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Effects of Nitroprusside on the Bradykinin Responsiveness of Human Fibroblasts

SU-CHEN TSAI, RONALD ADAMIK, BETTY E. HOM, VINCENT C. MANGANIELLO, and JOEL MOSS

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

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SUMMARY

The effects of agents that cause vasodilatation and hypotension, such as endogenously produced bradykinin (BK) or the drug nitroprusside (NP), appear to result from effects on cyclic nucleotides (cGMP, cAMP) and arachidonate metabolism. Cultured human fibroblasts, which possess B_2 BK receptors and respond to NP with an increase in cGMP, were used to study the interaction of these agents at the molecular level. Addition of BK or NP to cultured human fibroblasts caused a rapid increase in cGMP. The effect of NP was usually maximal within 30 sec, after which cGMP content declined. The increase in cGMP produced by BK reached a maximum at ~1 min and then fell; the rise with NP was more than 10 times that with BK. At 30 sec, cGMP content with NP plus BK was less than with NP alone. At later times, however, effects of BK and NP were slightly more than additive and maximal cGMP levels were reached at 90 sec. BK increased prostaglandin production by the fibroblasts; it is believed that the kinin-induced elevation in cAMP content is secondary to increased prostaglandin formation. NP caused a small, early increase in cAMP without significant effect on prostaglandin I_2 (PGI_2); after 2.5 min, effects on PGI_2 and cAMP were greater with BK and NP than with BK alone. To study further the roles of arachidonate metabolites in the fibroblast response to BK and NP, the cyclooxygenase inhibitor, indomethacin, and the combined lipoxygenase and cyclooxygenase inhibitor, 5,8,11,14-eicosatetraynoic acid (ETYA), were added to fibroblasts prior to BK or NP. Increases in cAMP or PGI_2 with BK or BK plus NP were blocked by indomethacin or ETYA. These effects of BK or BK plus NP on cAMP thus appear to be mediated through cyclooxygenase products of arachidonate metabolism. Indomethacin and ETYA did not affect cGMP in the presence of BK plus NP but enhanced NP-stimulated cGMP accumulation by 40–50%; effects of NP on cGMP may be independent of or perhaps inhibited by cyclooxygenase derivatives. Cellular responses to BK plus NP differed quantitatively and temporally from the sum of effects of BK and NP alone. Through interactions of this type, *in vivo* responses to drugs like NP may be influenced by levels of BK or similar endogenous mediators.

BK, a nonapeptide generated from kininogen by kallikrein, is believed to be involved in immune and inflammatory responses, and regulation of blood pressure, electrolytes, and fluid balance (1–5). BK and its analogues interact with specific cell surface receptors (6–8). Two types of BK receptors, B_1 and B_2, have been defined (6, 7). For B_1 receptors, des-Arg^9-BK is as potent an effector as is BK; for B_2 receptors, des-Arg^9-BK is either ineffective or very weak in competing for BK-binding sites or in eliciting biological responses (6, 7). B_2 receptors are found in smooth muscle and cultured human fibroblasts (7–9). The effects of BK on tissues appear to be mediated through cAMP, cGMP, activation of phospholipases, and release of arachidonate metabolites such as prostacyclin (10–17).

NP is a potent smooth muscle relaxant used clinically as a rapidly acting vasodilator (18–24), with effects on both the arterial and venous circulation (22, 23, 25–27); the precise biochemical pathways which mediate NP-induced smooth muscle relaxation have not been elucidated. Since in smooth muscle and other target tissues NP increases cGMP by enhancing guanylate cyclase activity, it has been proposed that cGMP is a critical intracellular intermediate in NP action (18, 19, 21, 24, 28–30). Arachidonate metabolism, which is affected by nitroglycerin and related compounds, may also participate in NP action (31).

Since both BK and NP may utilize similar cellular messengers and act on similar target tissues, we investigated in cultured human fibroblasts the interactions of the two agents at the cellular level, in terms of their effects on both cyclic nucleotide content and prostaglandin production.

Experimental Procedures

Materials

BK was purchased from Beckman (distributor for Peptide Institute, Protein Research Foundation, Osaka, Japan); sodium nitroprusside and indomethacin were from Sigma Chemical Co.; cGMP, cAMP, and prostacyclin radioimmunoassay kits were from New England Nuclear; fetal calf serum was from Hazleton. Eagle's minimal essential medium

ABBREVIATIONS: BK, bradykinin; NP, nitroprusside; ETYA, 5,8,11,14-eicosatetraynoic acid; TCA, trichloroacetic acid; PGI_2, prostaglandin I_2; PGE_2, prostaglandin E_2.

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was prepared by the National Institutes of Health media section. ETYA was a gift from Hoffmann-La Roche.

Methods

Subcultures of human fibroblasts (line HF-15) were grown to confluency in 6-cm dishes as previously described (32); growth medium was replaced every other day. On the day of the experiment, cells were washed three times with 3 ml of Hanks' balanced salt solution at 37° on a slow-moving shaker; then 2 ml of Hanks' solution were added and cells were equilibrated for 5–8 min before addition of Hanks' or various compounds.

Cells were incubated with or without additions for the indicated time. Data presented in Figs. 1–6 were obtained from representative experiments; these experiments were repeated three to five times. Samples were assayed for cGMP, cAMP, and prostacyclin content. In the figures, error bars represent the range of values (mean ± range of mean or standard error) from duplicate or triplicate samples, respectively; the absence of error bars indicates that the range falls within the symbols; the *t*-test was used for statistical evaluation.

Prostacyclin assays. Samples (300 μl) of medium were taken just before addition of TCA for radioimmunoassay of PGI_2 as its hydrolysis product 6-keto prostaglandin $F_{1\alpha}$ (9, 33).

cGMP and cAMP assays. Incubations were terminated by addition of 1 ml of 15% cold TCA and the cells were frozen in a dry ice-ethanol bath. After thawing, the supernatant was removed and the cell layer was washed with 1 ml of 5% TCA which was combined with the supernatant. TCA was extracted with Freon-tri-*n*-octylamine (34) and samples were taken for radioimmunoassay of cGMP and cAMP (New England Nuclear kit) as previously described (9).

Protein determination. Three to five dishes from each experiment were washed three times with 3 ml of Hanks' and then frozen after addition of 2 ml of Hanks' solution. After thawing, cells were harvested by scraping and solubilized in 0.1 N NaOH at 60–70° for 10 min. The protein content (400–600 μg/dish) was measured by the method of Lowry *et al.* (35).

Results

Incubation of cultured human fibroblasts with BK, NP, or both led to a rapid rise in cGMP (Fig. 1). BK alone caused only a slight increase in cGMP (Fig. 1A). NP was much more effective than BK, with the maximal increase in cGMP content usually occurring in <30 sec. Subsequently, cGMP content decreased gradually (Fig. 1, A and B).. The increase in cGMP produced by NP was more than 10 times that with BK alone (Fig. 1A). In the presence of BK and NP, the peak in cGMP occurred at ~1½ min and was higher than the summed effects of the two agents (Fig. 1, A and B). BK and NP also enhanced cAMP content of fibroblasts (Fig. 2). NP caused a small but significant increase in cAMP, with the maximal increase at 1 min (Fig. 2, *inset*). BK raised the cAMP content to a much greater extent. When NP was present with BK, cAMP levels were significantly higher than with BK alone at 6 min.

BK also caused a rapid increase in PGI_2 (Fig. 3). In the presence of BK and NP, PGI_2 formation at 2.5 and 6 min was greater than with BK alone (Fig. 3).

To examine further the role of arachidonate metabolites in the effects of BK and NP on cyclic nucleotides, the response to these agents was examined in the presence of indomethacin or ETYA, inhibitors of arachidonate metabolism. Both indomethacin and ETYA inhibited, in a parallel manner, the effects of BK or BK plus NP on PGI_2 formation and cAMP content or of BK on cGMP (Figs. 4–6). The drugs had no significant effect on cGMP content when BK was present with NP (Fig. 6). Indomethacin and, to a greater extent, ETYA increased cGMP in the presence of NP alone (Fig. 6).

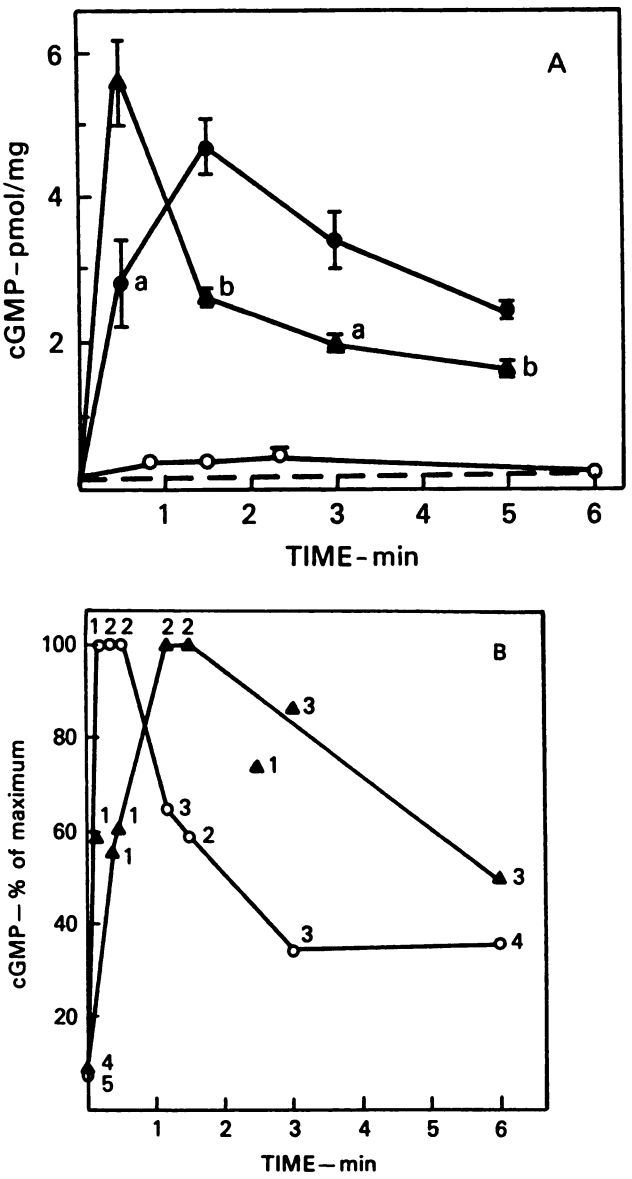

Fig. 1. A. Effects of NP, BK, or both on cGMP content of fibroblasts. Fibroblasts were incubated without and with 25 μM NP (▲), 80 nM BK (○), or both additions (●) for the indicated times prior to assay for cGMP content as described in Experimental Procedures. – – –, no addition. *a*, $p < 0.025$, and *b*, $p < 0.01$ for difference between NP and NP plus BK at the indicated time. B. Effects of NP or NP plus BK on cGMP content. Data from four experiments similar to that presented in A were plotted as percentage of the maximum. Maximal cGMP observed in the presence of NP (○) was 5.2 ± 0.9 pmol/mg (mean ± SD, $n = 4$). Maximal cGMP in the presence of NP plus BK (▲) was 4.0 ± 0.6 pmol/mg (mean ± SD, $n = 4$). The *numbers* next to the *symbols* are the number of experiments in which cGMP content was measured at the indicated time.

Discussion

Incubation of human fibroblasts with BK resulted in enhanced formation of prostacyclin (determined as its hydrolysis product 6-keto prostaglandin $F_{1\alpha}$) and PGE_2 (36) and accumulation of cAMP. Since prostacyclin and PGE_2 enhance cAMP formation, a finding similar to that observed in other systems (37–40), it has been postulated that the effects of BK on cAMP

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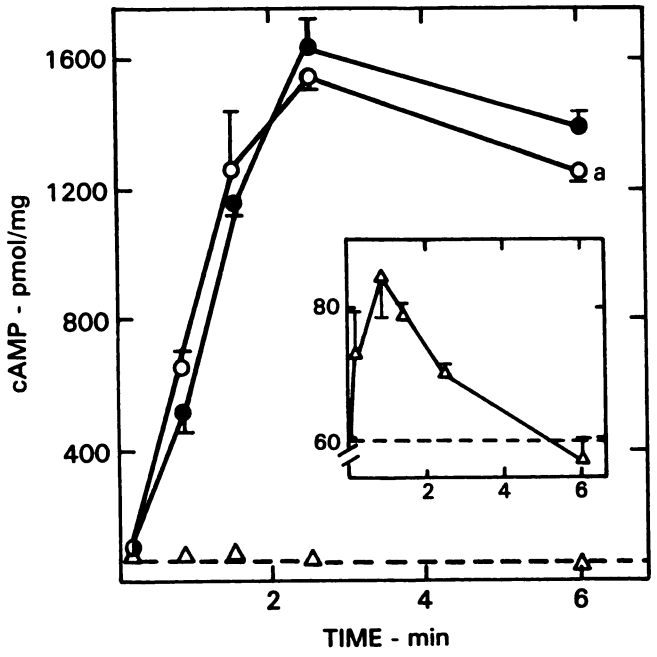

Fig. 2. Effects of NP, BK, or both on cAMP content of fibroblasts. Fibroblasts were incubated as described in Fig. 1 before assay of cAMP content as described in Experimental Procedures. BK (○), NP (△), or both additions (●) are indicated. – – –, no addition. *a*, $p < 0.05$ for difference between BK and BK plus NP at 6 min.

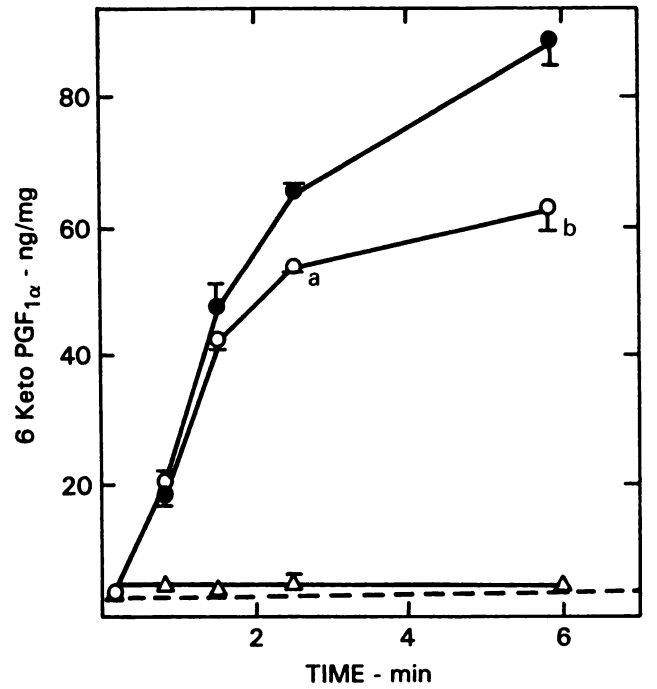

Fig. 3. Effects of BK or BK and NP on prostacyclin accumulation. Fibroblasts were incubated as described in Fig. 1 prior to assay of PGI_2 accumulation as described in Experimental Procedures. BK (○), NP (△), or both additions (●) are indicated. – – –, no addition. *a*, $p < 0.005$, and *b*, $p < 0.05$ for difference between BK and BK plus NP at the indicated time.

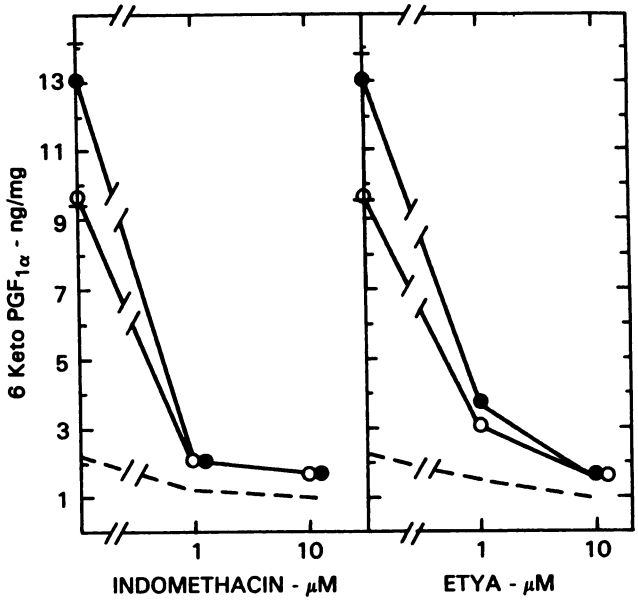

Fig. 4. Effects of indomethacin and ETYA on prostacyclin accumulation. Fibroblasts were incubated without or with the indicated concentrations of indomethacin and ETYA for 5–8 min prior to addition of 25 μM NP, 80 nM BK, or both. After 1 min with BK (○) or NP plus BK (●), samples of medium were taken for assay of prostacyclin as described in Experimental Procedures. – – –, no addition.

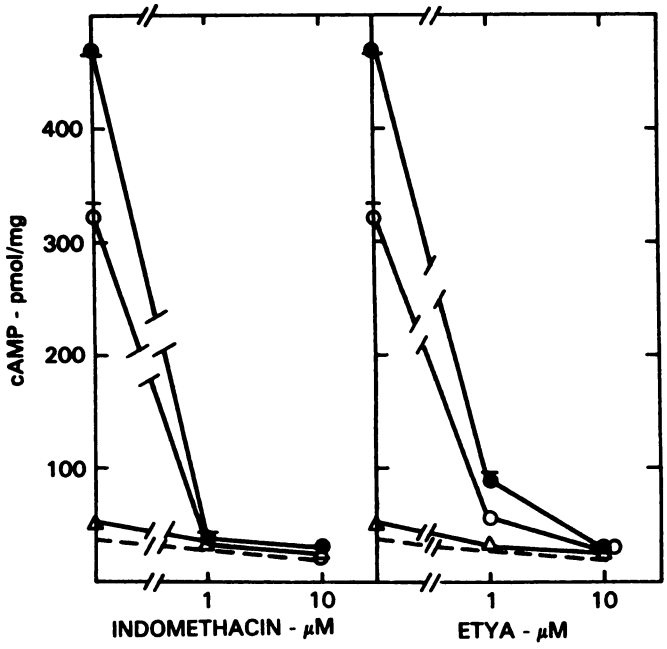

Fig. 5. Effects of indomethacin and ETYA on cAMP accumulation. Fibroblasts were incubated as described in Fig. 4 and assayed for cAMP content as described in Experimental Procedures. BK (○), NP (△), or both additions (●) are indicated. – – –, no addition.

are mediated through prostaglandin production. Both indomethacin, which at the concentrations used in these studies blocks the cyclooxygenase pathway, and ETYA, which inhibits both the lipoxygenase and cyclooxygenase pathways (41, 42), inhibited the stimulatory effects of BK on cAMP. Since both prostacyclin and PGE_2 are products of the cyclooxygenase pathway, it would appear that inhibition of the cyclooxygenase pathway suffices to block BK-stimulated accumulation of cAMP. The effects of BK on cGMP content which were much smaller than those on cAMP were also inhibited by indomethacin or ETYA. Thus, the cGMP response to BK in human fibroblasts may also be influenced by arachidonate metabolites of the cyclooxygenase pathway.

By itself, NP produces rather small effects on cAMP and

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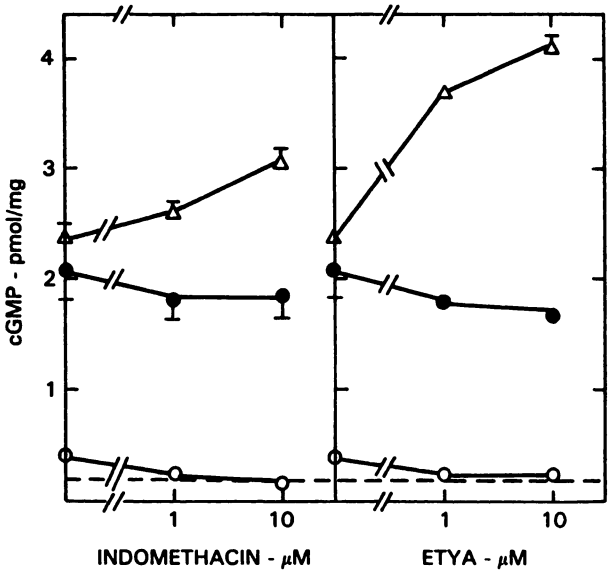

Fig. 6. Effects of indomethacin and ETYA on cGMP accumulation. The same samples as shown in Fig. 5 were assayed for cGMP content as described in Experimental Procedures. BK (○), NP (△), or both additions (●) are indicated. – – –, no addition.

PGI_2 production in human fibroblasts. NP does induce a marked but transient rise in cGMP. Whether activation of guanylate cyclase by NP is mediated by nitric oxide (28) or, as suggested by more recent information, proceeds via mechanisms other than liberation of nitric oxide or formation of *S*-nitrosothiols (43), our data strongly indicate that arachidonic acid metabolites can influence this cellular response to NP. Indomethacin and ETYA, which also inhibited effects of BK on cGMP, actually enhanced effects of NP on cGMP content, suggesting that cyclooxygenase products of arachidonic acid metabolism exert a suppressive effect on NP stimulation of guanylate cyclase. These putative cyclooxygenase products may have multiple targets. Conceivably, they could act directly on guanylate cyclase or, alternatively, at an earlier step critical to NP activation. Earlier reports have suggested that arachidonate metabolites are also involved in NP action in platelets (31).

Cellular responses to BK and NP together differed quantitatively and temporally from the sum of the effects of NP and BK alone. NP increased the effect of BK on cAMP content and prostaglandin formation. These NP-induced alterations in BK responsiveness seem to involve, or are at least ultimately controlled by, cyclooxygenase products, since they were blocked by both ETYA and indomethacin. The time course of cGMP accumulation was dramatically different in the presence of BK and NP than with either effector alone. In the presence of BK (which by itself exerted only a very small effect on cGMP content), accumulation of cGMP was maximal at ~1½ min and higher than the summed effect of either agent alone. The effect of BK on NP-induced accumulation of cGMP was not altered by ETYA or indomethacin, suggesting that some intracellular "second messenger" other than an eicosanoid produced in response to BK was affecting this cellular response to NP. Since BK can apparently activate the polyphosphoinositide signal cascade system in several cell types (44–51), conceivably a number of intracellular mediators, i.e., Ca^{2+}, diacylglycerol, arachidonic acid, etc., might be involved in BK regulation of the cGMP response to NP.

These cultured human fibroblasts provide a convenient model for investigating the interaction of BK with agents that alter cyclic nucleotide and arachidonate metabolism. It is interesting that, in the case of NP and BK, each agent seemed to modulate a major cellular response to the other. NP, with a very small effect on cAMP content, altered the effect of BK on cAMP content and prostacyclin formation. BK, with a small effect on cGMP content, altered the time course of cGMP accumulation in response to NP. These studies also point out the complexities in this mutual regulation which apparently involves cyclooxygenase products as well as other intracellular mediators. Through cellular interactions such as those described in this report, *in vivo* responses to drugs like NP may be influenced by levels of BK or similar endogenous mediators.

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References

1. Lewis, G. P. Kinins in inflammation and tissue injury. *Handb. Exp. Pharmacol.* **25**:516–530 (1979).
2. Authors. Kinins and blood pressure. *Lancet* **2**:663–665 (1978).
3. Mills, I. H. Kallikrein, kininogen, and kinins in control of blood pressure. *Nephron* **23**:61–71 (1979).
4. Cuthbert, A. W., and H. S. Margolius. Kinins stimulate net chloride secretion by the rat colon. *Br. J. Pharmacol.* **75**:587–598 (1982).
5. Manning, D. C., S. H. Snyder, J. F. Kachur, R. J. Miller, and M. Field. Bradykinin receptor-mediated chloride secretion in intestinal function. *Nature (Lond.)* **299**:256–259 (1982).
6. Regoli, D., J. Barabe, and W. K. Park. Receptors for bradykinin in rabbit aorta. *Can. J. Physiol. Pharmacol.* **55**:855–867 (1977).
7. Barabe, J., J.-N. Drouin, D. Regoli, and W. K. Park. Receptors for bradykinin in intestinal and uterine smooth muscle. *Can. J. Physiol. Pharmacol.* **55**:1270–1285 (1977).
8. Odya, C. E., and T. L. Goodfriend. Bradykinin receptors. *Handb. Exp. Pharmacol.* **25**(Suppl):287–300 (1979).
9. Roscher, A. A., V. C. Manganiello, C. L. Jelsema, and J. Moss. Receptors for bradykinin in intact cultured human fibroblasts. Identification and characterization by direct binding study. *J. Clin. Invest.* **72**:626–635 (1983).
10. Newcombe, D. S., J. V. Fahey, and Y. Ishikawa. Hydrocortisone inhibition of the bradykinin activation of human synovial fibroblasts. *Prostaglandins* **13**:235–244 (1977).
11. Bell, R. L., N. J. Baenzinger, and P. W. Majerus. Bradykinin-stimulated release of arachidonate from phosphatidyl inositol in mouse fibrosarcoma cells. *Prostaglandins* **20**:269–274 (1980).
12. McGiff, J. C., H. D. Itskovitz, A. Terragno, and P. Y.-K. Wong. Modulation and mediation of the action of the renal kallikrein-kinin system by prostaglandins. *Fed. Proc.* **35**:175–180 (1976).
13. Hong, S.-C. L., and L. Levine. Stimulation of prostaglandin synthesis by bradykinin and thrombin and their mechanisms of action on MC5-5 fibroblasts. *J. Biol. Chem.* **251**:5814–5816 (1976).
14. Fahey, J. V., C. P. Ciosek, Jr., and D. S. Newcombe. Human synovial fibroblasts: the relationships between cyclic AMP, bradykinin, and prostaglandins. *Agents Actions* **7**:255–264 (1977).
15. Stoner, J., V. C. Manganiello, and M. Vaughan. Effects of bradykinin and indomethacin on cyclic GMP and cyclic AMP in lung slices. *Proc. Natl. Acad. Sci. USA* **70**:3830–3833 (1973).
16. Moncada, S., K. M. Mullane, and J. R. Vane. Prostacyclin release by bradykinin *in vivo*. *Br. J. Pharmacol.* **66**:96P–97P (1979).
17. Bareis, D. L., V. C. Manganiello, F. Hirata, M. Vaughan, and J. Axelrod. Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts. *Proc Natl. Acad. Sci. USA* **80**:2514–2518 (1983).
18. Katsuki, S., W. P. Arnold, and F. Murad. Effects of sodium nitroprusside, nitroglycerin, and sodium azide on levels of cyclic nucleotides and mechanical activity of various tissues. *J. Cyclic Nucleotide Res.* **3**:239–247 (1977).
19. Katsuki, S., and F. Murad. Regulation of adenosine cyclic 3′,5′-monophosphate and guanosine cyclic 3′,5′-monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.* **13**:330–341 (1977).
20. Parmley, W. W., and K. Chatterjee. Vasodilator therapy. *Curr. Probl. Cardiol.* **2**:1–75 (1978).
21. Kukovetz, W. R., S. Holzmann, A. Wurm, and B. Pöch. Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. *Naunyn-Schmiedebergs Arch. Pharmacol.* **310**:129–138 (1979).
22. Fisher, J., S. Scheidt, M. Collins, and J. A. Borer. Cardiogenic shock: pathophysiology and therapy. *Prog. Cardiol* **11**:163–195 (1982).
23. Ribner, H. S., D. Bresnahan, A.-M. Hsieh, R. Silverman, C. Tommaso, A. Coath, and J. Askenazi. Acute hemodynamic responses to vasodilator therapy in congestive heart failure. *Prog. Cardiovasc. Dis.* **25**:1–42 (1982).

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24. Lincoln, T. M. Effects of nitroprusside and 8-bromo-cyclic GMP on the contractile activity of the rat aorta. *J. Pharmacol. Exp. Ther.* **224**:100–107 (1983).
25. Armstrong, P. W., D. C. Walker, J. R. Burton, and J. O. Parker. Vasodilator therapy in acute myocardial infarction. A comparison of sodium nitroprusside and nitroglycerin. *Circulation* **52**:1118–1122 (1975).
26. Miller, R. R., L. A. Vismara, D. O. Williams, E. A. Amsterdam, and D. T. Mason. Pharmacological mechanisms for left ventricular unloading in clinical congestive heart failure. Differential effects of nitroprusside, phentolamine, and nitroglycerin on cardiac function and peripheral circulation. *Circ. Res.* **39**:127–133 (1976).
27. Miller, R. R., W. H. Fennell, J. B. Young, A. R. Palomo, and M. A. Quinones. Differential systemic arterial and venous actions and consequent cardiac effects of vasodilator drugs. *Prog. Cardiovasc. Dis.* **24**:353–374 (1982).
28. Katsuki, S., W. Arnold, C. Mittal, and F. Murad. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin, and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J. Cyclic Nucleotide Res.* **3**:23–35 (1977).
29. Mittal, C. K., and F. Murad. Properties and oxidative regulation of guanylate cyclase. *J. Cyclic Nucleotide Res.* **3**:381–391 (1977).
30. Gerzer, R., F. Hofmann, and G. Schultz. Purification of a soluble, sodium nitroprusside-stimulated guanylate cyclase from bovine lung. *Eur. J. Biochem.* **116**:479–486 (1981).
31. Levin, R. I., B. B. Weksler, and E. A. Jaffe. The interaction of sodium nitroprusside with human endothelial cells and platelets: nitroprusside and prostacyclin synergistically inhibit platelet functions. *Circulation* **66**:1299–1307 (1982).
32. Manganiello, V. C., and J. Breslow. Effects of prostaglandin E_1 and isoproterenol on cyclic AMP content of human fibroblasts modified by time and cell density in subculture. *Biochem. Biophys. Acta* **362**:509–520 (1974).
33. Jaffe, B. M., J. W. Smith, W. T. Newton, and C. W. Parker. Radioimmunoassay for prostaglandins. *Science (Wash. D. C.)* **171**:494–496 (1971).
34. Riss, T. L., N. L. Zorich, M. D. Williams, and A. Richardson. A comparison of the efficiency of nucleotide extraction by several procedures and the analysis of nucleotides from extracts of liver and isolated hepatocytes by HPLC. *J. Liquid Chromatogr.* **3**:133–158 (1980).
35. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
36. Jelsema, C. L., J. Moss, and V. C. Manganiello. Effect of bradykinin on prostaglandin production by human fibroblasts. *Methods Enzymol.* **109**:480–503 (1985).
37. Claesson, H.-E. Prostaglandin I_2 synthesis and elevation of cyclic AMP levels in 3T3 fibroblasts. *Biochim. Biophys. Acta* **618**:399–406 (1980).
38. Gorman, R. R., and N. K. Hopkins. Agonist-specific desensitization of PGI_2-stimulated cyclic AMP accumulation by PGE_1 in human foreskin fibroblasts. *Prostaglandins* **19**:2–15 (1980).
39. Goldyne, M. E., J. Å. Lindgren, H.-E. Claesson, and S. Hammarström. Endogenous synthesis of prostaglandins E_1 and I_2 in 3T3 fibroblasts transformed by polyoma virus. *Prostaglandins* **19**:155–163 (1980).
40. Gorman, R. R., R. D. Hamilton, and N. K. Hopkins. Stimulation of human foreskin fibroblasts adenosine 3′:5′-cyclic monophosphate levels by prostacyclin (prostaglandin I_2). *J. Biol. Chem.* **254**:1671–1676 (1979).
41. Hamberg, M., and B. Samuelson. Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. *Proc. Natl. Acad. Sci. USA* **71**:3400–3404 (1974).
42. Salmon, J. A. Inhibition of AA metabolism in leukotrienes and prostacyclin. *NATO Adv. Study Inst. Ser. A Life Sci.* **54**:142–167 (1981).
43. Kreye, V. A. Direct vasodilators with unknown modes of action: the nitrocompounds and hydralizine. *J. Cardiovasc. Pharmacol.* **6**:S646–655 (1984).
44. Vincentini, L. M., and M. L. Villereal. Serum, bradykinin and vasopressin stimulate release of inositol triphosphates from human fibroblasts. *Biochem. Biophys. Res. Commun.* **123**:663–670 (1984).
45. Yano, K., H. Higashida, R. Inoue, and Y. Nozawa. Bradykinin-induced rapid breakdown of phosphatidyl 4,5-bisphosphate in neuroblastoma × glioma hybrid NG108-15 cells. *J. Biol. Chem.* **259**:10201–10207 (1984).
46. Murayama, T., and M. Ui. Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts. *J. Biol. Chem.* **260**:7226–7233 (1985).
47. Shayman, J. A., and A. R. Morrison. Bradykinin-induced changes in phosphatidyl inositol turnover in cultured rabbit papillary collecting tubule cells. *J. Clin. Invest.* **76**:978–984 (1985).
48. Speziale, N., E. H. Speziale, and J. Pasquine. Bradykinin stimulates phospholipase C in rat renal medullary slices. *Biochim. Biophys. Acta* **836**:14–18 (1985).
49. Yano, K., H. Higashida, H. Hattori, and Y. Nozawa. Bradykinin-induced transient accumulation of inositol triphosphate in neuron like cell line NG108-15 cells. *FEBS Lett.* **181**:403–406 (1985).
50. Yano, K., H. Higashida, and Y. Nozawa. Evidence for a Ca^{++} independent hydrolysis of phosphatidyl 4,5-bisphosphate in neuron-like cell line NG108-15 cells. *FEBS Lett.* **183**:235–239 (1985).
51. Higashida, H., R. Streaty, W. Klee, and M. Nirenberg. Bradykinin-activated transmembrane signals are coupled via N_o or N_i to production of inositol 1,4,5-triphosphate, a second messenger in NG108-15 neuroblastoma × glioma hybrid cells. *Proc. Natl. Acad. Sci. USA* **83**:942–946 (1986).

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