Activation of Protein Formation and Cell Division by Bradykinin and Des-Arg⁹-bradykinin*

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We employed des-Arg⁹-bradykinin to investigate the relation between bradykinin-induced prostaglandin (PG) synthesis and bradykinin-induced protein accumulation. In this feedback control system, bradykinin-induced PG synthesis limits bradykinin-induced protein production. At low concentration (5 x 10⁻⁶ M), des-Arg⁹-bradykinin was significantly less active than bradykinin in stimulating the formation of prostaglandins by human fetal lung fibroblasts in culture. At high concentration (5 x 10⁻⁴ M), bradykinin induced a 24% increase in protein formation, while des-Arg⁹-bradykinin induced a 61% increase in collagen formation and an 80% increase in total protein accumulation. In the presence of indomethacin, bradykinin-induced protein formation was increased further, whereas des-Arg⁹-bradykinin-induced protein formation was unchanged. The bradykinin derivative increased the production of types I and III procollagens without affecting the distribution of procollagen types. The incorporation of [³H]thymidine into DNA in lung fibroblast cultures was increased 3-fold by des-Arg⁹-bradykinin alone or by bradykinin in combination with indomethacin. Des-Arg⁹-[Leu⁵]bradykinin inhibited the des-Arg⁹-bradykinin-induced protein formation and cell division; des-Arg⁹-bradykinin alone stimulates protein formation and cell division without activating PG synthesis and PG feedback control.

Bradykinin is generated during inflammatory reactions by the action of plasma kallikrein. The hormone stimulates prostaglandin synthesis by many cell types including fibroblasts and endothelial cells (1–3). These PGs' mediate many responses not all of the bradykinin-induced cellular responses. We determined previously that bradykinin-induced PG synthesis in fibroblast cultures was maximally stimulated when the bradykinin concentration was 5 x 10⁻⁸ M or greater (2). Bradykinin at 5 x 10⁻⁸ M also induced a slight increase in protein accumulation. This bradykinin-induced protein accumulation was increased further when bradykinin was used at higher concentrations. In addition, when bradykinin-induced PG synthesis was inhibited with indomethacin, the increase in protein formation induced by bradykinin was considerably higher. These data point to a feedback system in which PG synthesis limits the bradykinin-induced increase in protein production.

The effect of bradykinin in vivo may result in part from the generation of active metabolites. For example, cleavage of the C-terminal arginine residue results in the formation of the biologically active metabolite des-Arg⁹-bradykinin (4, 5). Although this metabolite is less active than bradykinin in stimulating PG synthesis by endothelial cells in culture (6), certain vascular tissues appear to have surface receptors which are particularly activated by exposure to des-Arg⁹-bradykinin (4, 5). Our data indicate that des-Arg⁹-bradykinin is less active than bradykinin in stimulating PG synthesis by lung fibroblasts in culture; however, this bradykinin derivative dramatically stimulates protein formation and DNA synthesis.

MATERIALS AND METHODS

Human embryonic lung fibroblasts (IMR-90, Institute for Medical Research, Camden, NJ) were grown in Dulbecco's modified Eagle's medium with 0.37 g of sodium bicarbonate/100 ml, 10% fetal bovine serum, 100 units of penicillin/ml, 10 μg of streptomycin/ml, and 0.1 mM nonessential amino acids. The cells were maintained in a humidified 5% CO₂, 95% air incubator at 37 °C. The cell cultures were grown to confluence in Falcon P-35 dishes (prostaglandin determinations) or in Falcon P-60 dishes (protein determinations). After confluence was reached, the cells were placed into the quiescent state by reducing the serum content of the medium to 0.4%. These cells remain viable and can be restimulated to divide by increasing the serum content of the culture medium (7).

Prostaglandin Determination—Prostaglandins are synthesized within cells and rapidly extruded into the extracellular space; they are not stored (8). Prostaglandin production was determined by direct radioimmunoassay of the culture medium; preliminary extraction was not necessary (9). Production of prostacyclin and thromboxane A₂ was determined by assay for their degradative products, 6-keto-PGF₁α and thromboxane B₂, respectively. Antibodies to PGE₂, 6-keto-PGF₁α, and thromboxane B₂ were developed and used in standard radioimmunoassay procedures (2, 10). The cross-reactivities of these antisera for the non-targeted PG did not exceed 4%.

Collagen and Total Protein Production—Collagen and total protein production was evaluated by determining the amount of nondialyzable (¹⁴C)hydroxyproline (collagen) and (¹⁴C)proline (total protein) present in the culture medium and cell layer. The culture medium was replaced with serum-free, proline-free medium containing ascorbate (50 μg/ml). The cells were incubated for 1 h at 37 °C. The medium was replaced with serum-free medium containing 1 μCi/ml of (¹⁴C)proline (specific activity, 290 μCi/mmol; Schwarz/Mann). After 24 h, the media and the cell layers were lyophylized. The samples were placed in 6 N HCl and hydrolyzed for 24 h in vacuo at 107 °C. The hydrolysates were analyzed for (¹⁴C)hydroxyproline and (¹⁴C)proline using a Beckman automatic amino acid analyzer fitted with a stream-splitting device and a fraction collector. The appropriate fractions were placed in scintillation fluid, and radioactivity was determined. To determine the nanomoles of hydroxyproline in the

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‡The abbreviations used are: PGs, prostaglandins; DAL, des-Arg⁹-[Leu⁵]bradykinin.
hydrolysat,e the amino acids were reacted with ninhydrin and assayed colorimetrically.

Polyacrylamide Gel Electrophoresis and Autoradiography—Confluent quiescent fibroblast cultures in the presence or absence of des-Ar^2-bradykinin (5 x 10^{-6} M) were labeled with [3H]arachidonic acid for 24 h. After the pulse-labeling period, a solution of protease inhibitors was immediately added to the pooled medium fractions to yield a final concentration of 10^{-5} M p-hydroxymercurobenzoate, 10^{-5} M phenyl-

methanesulfonfluryl fluoride, and 2 mM EDTA. The medium was then dialyzed against H_2O at 4 °C to remove free [3H]arachidonic acid and lyophilized. Polyacrylamide gel electrophoresis of medium fractions in the presence or absence of dithiothreitol was performed on a 5% gel (11). Autoradiography was performed according to the method of Bonner and Laskey (12).

Release of Arachidonic Acid and Its Metabolites—Confluent quiescent fibroblasts were maintained in medium containing 0.4% serum for 2 days. These cultures were then labeled with [3H]arachidonic acid (specific activity, 867.4 Ci/mmol; New England Nuclear). After 12 h, the medium was removed and the cultures were rinsed twice with serum-free medium. The cultures were then incubated for 30 min with serum-free medium containing 2 mg/ml of fatty acid-free bovine serum albumin with no other additions as controls. Additional cultures received either 5 x 10^{-6} M bradykinin or 5 x 10^{-6} M des-Ar^2-bradykinin. Radioactivity was determined in aliquots of the cultures received either after 12 h, and the final incubation medium. The difference between the initial counts/min of [3H]arachidonic acid present in the culture medium and that remaining after 12 h was used to calculate percentage of [3H]arachidonic acid incorporated by the cells. The percentage of incorporation was determined by dividing the counts/min released into the culture medium by counts/min of [3H]arachidonic acid incorporated into the cell layer.

DNA Synthesis—DNA synthesis was evaluated by measuring [3H]thymidine incorporation into a trichloroacetic acid-insoluble cell fraction. The culture medium was replaced with serum-free medium containing 0.1 Ci/ml of [methyl-3H]thymidine (specific activity, 51 Ci/mmol; Amersham Corp.). After 24 h, the medium was removed and the cells were washed twice with saline. The cell layer was then exposed to 1 ml of 1% Triton X-100 and harvested into glass tubes. The suspension was centrifuged following the addition of 20% trichloracetic acid. The trichloracetic acid precipitated material was solubilized by heating to 70 °C for 20 min, and radioactivity was determined by scintillation counting.

Statistics—Statistical evaluation of the data was carried out using a Student's t test for means of unequal size (13). Probability values <0.05 were considered significant.

RESULTS

The effects of des-Ar^2-bradykinin and bradykinin on PG production by lung fibroblasts are shown in Table I. Des-Ar^2-bradykinin was significantly more active than bradykinin in stimulating PG synthesis by lung fibroblasts in culture. At a concentration of 5 x 10^{-5} M, des-Ar^2-bradykinin stimulated a small increase in PG synthesis. This increase was significantly greater than the stimulated increase in controls to 11.41 nmol/flask (mean ± S.E., n = 4) in untreated cultures, indicating that PG synthesis was stimulated by bradykinin together with indomethacin resulted in a 51% increase in collagen formation and a 98% increase in total protein formation, confirming our previous results (2). In contrast, des-Ar^2-bradykinin alone at 5 x 10^{-6} M stimulated a 61% increase in collagen and an 80% increase in total protein accumulation. The presence of indomethacin did not affect the level of des-Ar^2-bradykinin-induced collagen or total protein formation.

To ensure that des-Ar^2-bradykinin-induced increase in [3H]hydroxyproline reflected an increase in collagen formation and not a change in amino acid pool size, we measured the radioactivities of nondialyzable hydroxyproline in the culture medium. The total hydroxyproline level increased from 8.34 ± 0.66 nmol/flask (mean ± 1 S.E., n = 4) in controls to 11.41 ± 0.30, p < 0.05, in des-Ar^2-bradykinin-treated cultures and 11.56 ± 0.52, p < 0.05, in des-Ar^2-bradykinin- and indomethacin-treated cultures.

The amount and distribution of procollagens present in the medium were determined by autoradiograms of polyacrylamide gels (Fig. 2). Autoradiograms also indicated that des-Ar^2-bradykinin induced a 2-fold increase in the total amount of procollagens synthesized.

Bradykinin and des-Ar^2-bradykinin were examined for their effects on cell division. This was determined by evaluating the incorporation of [3H]thymidine into DNA in fibroblast cultures (Fig. 3). In these cell cultures, bradykinin alone did not stimulate an increase in [3H]thymidine incorporation into DNA. However, when prostaglandin synthesis was inhibited by indomethacin, bradykinin induced a 3-fold increase in [3H]thymidine incorporation into DNA. In contrast, 5 x 10^{-6} M des-Ar^2-bradykinin alone stimulated a 3-fold increase in [3H]thymidine incorporation. The increase in cell division induced by des-Ar^2-bradykinin was not affected by the presence of indomethacin. In separate experiments, at 1 x 10^{-6} M, des-Ar^2-bradykinin induced maximal [3H]thymidine incorporation into DNA.

Des-Ar^2-[Leu^5]bradykinin competitively inhibits the effects of des-Ar^2-bradykinin on smooth muscle preparations (4). The effect of DAL on bradykinin-induced PG synthesis is shown in Table III. DAL alone, at 5 x 10^{-6} M, did not stimulate a detectable increase in PG synthesis, and the presence of DAL did not inhibit bradykinin-induced PG synthesis.

We assessed the effect of DAL on des-Ar^2-bradykinin-induced cell division (Fig. 4). The addition of DAL (5 x 10^{-6} M) resulted in an 80% decrease in the des-Ar^2-bradykinin-induced incorporation of [3H]thymidine into DNA. This decrease in [3H]thymidine incorporation into DNA was not affected by the presence or absence of indomethacin. DAL alone did not affect cell division; the incorporation of [3H] thymidine into the trichloroacetic acid-insoluble cell fraction was 280 ± 23 cpm (mean ± 1 S.E., n = 4) in untreated cultures and 311 ± 10 cpm in cultures treated with 5 x 10^{-6} M DAL.

Collagen and total protein production were not affected by DAL alone (Table IV). However, the addition of DAL resulted in a concentration-dependent decrease in des-Ar^2-bradykinin-induced collagen and total protein formation. The presence of DAL at 5 x 10^{-6} M inhibited des-Ar^2-bradykinin-induced stimulation of total protein formation. This inhibi-
Fatty acids. In order to evaluate this possibility, we examined the ability of des-Arg-bradykinin to activate collagen-related peptides stimulate protein accumulation through an optimal bradykinin-induced PG synthesis.

The cultures were labeled with [3H]arachidonic acid for 12 h. The medium was removed 30 min after the addition of bradykinin or des-Arg-bradykinin, and PG levels were determined. Data are expressed as mean ± 1 S.E. (n = 4).

| Additions                          | PGE₂ | 6-Keto-PGF₁α | PGF₂₀ | Thromboxane B₂ |
|-----------------------------------|------|--------------|-------|---------------|
| None (control)                    | <0.05| <0.05        | <0.05 | <0.05         |
| Des-Arg⁴-bradykinin               | 0.84 ± 0.06 | <0.05 | 0.17 ± 0.05 | 0.19 ± 0.16 |
| 5 × 10⁻⁶ M                        | 0.82 ± 0.06 | <0.05 | 0.95 ± 0.01 | <0.05        |
| Des-Arg⁴-bradykinin (5 × 10⁻⁴ M)  | <0.05| <0.05        | <0.05 | <0.05         |
| Bradykinin (5 × 10⁻⁶ M) and indomethacin | 0.84 ± 0.06 | <0.05 | 0.17 ± 0.05 | 0.19 ± 0.16 |
| Bradykinin (5 × 10⁻⁴ M) and indomethacin | 0.82 ± 0.06 | <0.05 | 0.95 ± 0.01 | <0.05        |

*Significantly different from control cultures (p < 0.05).

Discussion

In this report, we examine the hypothesis that bradykinin-related peptides stimulate protein accumulation through a cellular mechanism different from that involving PG production. It is known that bradykinin activates phospholipase A₂ and perhaps other lipases which in turn liberate arachidonic acid from lipid stores (1, 14, 15). The arachidonate is then converted to PGs by a series of enzymes. Our data (Tables I and II) and that of others (6) indicate that des-Arg⁴-bradykinin is significantly less active than bradykinin in stimulating the release of fatty acids and the synthesis of prostaglandins. The C-terminal arginine residue appears to be required for optimal bradykinin-induced PG synthesis.

Protein formation induced by bradykinin may well be mediated through a mechanism that does not involve the release of fatty acids. In order to evaluate this possibility, we examined the ability of des-Arg⁴-bradykinin to activate collagen and total protein formation by fibroblasts. Although des-Arg⁴-bradykinin (5 × 10⁻⁴ M) only minimally stimulated PG synthesis, this bradykinin metabolite induced an 80% increase in protein accumulation and a 61% increase in collagen formation; the peptide equally increased the production of both type I and type III procollagens.

We previously demonstrated (2) that bradykinin-induced protein formation was markedly increased by the presence of indomethacin (inhibition of PG synthesis). These data indicate that bradykinin-induced PG synthesis provides a feedback control system for protein accumulation, particularly collagen. In the present studies, we found that des-Arg⁴-bradykinin alone increased collagen and total protein accumulation to levels similar to that produced by the combination of bradykinin and indomethacin. Since des-Arg⁴-bradykinin only minimally stimulates PG synthesis, protein formation induced by des-Arg⁴-bradykinin was not limited by PG feedback control. Indeed, the presence of indomethacin did not affect the level of des-Arg⁴-bradykinin-induced collagen and total protein formation (Fig. 1).

The increase in total protein production induced by both bradykinin and des-Arg⁴-bradykinin was localized primarily in the cell layer fraction. This suggested that the increase in total production might be associated with an increase in cell division. We examined the effect of bradykinin and des-Arg⁴-bradykinin on cell division by evaluating the incorporation of [%H]thymidine into DNA (Fig. 3). Des-Arg⁴-bradykinin at 5 × 10⁻⁴ M stimulated a 5-fold increase in the incorporation of [%H]thymidine into DNA. In the presence of indomethacin, bradykinin itself stimulated a 3-fold increase in DNA synthesis. Other workers have reported that bradykinin has mitogenic properties when used at high concentrations. Whitfield and associates (16) indicated that bradykinin at 1 μM stimulated DNA synthesis in rat thymocyte cultures. Furthermore, Lys-bradykinin has been reported to induce DNA synthesis perhaps by stimulating Na⁺ influx (17).

PG synthesis induced by bradykinin appeared to inhibit the bradykinin-induced [%H]thymidine incorporation in DNA. Therefore, bradykinin-induced PG synthesis limits both protein production and cell division. Indeed, the addition of PGE₂ to fibroblast cultures is known to inhibit both protein formation and cell growth (18, 19). In contrast, des-Arg⁴-bradykinin at 5 × 10⁻⁴ M only minimally stimulates PG synthesis, and the presence of indomethacin did not affect des-Arg⁴-bradykinin.
Bradykinin- and Des-Arg9-bradykinin-induced Protein Formation

**Fig. 1.** Effect of bradykinin and des-Arg9-bradykinin on collagen and total protein accumulation by lung fibroblasts. Confluent fibroblast cultures were maintained in a medium containing 0.4% bovine fetal serum for 3 days. The medium was replaced with fresh serum-free medium containing \(^{14}C\)proline. Control cultures (C) received no additions. As indicated, additional cultures received \(5 \times 10^{-6}\) M indomethacin (INDO), \(5 \times 10^{-6}\) M bradykinin (BK), or \(5 \times 10^{-4}\) M des-Arg9-bradykinin (DA). After 24 h, the medium and cell layer were harvested, and nondialyzable \(^{14}C\)hydroxyproline and \(^{14}C\)proline were determined. Data are expressed as mean ± 1 S.E. (n = 4).

bradykinin-induced cell division and protein formation.

Des-Arg9-bradykinin is a octapeptide with a phenylalanine residue in the C-terminal position. Substitution of the terminal phenylalanine with a leucine residue forms the bradykinin analogue des-Arg9-[Leu9]bradykinin. This molecule did not stimulate total protein formation or cell division. However, the presence of DAL inhibited the des-Arg9-bradykinin-induced increase in protein formation and cell division (Table IV and Fig. 4). The dose-concentration relation for DAL-induced inhibition suggest that des-Arg9-bradykinin and DAL competitively bind to a membrane receptor.

Taken together, these results suggest the presence of two distinct systems activated by bradykinin. One system activated at \(5 \times 10^{-6}\) M generates the synthesis of PGs and requires the intact bradykinin molecule. A second system activated at higher concentration by both bradykinin and the bradykinin derivative des-Arg9-bradykinin stimulates protein formation and cell division. The activation of protein formation and cell division by bradykinin-related peptides may well be mediated through a different receptor than that involved with PG synthesis. This is suggested by the ability of des-Arg9-bradykinin to stimulate protein formation and cell division without stimulating the release of arachidonic acid.

**Fig. 2.** Autoradiograph of 5% polyacrylamide gel electrophoresis of lung fibroblast culture medium after limited pepsin digestion. Confluent quiescent cultures were pulsed with \(^{35}S\)proline for 24 h. Lanes 1 and 3 received medium samples from untreated cultures; lanes 2 and 4 received medium from des-Arg9-bradykinin (5 \(\times 10^{-6}\) M)-treated cultures. Lanes 1 and 2 were not reduced; lanes 3 and 4 were reduced with dithiothreitol.
Bradykinin- and Des-Arg\(^9\)-bradykinin-induced Protein Formation

**TABLE III**

*Effect of des-Arg\(^9\)-[Leu\(^8\)]bradykinin on bradykinin-induced stimulation of prostaglandin production*

All cells were maintained in Dulbecco's modified Eagle's medium containing 0.4% bovine fetal serum for 3 days. The medium was replaced with serum-free medium without additions as controls. Additional cultures received 5 × 10\(^{-8}\) M bradykinin (BK) or DAL as indicated. After 30 min, the medium was removed, and prostaglandin levels were determined. Data are expressed as mean ± 1 S.E. (n = 4). The PG levels in cultures treated with the combination of bradykinin and DAL were not significantly different (p > 0.05) from bradykinin alone.

| Additions            | PGE\(_2\)    | 6-Keto-PGF\(_{1\alpha}\) | PGF\(_{2\alpha}\) | Thromboxane B\(_2\) |
|----------------------|-------------|--------------------------|------------------|---------------------|
| None (control)       | 0.18 ± 0.04 | <0.05                    | <0.05            | <0.05              |
| BK (5 × 10\(^{-8}\) M) | 2.58 ± 0.23 | 0.58 ± 0.04              | 0.70 ± 0.11      | 1.88 ± 0.08        |
| DAL (5 × 10\(^{-8}\) M) | <0.05      | <0.05                    | <0.05            | <0.05              |
| BK + DAL (5 × 10\(^{-8}\) M) | 3.39 ± 0.11 | 0.66 ± 0.05              | 0.85 ± 0.09      | 2.33 ± 0.22        |
| BK + DAL (5 × 10\(^{-7}\) M) | 3.38 ± 0.19 | 0.63 ± 0.05              | 0.84 ± 0.05      | 2.43 ± 0.29        |
| BK + DAL (5 × 10\(^{-6}\) M) | 2.52 ± 0.20 | 0.69 ± 0.03              | 0.70 ± 0.03      | 1.65 ± 0.53        |

**TABLE IV**

*Effect of des-Arg\(^9\)-[Leu\(^8\)]bradykinin on nondialyzable \([^{14}C]\)hydroxyproline and \([^{14}C]\)proline levels in untreated and des-Arg\(^9\)-bradykinin-treated fibroblast cultures*

All cells were maintained in Dulbecco's modified Eagle's medium containing 0.4% bovine fetal serum for 3 days. The medium was removed and replaced with 1 ml of serum-free medium without additions as controls. Additional cultures received 5 × 10\(^{-8}\) M des-Arg\(^9\)-bradykinin or DAL. Other cultures received des-Arg\(^9\)-bradykinin (5 × 10\(^{-8}\) M) and DAL, at the indicated concentrations. After 24 h, the cultures were harvested and assayed for nondialyzable \([^{14}C]\) hydroxyproline and \([^{14}C]\)proline. Data are expressed as mean ± 1 S.E. (n = 4).

| Additions      | \([^{14}C]\)Hydroxyproline | \([^{14}C]\)Proline |
|----------------|---------------------------|-------------------|
| None (control) | 130 ± 5                   | 50 ± 1.3          |
| Des-Arg\(^9\)-bradykinin | 235 ± 14\(^b\)   | 108 ± 4.3\(^b\)   |
| DAL (5 × 10\(^{-8}\) M) | 109 ± 11                  | 47 ± 5.5          |
| Des-Arg\(^9\)-bradykinin and DAL (5 × 10\(^{-8}\) M) | 143 ± 8\(^b\)  | 48 ± 5.9          |
| DAL (5 × 10\(^{-7}\) M) | 152 ± 8\(^b\)   | 65 ± 2.9\(^b\)   |
| DAL (5 × 10\(^{-6}\) M) | 243 ± 18\(^b\)  | 113 ± 15.3\(^b\) |

\(^b\)Significantly different from untreated cultures (p < 0.05).

Moreover, DAL inhibited the increase in cell division and protein production by des-Arg\(^9\)-bradykinin without inhibiting the bradykinin-induced PG synthesis. However, DAL was less effective in inhibiting the increase in collagen and protein formation induced by bradykinin or the combination of bradykinin and indomethacin. Thus, it is not yet certain whether bradykinin and des-Arg\(^9\)-bradykinin activate a single receptor for cell division and protein accumulation.

Studies by others also indicate that bradykinin has multiple effects on the same tissue (4, 20). These actions may result from the activation of separate membrane receptors. For example, bradykinin and its metabolite des-Arg\(^9\)-bradykinin have a different pattern of activity on smooth muscle preparations. Bradykinin stimulates the contraction of guinea pig ileum, whereas des-Arg\(^9\)-bradykinin is more effective in stimulating the contraction of rabbit aorta. These data suggest that bradykinin has at least two membrane receptors. Receptor binding studies employing \([^{3}H]\)bradykinin indicate a dissociation constant of 5 × 10\(^{-8}\) M for membranes derived from the guinea pig ileum (21). The binding constant for des-Arg\(^9\)-bradykinin on isolated membranes is unknown. Studies of intact rabbit mesenteric vein indicate a binding constant of approximately 1–1.6 × 10\(^{-7}\) M (22, 23). The relation between these smooth muscle receptors and bradykinin-induced prostaglandin synthesis and protein production in cell culture is not known.

Similar to the intact bradykinin molecule, platelet-derived growth factor and certain lymphokines increase collagen production, cell division, and PG synthesis by fibroblasts in culture (24–26). These effectors are larger molecules than bradykinin. It is unclear whether these effectors also function through two or more activation systems.

Bradykinin is degraded to an inactive metabolite by the angiotensin-converting enzyme, which removes the C-terminal dipeptide Phe\(^8\)-Arg\(^8\) (4). This enzyme is present on the luminal surface of endothelial cells (27). A decrease in angiotensin-converting enzyme activity appears to occur in several animal models of fibrogenic lung injury, perhaps as a result of endothelial cell damage and tissue hypoxia (28–31). The
that results in cell proliferation and excess connective tissue accumulation in the lung. Our studies suggest that the presence of des-Arg⁹-bradykinin could contribute to the fibrotic process by stimulating cell division and collagen production without inducing PG synthesis.

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FIG. 5. The effect of des-Arg⁹-[Leu⁹]bradykinin on des-Arg⁹-bradykinin- and bradykinin-induced protein formation. Cultures were confluent and maintained in medium containing 0.4% bovine fetal serum for 2 days. The medium was replaced with fresh serum-free medium containing [¹⁴C]proline. Control cultures (C) received no additions. All effectors were added at 5 × 10⁻⁷ M. A, as indicated, cultures received des-Arg⁹-bradykinin (DA) alone or together with DAL and indomethacin (INDO). B, additional cultures received bradykinin (BK) alone or together with DAL and indomethacin. After 24 h, medium and cell layer were harvested, and nondiaryzable [¹⁴C]proline was determined. The data are expressed as mean ± 1 S.E. (n = 4). The asterisk denotes significant difference from bradykinin or des-Arg⁹-bradykinin alone (p < 0.05). The double asterisk denotes significant difference from bradykinin plus indomethacin or des-Arg⁹-bradykinin plus indomethacin (p < 0.001).