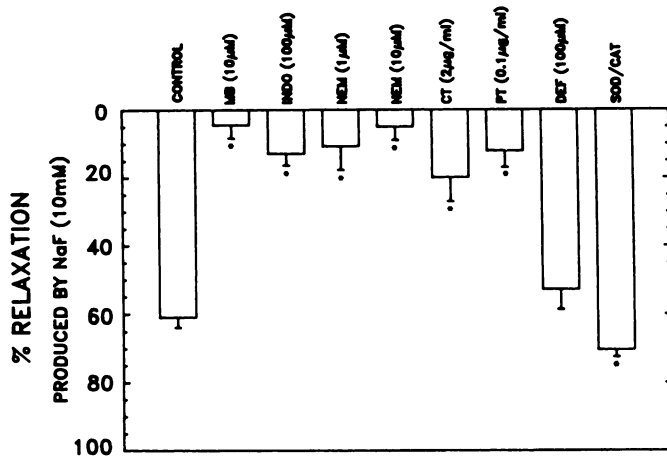

Fig. 3. Effect of various agents on NaF (10 mM)-produced relaxation in porcine coronary rings with an intact endothelium precontracted with $PGF_{2\alpha}$. Data are the mean ± S.E. from at least 8 to 17 vascular rings taken from 4 to 12 hearts. * Values significantly different from control (P < .05; Student's t test).
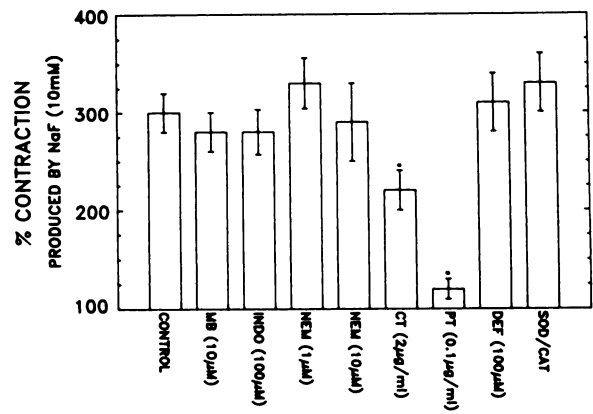

Fig. 4. Effect of various agents on NaF-produced contraction in porcine coronary rings with an intact endothelium precontracted with $PGF_{2\alpha}$. Data are the mean ± S.E. from 8 to 27 vascular rings taken from 8 to 14 hearts. * Values significantly different from control (P < .05; Student's t-test).

TABLE 2

Effect of MB and INDO on BK (100 nM)-induced endothelium-dependent relaxation in porcine coronary rings precontracted with $PGF_{2\alpha}$

Data are the mean ± S.E. from 16 rings taken from eight hearts.

Treatment	% Relaxation
Control	88.1 ± 2.8
MB (10 μM)	61.2 ± 4.9*
INDO (100 μM)	82.6 ± 4.5

* Values significantly different from control (P < .05; Student's t test).

the *alpha* value was set *a priori* at .05. Relaxations and contractions in porcine arteries were represented as percentage of the contraction generated by $PGF_{2\alpha}$ (10 μM). Tracings for responses in human, canine, porcine and bovine rings were representative of 12 to 16 rings taken from two to eight hearts.

Results

NaF caused endothelium-dependent relaxation and endothelium-independent contraction in porcine, bovine, canine and human coronary artery. In coronary rings, with an intact endothelium, precontracted with either $PGF_{2\alpha}$ (porcine, bovine or canine) or KCl (human) NaF (10 mM) caused transient relaxation followed by sustained contraction (fig. 1). After removal of the endothelium the relaxing effect to NaF was abolished completely whereas the contractile effect was unaltered (fig. 1). Under basal tone, addition of NaF to porcine coronary rings with an intact endothelium resulted in only contraction (table 1).

Effect of various modulators of endothelial function and G proteins on NaF responses in porcine coronary artery. NaF caused concentration-dependent relaxation in porcine coronary rings with an intact endothelium (fig. 2 upper panel). At 1 mM, NaF caused modest contraction (fig. 2 upper panel). The 10 mM concentration of NaF was selected for further investigation of the relaxations. Preincubation with MB (10 μM), INDO (100 μM), NEM (1–10 μM), CT (2 μg/ml) and PT (100 ng/ml) resulted in significant attenuation of the relaxing response of 10 mM NaF (fig. 3). Treatment of rings with DEF (100 μM) had no effect on the relaxing response to NaF whereas SOD/CAT (0.75 μg/ml:0.1 mg/ml) treatment modestly, but significantly, augmented NaF relaxation (fig. 3). It should be mentioned that treatment with indomethacin,

SOD/CAT, NEM (1 μM), DEF and PT did not effect basal tone or the contraction response to PGF$_{2\alpha}$. However, MB treatment enhanced PGF$_{2\alpha}$ contraction and treatment with CT and NEM (10 μM) modestly reduced PGF$_{2\alpha}$ tone but had no effect on basal tone.

In PGF$_{2\alpha}$ contracted porcine coronary rings without the endothelium, NaF did not cause any relaxation and produced contractions which were concentration-dependent (fig. 2, lower panel). The 10 mM concentration of NaF was selected for further investigation of the contractions. The magnitude of the contraction produced by 10 mM NaF in denuded vessels was comparable to that of the contraction phase seen in rings with an intact endothelium suggesting that contraction was independent of the endothelium (table 1). For this reason all subsequent experimentation with respect to contraction was done in vessels without removing the endothelium.

Pretreatment of porcine coronary rings with MB (10 μM), INDO (100 μM), NEM (1-10 μM), DEF (100 μM) and SOD/CAT (0.75 μg/ml:0.1 mg/ml) did not alter the contractile response to NaF (fig. 4). However, pretreatment with CT or PT resulted in a significant attenuation of the contraction (fig. 4).

Effect of MB and INDO on BK-induced endothelium-dependent relaxation. In porcine vessels with an intact endothelium, BK (100 nM) caused relaxation (table 2) and removal of the endothelium abolished this relaxation completely (data not shown). Pretreatment with MB (10 μM) significantly reduced the relaxation produced by BK (table 2). However, pretreatment with INDO (100 μM) did not alter the BK response (table 2).

Discussion

Relaxation response. The results from this study indicated that fluoride caused endothelium-dependent relaxation followed by endothelium-independent contraction in coronary artery. The relaxation appeared to be due to the release of more than one EDRF. The first of these EDRF's was sensitive to MB treatment indicating that its action was on the soluble form of guanylate cyclase as described by Ignarro (1989). This EDRF is similar to the EDRF released by BK as MB also attenuated BK-induced relaxation and may represent nitric oxide (Ignarro, 1989). The other EDRF released by NaF, but not by BK, may be a prostanoid compound based on its sensitivity to INDO treatment. Furthermore, this relaxation is likely the result of fluoride activation of a G protein inasmuch as NEM, PT and CT all attenuated the relaxation.

The suggestion that fluoride caused relaxation by interaction with an endothelial G protein was based on the information that fluoride is an activator of G proteins (Bigay et al., 1985). Furthermore, the effects of CT, PT and NEM were also investigated to determine G protein involvement in the relaxing effect of fluoride. CT is known to ADP-ribosylate G$_s$ whereas PT ADP-ribosylates various types of G$_i$ (see Gilman, 1987 for review). NEM on the other hand causes nonspecific alkylation of sulfhydryl groups contained in G proteins and is not specific for either G$_s$ or G$_i$ (Korner et al., 1982). All of these agents attenuated fluoride-induced relaxation and, therefore, it is difficult to discern which G protein(s) (G$_s$, G$_i$ or others) mediated the relaxing effect of fluoride. The PT and MB data are supported by the preliminary investigation of Flavahan and Vanhoutte (1989) in canine coronary artery. They reported attenuation of fluoride-induced relaxation by both MB and PT.

Pretreatment with SOD/CAT was used to test the possible involvement of a radical type EDRF (possibly nitric oxide) in the relaxation produced by fluoride. SOD inactivates superoxide anion whereas CAT eliminates hydrogen peroxide (Katusic and Vanhoutte, 1989). Furthermore, SOD is known to potentiate the actions of EDRF by slowing the rate of its degradation (Gryglewski et al., 1986; Warner et al., 1989; Ignarro, 1989). Because SOD/CAT significantly potentiated the relaxation produced by fluoride in the present study, it can be postulated that one released EDRF may indeed be nitric oxide.

Boulanger et al. (1989) reported that cultured porcine aortic endothelial cells release both nitric oxide and prostacyclin when exposed to various agents and provided evidence for the release of two relaxing factors. One of these factors was indistinguishable from nitric oxide and the other was not nitric oxide and may very likely be a prostanoid. Similar conclusions were also presented by Palmer et al. (1987). In the present study INDO was used to investigate the possible role of a cyclooxygenase product in the relaxing effect of fluoride. It should be mentioned that treatment with INDO did not effect basal tone or the contraction produced by PGF$_{2\alpha}$. Because pretreatment with INDO attenuated fluoride-induced relaxation, it is likely that a prostanoid compound derived from the endothelium may be partially responsible for fluoride relaxation. However, we did not establish in these studies that INDO was acting solely by inhibition of cyclooxygenase. In contrast, INDO was ineffective in inhibiting the relaxation produced by BK. This suggests that there may very likely be more than one EDRF released by fluoride. Other data presented in this study demonstrated that DEF, an agent which inhibits the production of hydroxyl radicals (Katusic and Vanhoutte, 1989) was without effect on the relaxation (or contraction) produced by fluoride suggesting that these radicals were not involved in the fluoride responses.

Contraction response. With respect to the contractile effect of fluoride, the first objective was to determine whether or not this phenomenon was dependent on the endothelium. Because fluoride caused contraction of equal magnitude in vessels with and without endothelium, it was apparent that the contraction was independent of the endothelium. With the exception of CT and PT, none of the other agents examined had any significant effect on the contractile response of fluoride. Specifically, DEF, SOD/CAT and INDO did not attenuate fluoride contraction. The lack of an effect by SOD, which eliminates superoxide radicals, further suggests that contraction was independent of the endothelium on the basis that O$_2^-$ has been identified as an endothelium-derived contracting factor (Katusic and Vanhoutte, 1989). The observation that CT and PT significantly attenuated the contracting effect of fluoride suggested a G protein involvement. However, the G protein responsible for this effect is difficult to identify as both toxins blocked the contraction whereas NEM was ineffective.

In summary, fluoride is capable of producing biphasic responses in human, bovine, canine and porcine coronary artery. Specifically, an endothelium-dependent relaxation and an endothelium-independent contraction. Fluoride appeared to release an EDRF with similar characteristics to nitric oxide (Ignarro, 1989; Boulanger et al., 1989). Fluoride also released one prostanoid type EDRF that was sensitive to INDO treatment and may be similar to that reported by Boulanger et al. (1989). The data further suggested that fluoride can interact with a G protein(s) to cause release of these EDRFs; however, the identity of this G protein is unknown. Furthermore, fluoride

produced contraction of coronary artery by an endothelium-independent mechanism and this effect may have been the result of G protein activation based on its sensitivity to CT and PT.

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