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Role of endothelial cells in relaxation of isolated arteries by bradykinin

(prostaglandins/vasodilation/acetylcholine/5,8,11,14-icosatetraynoic acid/quinacrine)

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ABSTRACT **Bradykinin elicits relaxation of isolated transverse rings of canine coronary, celiac, superior mesenteric, renal, splenic, pulmonary, gastric, and femoral arteries. After endothelial cells of the vessel wall are removed by rubbing of the intimal surface, canine arteries fail to relax upon addition of bradykinin. The endothelium-dependent relaxation of canine arteries remains intact after treatment with cyclooxygenase inhibitors (indomethacin and flurbiprofen), and this argues against mediation by prostaglandins. When they are stimulated with bradykinin, endothelial cells of canine arteries appear to release a substance mediating vascular smooth muscle relaxation. In contrast, preparations of arteries of cats (superior mesenteric) and rabbits (superior mesenteric and celiac) may be rubbed on the intimal surface without a consistent loss of sensitivity to the relaxing effects of bradykinin. In addition, relaxation of the cat and rabbit arteries is completely blocked by cyclooxygenase inhibitors. Preliminary studies indicate that bradykinin relaxes human arteries in an endothelium-dependent manner and that this effect is not mediated by prostaglandins. We have previously reported that arteries of all species tested require the presence of endothelial cells for relaxation in response to acetylcholine and we have also demonstrated, using the rabbit aorta, that this effect is mediated by the release of an uncharacterized substance from these cells that relaxes vascular smooth muscle. We conclude that bradykinin relaxes canine and human arteries via a similar mechanism but that it relaxes cat and rabbit arteries by stimulating release of prostaglandins from as yet undefined cell types.**

One of the chief physiological properties of bradykinin (BKN) is its ability to produce a fall in blood pressure when injected intravenously in minute amounts. With the purification and characterization of the molecule (1, 2) it became apparent that on a molar basis the nonapeptide was one of the most potent vasodilator substances known. However, its vasodilator activity is not consistently reflected in studies performed on isolated blood vessels. Depending on the species and anatomical origin of the blood vessel, application of BKN may elicit relaxation, contraction, or no response at all (3–5).

It has been argued that at least part of the vasodilator response to BKN is an indirect effect mediated by release of prostaglandins (PGs) (6), and support is given by the observed effects of the peptide on vascular PG synthesis (7). We have recently reported that other potent vasodilator agents relax vascular smooth muscle in an indirect manner by stimulating the release of a relaxing substance from endothelial cells. This endothelium-derived relaxing factor (EDRF) is not a PG (8–11). Acetylcholine (AcCho) and substance P (SP) act exclusively by this mechanism in producing relaxation of isolated arteries (8–10), and ATP and ADP exert their relaxing effects predominantly by stimulating endothelial cells and, to a lesser degree, by a direct action on smooth muscle (9, 11, 12). The current investigation was undertaken to explore the relative contribution of PGs and EDRF in the relaxation of isolated arteries by BKN. Our results indicate that this relaxation is mediated by PGs in the arteries of cats and rabbits but is mediated by EDRF in the arteries of dogs and humans. A preliminary note on some of these results has already appeared (13).

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MATERIALS AND METHODS

Preparation of Arterial Rings. Cats and mongrel dogs of either sex were anesthetized with pentobarbital. Male New Zealand White rabbits of 2–3 kg were killed by a blow to the head. From these animals selected arteries were carefully dissected, applying minimal traction to avoid stretching and taking care not to subject the intima to rubbing either with instruments or upon itself. In the case of canine coronary and pulmonary arteries, dissection was carried out after excision of the heart and lungs from the animal. Small segments of arteries of human mesentery were obtained from tissue removed in the course of surgery for resection of the bowel. After dissection, arteries were quickly immersed in Krebs solution. Transverse rings of arteries were prepared in a manner similar to that described previously (8, 9). In brief, the arteries were carefully cleaned of any adherent fat and connective tissue and cut into 2.5-mm ring segments with a razor blade slicing device. The rings were mounted on pairs of stainless steel wire hooks and placed in 20-ml all-glass muscle chambers containing Krebs solution at 37°C.

Intimal endothelial cells were removed either prior to introduction of the rings in the chambers or after testing with vasoactive agents. In either case, the ring was left on the hooks and a 2-g weight was attached to the lower hook. A small wooden stick was then inserted into the lumen and rubbed gently on the intimal surface for 30 to 60 sec. To test the effectiveness of this treatment in removing endothelial cells, we sometimes made histological observations of the intimal surface of rubbed and unrubbed preparations at the end of an experiment, using a silver staining technique (8). We have previously shown that relaxation of isolated arteries by AcCho is directly related to the presence of endothelial cells. Therefore, in all experiments the effectiveness of the rubbing procedure in removing endothelial cells was ascertained by a functional test using AcCho. The complete loss of the relaxing response to this agent indicated an essentially complete loss of endothelial cells.

Bathing Solution and Drugs. The bathing fluid was Krebs solution of the following composition (mM): NaCl, 118; KCl,

Abbreviations: BKN, bradykinin; PG, prostaglandin; NE, *l*-norepinephrine; AcCho, acetylcholine; Q, quinacrine; ETYA, 5,8,11,14-icosatetraynoic acid; SP, substance P; EDRF, endothelium-derived relaxing factor; IND, indomethacin; FBP, flurbiprofen.

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4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 24; glucose, 11; Na_2EDTA, 0.03. The solution was continuously gassed with 95% O_2/5% CO_2, resulting in a pH of 7.4.

Drugs used in this study were: *l*-norepinephrine (NE) bitartrate, AcCho chloride, indomethacin (IND), BKN triacetate, quinacrine (Q) dihydrochloride (Sigma); 5,8,11,14-icosatetraynoic acid (ETYA) (Hoffmann–La Roche), sodium flurbiprofen (FBP) (Allergan, Irvine, CA), and the sodium salt of prostacyclin and the tromethamine salt of $PGF_{2\alpha}$ [gifts of Dr. John Pike (Upjohn)]. All drugs were prepared as aqueous solutions except for ETYA and indomethacin, which were dissolved in absolute ethanol to make, respectively, 33 and 40 mM stock solutions.

Recording. Tension changes induced in arterial preparations were measured with FT-03 isometric strain gauges and recorded on a four-channel model 7 polygraph (Grass). Resting tension was adjusted by means of a rack-and-pinion clamped to the strain gauge. Baseline tension was set at 2 g after an equilibration period of at least 90 min (1 g tension = 9.8 millinewtons).

RESULTS

Relaxation of Isolated Arteries by BKN. BKN elicited relaxation of transverse rings of superior mesenteric arteries from cats, superior mesenteric and celiac arteries from rabbits, and superior mesenteric, celiac, coronary, renal, splenic, pulmonary, gastric, and femoral arteries from dogs. BKN also relaxed preparations of arteries obtained from human mesentery. Two notable exceptions were the aorta and renal artery of rabbits. BKN did not induce any relaxation, and at high concentrations it induced contraction of these arteries (not shown). In order to observe relaxation by BKN and other agents, it was first necessary to induce contraction of the blood vessels used, because they had very low intrinsic tone. Arteries were induced to contract with NE except in the case of coronaries, for which $PGF_{2\alpha}$ was used. NE was not suitable for inducing contraction in coronary arteries because it has significant efficacy for stimulating the β_1-adrenergic receptors present, which mediate vascular smooth muscle relaxation.

The threshold concentration of BKN required to induce relaxation was somewhat variable, but canine and human arteries were uniformly more sensitive than those of cat and rabbit. The threshold concentration of BKN for relaxation of canine and human arteries ranged between about 0.1 and 1.0 nM, whereas the threshold of cat and rabbit arteries was usually 10 to 100 nM.

Effect of Cyclooxygenase Inhibition. There is some evidence that indicates a role for PGs in the vascular actions of kinins (6, 7, 14, 15). We have studied the effects of inhibitors of cyclooxygenase, the enzyme that catalyzes the first committed step in PG synthesis, on the relaxation of isolated arteries by BKN. Fig. 1 illustrates the inhibitory effects of IND on relaxation of cat superior mesenteric and rabbit celiac arteries by BKN. Although relaxation by BKN is abolished after inhibition of PG synthesis, relaxation induced by other vasoactive agents is not. Thus, after treatment with IND, relaxation of the rabbit mesenteric artery was evoked by SP at 10 pM, an effect we have shown is dependent on the presence of endothelial cells (9). Another frequently observed effect of treatment with IND is a shift in the NE concentration–effect relationship such that less NE is required to elicit a given level of contraction. This presumably occurs because the isolated arteries are synthesizing PGs with relaxing activity that opposes the contraction induced by NE. Interestingly, this effect is never observed in the rabbit renal artery and aorta, two preparations that do not relax in response to addition of either BKN or prostacyclin (PGI_2).

The relaxation of canine arteries by BKN differs from that observed with cat and rabbit arteries in that relaxation is unaffected by inhibitors of cyclooxygenase. Isolated rings of canine splenic, gastric, celiac, femoral, renal, coronary, pulmonary, and superior mesenteric arteries were preincubated with the cyclooxygenase inhibitors IND or FBP. Such treatment did not inhibit the response to BKN of any of these arteries. Responses of some of these arteries to BKN before and after treatment with IND or FBP are shown in Fig. 2. IND or FBP were added at least 10 min prior to the second test. In the case of the pulmonary, a slight decrease in sensitivity to BKN was seen, but a similar decrease in sensitivity was observed in a control preparation not treated with FBP (not shown). Treatment of canine arterial rings with inhibitors of cyclooxygenase potentiated contraction induced by NE or, in the case of coronary arteries, $PGF_{2\alpha}$, an effect consistent with that observed with arteries of the cat and rabbit.

Effect of Removal of Endothelial Cells. Fig. 3 illustrates BKN-induced relaxation of some representative arteries of the rabbit, cat, and dog. Removal of endothelial cells by rubbing of the intimal surface resulted in the complete loss of relaxing response to BKN of arteries obtained from dog, in sharp contrast with the lack of effect on arteries of the rabbit and cat. It should be noted that preparations of superior mesenteric arteries of the

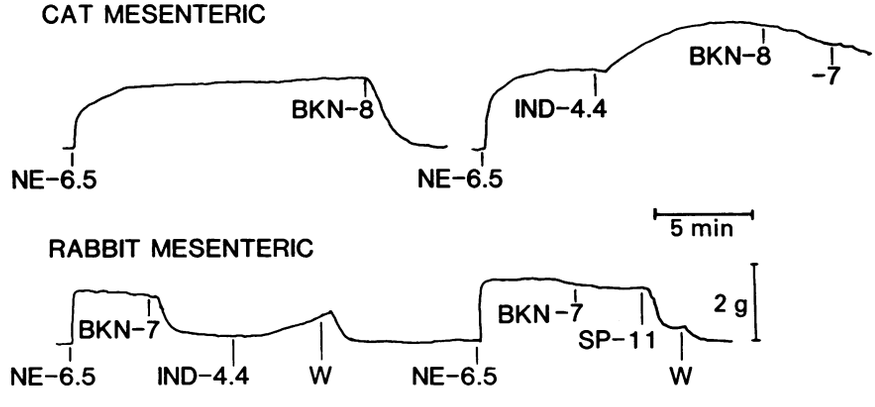

FIG. 1. Inhibition by IND of BKN-induced relaxation of cat and rabbit superior mesenteric arteries. The tracings are of polygraph recordings of the mechanical responses of the arterial rings to application of NE and BKN. The tracings show the point at which each drug addition was made, and the corresponding cumulative concentration of the drug in the chamber, expressed as the logarithm of the molar concentration. W indicates washout of the chamber. IND completely blocked relaxation by BKN of the NE-induced contraction of arteries of cat (n = 10) and rabbit (n = 5). Note that IND did not interfere with relaxation of the rabbit artery induced by SP. IND also did not interfere with relaxation of cat and rabbit arteries by AcCho (not shown).

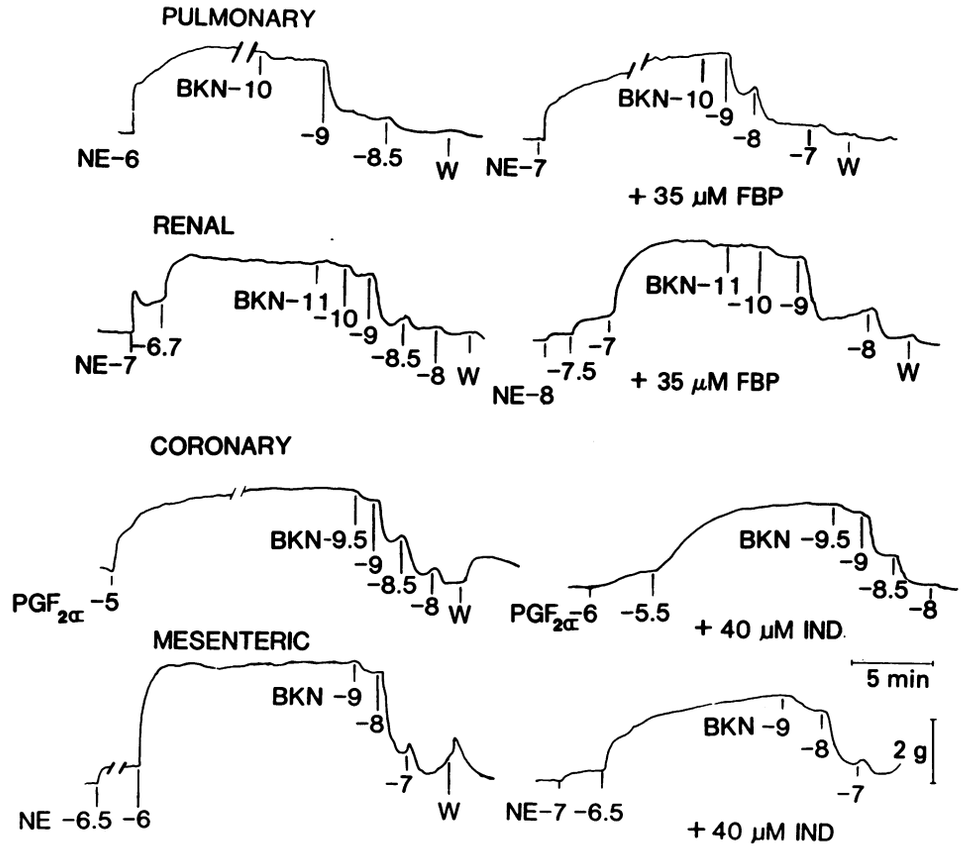

FIG. 2. Lack of effect of cyclooxygenase inhibition on BKN-induced relaxation of rings of arteries of the dog. IND at 40 μM or FBP at 35 μM, added after the first trial (left) and at least 10 min prior to the second trial (right), failed to alter sensitivity to BKN of all canine arteries tested. These drugs potentiated NE- and $PGF_{2\alpha}$-induced contraction, presumably by blocking synthesis of PGs with vascular smooth muscle-relaxing activity. The coronary artery used here was the circumflex. Results similar to those shown were obtained in nine experiments with pulmonary, five with renal, three with coronary, seven with superior mesenteric, and one each with splenic, gastric, and femoral arteries.

cat occasionally failed to relax in response to BKN after removal of endothelial cells. Nevertheless, unrubbed rings from these arteries that initially relaxed in response to BKN did not do so again after cyclooxygenase inhibition (see above).

Effect of Q and ETYA on Relaxation of Canine Arteries. The relaxation of canine arteries induced by BKN is not dependent on PG synthesis, and it shares the requirement for endothelial cells with the relaxation induced by AcCho. Thus it was of interest for us to examine the effect of two inhibitors of AcCho-induced relaxation (8, 9), Q and ETYA, on BKN-induced relaxation of canine arteries. Fig. 4 is a comparison of the acute antagonism by these two agents of AcCho- and BKN-induced relaxation of canine superior mesenteric artery. Q and ETYA do not by themselves induce contraction of isolated arteries, but they actually inhibit contractions induced by agents such as NE. This inhibition of contraction may account for the observed incomplete reversal of AcCho- and BKN-induced relaxations by Q and ETYA.

BKN Relaxation of Human Arteries. BKN was tested on rings from a branch of the inferior mesenteric artery of a 74-year-old woman and on rings from a branch of the superior mesenteric artery of a 23-year-old man. In both experiments it induced relaxation that was strictly dependent on the presence of endothelial cells. Cyclooxygenase blockade with either FBP or IND did not inhibit BKN-induced relaxation; indeed, such treatment appeared to enhance the degree of relaxation. Records from one experiment are shown in Fig. 5. Thus, the mechanism of BKN relaxation in human arteries appears to be similar to that in canine arteries.

DISCUSSION

Our experiments have demonstrated that relaxation of isolated arteries by BKN is an indirect effect mediated by the release of other vasoactive substances. Relaxation of canine and human arteries by BKN is mediated by EDRF, an uncharacterized factor released from endothelial cells, whereas relaxation of arteries from cats and rabbits by BKN is mediated by PGs.

The role of PGs in relaxation of cat and rabbit arteries by BKN is supported by the ability of cyclooxygenase inhibitors to block this effect. Aiken (14) has reported that IND completely inhibits relaxation of isolated rabbit celiac arteries by BKN, an effect that we have duplicated. With respect to rabbit arteries, those that are relaxed by BKN are also relaxed by prostacyclin (PGI_2) (16). Those rabbit arteries that are not relaxed by BKN—namely, the aorta and the renal artery—also fail to relax in response to PGI_2 (ref. 16 and unpublished observations). In these latter arteries, BKN may well stimulate release of PGI_2 but not elicit relaxation because of a lack of responsiveness of the smooth muscle cells of these arteries to PGI_2. Our experiments cannot determine the cellular elements that release the PG upon stimulation with BKN. Although most arteries of cats and rabbits relax in response to BKN after removal of endothelial cells, this does not rule out a contribution of these cells to the total PG released. Indeed, the occasional loss of BKN relaxation after rubbing of the intimal surface of arteries of the cat might be explained if in these particular preparations endothelial cells produced the major portion of the PGs. However, the more usual result indicates that other vascular cell types respond to stimulation with

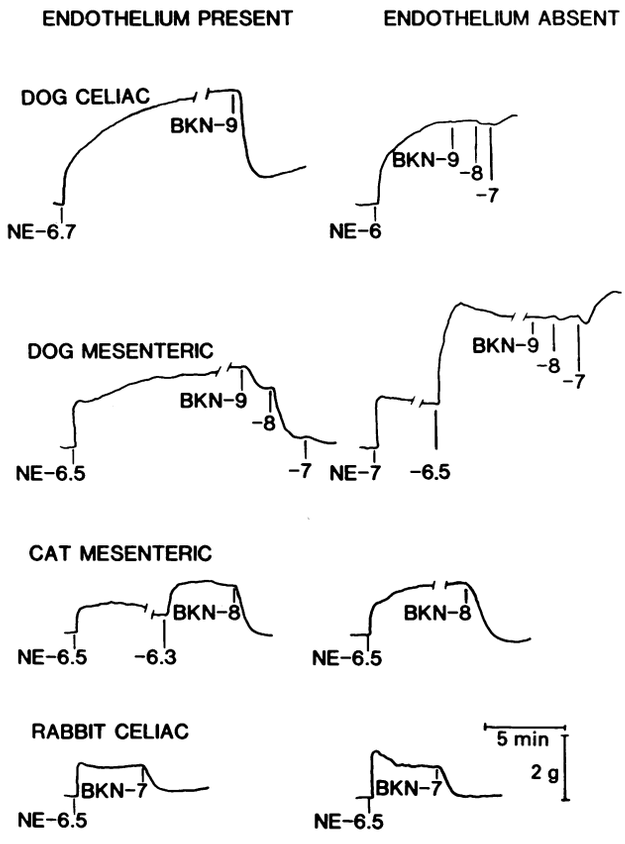

FIG. 3. Effect of removal of endothelial cells on BKN-induced relaxation of arteries of rabbit, cat, and dog. Comparisons are shown between intact arterial rings and rings rubbed on the intimal surface to remove endothelial cells. For each comparison, rings were cut from adjacent areas of the same artery. Prior to testing with BKN, all preparations were tested with AcCho. Only preparations with endothelial cells relaxed upon addition of AcCho, regardless of the species from which they were obtained (not shown). The preliminary test with AcCho ensures that the rubbing of the intimal surface has removed essentially all endothelial cells. Removal of endothelial cells had no effect on the sensitivity of arterial rings from rabbit ($n = 4$) and cat ($n = 3$). Removal of endothelial cells eliminated relaxation induced by BKN in the rings of canine arteries. Results similar to those shown were obtained in all of 19 experiments on a variety of canine arteries, including superior mesenteric, celiac, renal, splenic, pulmonary, and coronary.

BKN by releasing PGs that relax smooth muscle.

In marked contrast to the cat and rabbit, BKN-induced relaxation of canine and human arteries is entirely dependent on the presence of endothelial cells. We have found that BKN relaxation in these species is not affected by inhibition of the cyclooxygenase pathway with IND or FBP. In contrast, Toda (5) found that IND reduced sensitivity of canine coronary artery to BKN to a slight degree, and McGiff *et al.* (6), in studies of the perfused dog kidney, found a significant attenuation of the vasodilation induced by BKN with such treatment. The results in the perfusion system might be explained by the contribution of extravascular elements in the kidney that release PGs when stimulated with BKN. Our studies cannot exclude the possibility that BKN stimulates the release of some PGs in human and canine arteries, but they do suggest that PGs make little or no contribution to the relaxing effect of the peptide. We have concluded that EDRF is more important than PGs as mediator of the vascular effects of BKN at the local level of the artery in the dog and human.

Relaxation induced by AcCho in all species tested and relaxation induced by BKN in the dog are sensitive to Q, an antimalarial drug that has been reported to inhibit phospholipase A_2 activity (17). Relaxation that is endothelium dependent is also inhibited by ETYA, an acetylenic analog of arachidonic acid known to inhibit both the cyclooxygenase and lipoxygenase

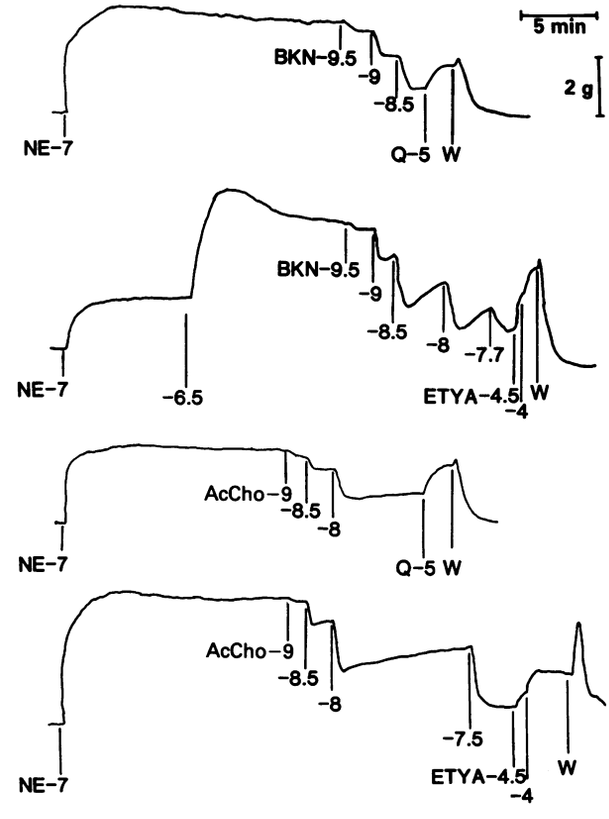

FIG. 4. Effect of Q and ETYA on AcCho- and BKN-induced relaxation of rings of canine superior mesenteric artery. Q and ETYA produce acute reversal of relaxation induced by agents whose effects are mediated by endothelial cells. Q and ETYA do not induce contraction when added to either a preparation induced to contract by NE or at resting tension in the absence of contraction-inducing agents. Similar results were obtained with four preparations of canine arteries.

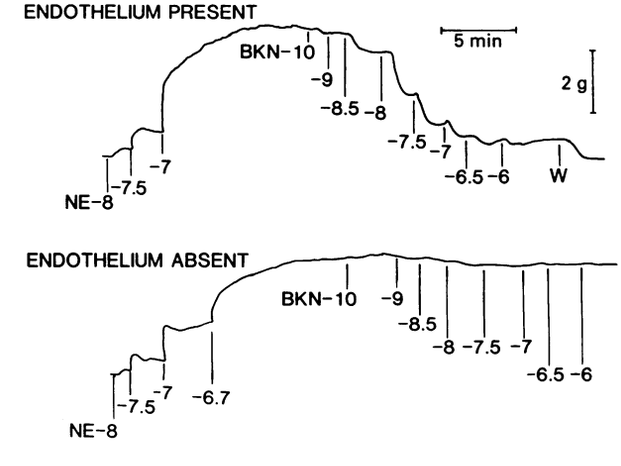

FIG. 5. Endothelium-dependent relaxation of human artery by BKN. The records are from two rings from a branch of the superior mesenteric artery of a 23-year-old man. These preparations were preincubated for 20 min with 40 μM IND, and the drug was also present during testing with NE and BKN. IND did not inhibit relaxation induced by BKN. Removal of endothelial cells by rubbing of the intimal surface resulted in loss of relaxing response to BKN.

pathways for oxidation of unsaturated fatty acids (18). Thus, endothelium-dependent relaxation may require activation of enzyme systems involved in lipid metabolism.

In the dog, BKN appears to act like AcCho and other agents (SP, ATP) that cause relaxation of arteries via the release of EDRF, an as yet uncharacterized substance released from vascular endothelial cells (8–11). BKN appears to cause relaxation of human arteries in a similar manner. EDRF may be a product of the release and subsequent oxidation of fatty acids, but it is clearly not a PG.

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