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Characterization of binding and receptors for epidermal growth factor in smooth muscle

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Summary. The mitogenic and differentiation-inducing activities of epidermal growth factor (EGF) in epithelial tissues have been well described. Since non-mitogenic effects of EGF, especially in mesenchymal tissues such as smooth muscle are not well-known (Nanney et al. 1984), we have examined EGF-binding and receptors in smooth muscle from many sites. Specific EGF binding sites were detected by incubating small pieces of tissue with ^{125}I-EGF; immunoreactive EGF receptors were detected by immunohistochemistry. In-situ localization of ^{125}I-EGF binding sites and immunoreactive EGF receptors of smooth muscle cells in intact mammalian tissues were identical using either ^{125}I-EGF autoradiography or anti-EGF receptor antibody in an immunoperoxidase method. Cultured rat aortic smooth muscle also contained specific EGF receptors as detected by their biological response to EGF-binding and internalization of ^{125}I-EGF, as well as EGF-stimulated phosphorylation of a 170K protein. The presence of EGF receptors in a well-differentiated smooth muscle cell indicates that EGF may play a physiological, but non-mitogenic role in mammalian tissues in vivo.

Key words: Epidermal growth factor – Smooth muscle – Receptors – Tissue culture (man, rat)

Epidermal growth factor (EGF), a well-defined mitogenic peptide (Stoscheck and King 1986a, b), stimulates growth and differentiation of a wide variety of cells (Carpenter 1981; King and Carpenter 1983). Whether or not a cell responds to EGF is dependent upon the presence of a membrane-bound receptor which has integral tyrosine kinase activity (Buhrow et al. 1982; Cohen et al. 1982; Sefton et al. 1980). Not all cells respond to an EGF stimulus by initiating DNA synthesis. For example, EGF enhances production of chorionic gonadotropin and progesterone in choriocarcinoma cells (Bahn et al. 1980; Beneviste et al. 1978; Huot et al. 1981; Lai and Guyda 1984); a similar role for EGF has been postulated in placental syncytiotrophoblast cells (Huot et al. 1981; Lai and Guyda 1984; Magid et al. 1986). Other cells respond to an EGF stimulus by transporting EGF across epithelial surfaces. Accordingly, high levels of EGF are found in sweat, milk, and urine (Starkey and Orth 1977), and EGF receptors (EGF-R) have been morphologically demonstrated on eccrine sweat ductal cells (Nanney et al. 1984a).

Our interest in investigating the distribution of EGF-R on smooth muscle cells (SMC) began with our initial observation that high levels of EGF-R were present on smooth muscle cells SMC in human skin (Nanney et al. 1984a, c). SMC in-vivo rarely undergo mitosis except during tissue repair and atherosclerosis (Björkerud 1979, 1985; Chamley-Campbell et al. 1981; Ross and Kariya 1980). Thus, the role of EGF in these normal, adult cell populations is unclear.

In this report we show that EGF receptors in SMC occur in several tissues, including the coronary arteries. The level of immunoreactive EGF receptors and EGF-binding sites in SMC varies at different sites and may reflect the differences in tissue or cellular metabolic activity. Finally, we show that the response to EGF of the EGF receptor in SMC appears biologically similar to that seen in other tissues.

Materials and methods

Reagents

EGF was purchased from Collaborative Research (Waltham, MA), and ^{125}I and ^{32}P-ATP were obtained from New England Nuclear (Boston, MA). NTB-2 autoradiography emulsion was obtained from Eastman Kodak (Rochester, NY). Goat antirabbit IgG conjugated to horseradish peroxidase (HRP) was purchased from Miles Laboratories (Elkhart, IN). The supply of 3,3-diaminobenzidine (DAB) and bovine serum albumin (BSA) was from Sigma Chemical Co. (St. Louis, MO), and 4-(2-hydroxyethyl)-1-piperazine-ethane sulfonic acid (HEPES) was from Calbiochem (San Diego, CA). Calf serum and Dulbecco's modified Eagle's medium (DMEM) and Hanks' balanced salt solution (HBSS) were purchased from GIBCO (Grand Island, NY). For 3-step immunohistochemistry, PAP kits were purchased from Dako Corporation (Santa Barbara, CA).

Acquisition of tissues

Normal muscle specimens from various body regions were collected 1–6 h after post-mortem examinations at Vanderbilt University or Veterans Administration Medical Centers in Nashville, TN. Smooth muscle cells from rat aorta were kindly supplied by Dr. Tadashi Inagami.

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Localization of ^{125}I-EGF binding by autoradiography

EGF was labeled with ^{125}I by the chloramine-T method, as previously described by Carpenter and Cohen (1976), to a specific activity of 1.5×10^8 counts min/μg. The ^{125}I-EGF-bound to A-431 cells and fibroblasts in a concentration-dependent, saturable, and reversible manner (Carpenter and Cohen 1976; Comens et al. 1982). Excess unlabeled (2 μg) EGF was added after ^{125}I-EGF-binding to show the extent of nonspecific or nondisplaceable ^{125}I-EGF.

Localization of ^{125}I-EGF-binding sites by autoradiography in sections of human muscle was performed as previously described. In brief, muscle samples were sliced into very small pieces (1 mm^2). Samples were incubated for 90 min at 25° C with shaking in a medium consisting of 0.5 ml HBSS containing 1 mg/ml BSA, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4 (binding buffer), and 10 ng/ml ^{125}I-EGF. Unbound EGF was removed by repeated washes in the binding buffer at 25° C. All skin samples were fixed for 4 h in 10% neutral buffered formalin and processed for light microscopy in paraffin. Sections (6 μM) were dipped in a 1:1 mixture of NTB-2 emulsion and distilled water. Following exposure at 4° C for 4–12 weeks, the emulsion was developed for 2 min in Kodak D-19 developer at 10° C. Sections were stained with hematoxylin and eosin and mounted in Permount. Photographs were made using a Nikon microscope with both bright- and dark-field illumination.

EGF receptor antiserum – production and characterization

The affinity-purified EGF receptor preparation, for immunization of rabbits to produce a specific antiserum to the receptor, was prepared as previously described (Stoscheck and Carpenter 1983a, b). Briefly, the EGF receptors from A-431 human epithelioid carcinoma membrane vesicles were solubilized and placed on an EGF affinity column. The receptor was eluted with 5 mM ethanolamine, pH 9.7, containing 10% glycerol and 0.2% Triton X-100. Approximately 5 μg of receptor protein was injected into the rabbit in complete Freund's adjuvant followed 1 month later by an additional 5 μg in incomplete Freund's adjuvant. One week after the latter injection, antiserum collection was begun. The antiserum (No 451) cross-reacted and immunoprecipitated native occupied or unoccupied EGF receptors from a number of normal tissues, including normal human skin (Nanney et al. 1984a, b, 1986) and placenta (Magid et al. 1986). The antisera also cross-reacted with precursor and intracellular degradation forms of the EGF receptor (Stoscheck and Carpenter 1983a, b).

Localization of immunoreactive EGF receptor molecules

Immunoreactive EGF receptor molecules were localized utilizing either 2- or 3-step immunoperoxidase staining. For the 2-step procedure, cryostat-sectioned tissue sections (4–6 μm) were fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS), pH 7.4, for 15 min at 25° C. The sections were rinsed and then incubated 15 min with 3% goat serum and 1 mg/ml glycine in PBS. Sections were treated for 4 h at 25° C with either purified normal rabbit serum or anti-EGF receptor serum diluted 1:200 in goat serum/glycine/PBS. After extensive washing, the sections were incubated 1 h with goat anti-rabbit IgG conjugated to horseradish peroxidase diluted to 1:100 in goat serum/glycine/PBS. After rinsing, tissue sections were reacted with 0.05% diaminobenzidine (DAB) and 0.01% H_2O_2 in 0.05 Tris buffer at pH 7.6.

For the 3-step immunoperoxidase procedure, tissues were routinely fixed in 10% neutral buffered formalin and embedded in paraffin at temperatures not greater than 56° C. Sections (7 μm) were incubated according to the instructions supplied with the Dako PAP kit. The primary antibody (anti-EGF receptor antibody) was applied for 4 h at a dilution of 1:200 in goat serum/glycine/PBS. After the 3-step incubations in antibodies, the sections were reacted for 45 min in the 9-amino-9-ethylcarbazole (AEC) supplied in the kit. Sections were briefly counterstained for 1 min in Mayer's hematoxylin and coverslipped in Dako Glycerogel. Sections were photographed the next day before fading of the red AEC chromagen occurred.

EGF-stimulated phosphorylation

Cultured smooth muscle cells from rats were scraped from culture dishes in ice-cold PBS and pelleted by centrifugation at $8000 \times g$ for 5 min. The cells were then homogenized in a 10 × volume of 3 mM ethylene glycol bis(β-aminoethyl ether)N,N,N,N tetra-acetic acid (EGTA), 3 mM iodoacetic acid in 20 mM HEPES buffer, pH 7.4, using a ground glass homogenizer. Nuclei and unbroken cells were precipitated by centrifugation at $600 \times g$ for 5 min. The membrane fraction of the supernatants was precipitated by centrifugation at $100000 \times g$ for 1 h. The pellet was resuspended in 20 mM HEPES buffer, pH 7.4. Monolayers of A-431 cells were washed 3 times with PBS, scraped and homogenized in the homogenization buffer. The membrane fraction of the supernatant was precipitated and suspended as described above.

The phosphorylation assay mixture contained 75 μg smooth muscle cell membranes or 15 μg A-431 membranes, 0.1% Triton X-100, 2 mM $MnCl_2$, 10 μM o-vanadate, 0.25 mg/ml BSA, 1 μCi [γ-32]ATP (5 Ci/μmol) (ICN, Irvine, CA) in 20 mM Hepes, pH 7.4, in the absence or presence of 1.0 ng/μl EGF in a total volume of 60 μl. The reaction proceeded for 10 min at 0° C and was stopped by the addition of 60 μl of 2 × Laemmli sample buffer (King and Carpenter 1983). After heating the samples to 95° C for 5 min, they were placed on a 6% polyacrylamide gel, electrophoresed, and the dried gel placed against a sheet of Kodak X-O Omat AR film. The film was exposed at −70° C with a Dupont Lightening Plus screen for 1 day.

Results

Autoradiography of ^{125}I-EGF binding sites

To determine whether the presence of silver grains represented the binding of ^{125}I-EGF to its receptor (EGF-R), the following control experiments were performed as previously described for human skin (Nanney et al. 1984a, b, 1986). A 100-fold excess of unlabeled EGF was added to the ^{125}I-EGF incubation media. In other controls, reversibility of binding was evaluated by adding excess unlabeled EGF to the incubation media after the tissue had been incubated with ^{125}I-EGF. The number of silver grains detected by autoradiography in the tissue sections under the conditions used was considered to be nonspecific binding or background radioactivity (and was always less than 5%).

Since the EGF autoradiography techniques were originally tested in human skin (Nanney et al. 1984a, b, 1986), we included skin specimens in each autoradiography experiment to serve as positive and negative control specimens.

To evaluate the significance of the presence of EGF receptors in smooth muscle, we determined the morphological distribution of EGF-binding/receptors in smooth muscle from different tissues in human and other mammalian species. Representative samples of ^{125}I-EGF-binding studies are shown in Fig. 1A–H. The silver grains indicating the sites of ^{125}I-EGF binding were localized mostly over the smooth muscle regions of human urinary bladder and not in the adjacent connective tissue regions (Fig. 1A, B). A specific or non-random distribution of silver grains was detected overlying the smooth muscle cells of the bladder wall. By contrast, the number of silver grains detected over the adjacent submucosal or adventitial regions of the bladder was negligible (data not shown).

^{125}I-EGF-binding sites were readily visualized over smooth muscle cells in human skin. A representative view of the arrector pili muscle from adult human skin is shown in Fig. 1C and D. The amount of ^{125}I-EGF-binding by these smooth muscle cells, which are a functional portion of the pilosebaceous apparatus, is much higher than in adjacent fibroblasts. Specific binding of ^{125}I-EGF to cells in the muscular layer of a small dermal artery is shown in Fig. 1E and F. The number of silver grains is highest over the tunica media and a decreased number is detectable over the tunica adventitia and adjacent dermal connective tissue. The sensitivity of the autoradiographic method or the level of ^{125}I-EGF-binding did not allow us to determine if SMC associated with dermal veins had specific EGF-binding sites. Also of interest was the observation that the adjacent, fully-differentiated skeletal muscle lying underneath the adult human skin did not contain detectable levels of ^{125}I-EGF binding sites or immunoreactive EGF-R (data not shown).

Smooth muscle cells from arteries from different anatomical sites had varying levels of detectable ^{125}I-EGF-binding. Porcine coronary arteries from the heart muscle of Sinclair mini-pigs had a high concentration of ^{125}I-EGF binding sites over the smooth muscle cells of the tunica media (Fig. 1G, H). However, the tunica adventitia and intima only had a small number of ^{125}I-EGF-binding sites which were appreciably different from the adjacent tissue. In human aorta specimens, the concentration of ^{125}I-EGF-binding sites associated with SMC was much lower than the coronary arteries and only slightly higher than background. Little or no staining of SMC in the ductus deferens of the male reproductive tract was observed (data not shown). These findings may be a reflection of the cellular and/or metabolic heterogeneity of the SMC from different locations in the vascular system (Björkerud 1985).

Localization of immunoreactive EGF receptors

Anti-EGF receptor properties. The polyclonal antiserum against the human EGF receptor was produced in a rabbit using affinity-purified EGF receptor (Stoscheck and Carpenter 1983b). As previously described (Stoscheck and Carpenter 1983a, b), this antiserum specifically immunoprecipitates both the Mr = 170000 and Mr = 150000 forms of the EGF receptor as well as the intracellular precursor form of EGF receptor, but not ^{125}I-EGF. The anti-EGF-R antibody blocked ^{125}I-EGF-binding to EGF-R in human, rat, and mouse, but not rabbit tissues (Stoscheck and Carpenter 1984a). However, most importantly, ^{125}I-EGF-binding to unoccupied EGF-R did not prevent binding of and immunoprecipitation by the anti-EGF-R antibody.

Control incubations. To ascertain whether the presence of either a brown DAB reaction product or red AEC precipitate represented the location of immunoreactive EGF-R, both positive and negative control experiments were conducted. As negative controls, the primary antiserum was replaced with PBS to determine the endogenous background of nonspecific reaction product (data not shown), or the antiserum was replaced with normal rabbit serum (Fig. 2B, D). In some cases, some human IgG deposition was detected in clinically normal skin. However, this IgG deposition produced only a faint reaction product compared to the marked precipitate noted when anti-EGF-R antibody was used. In a few experiments in which antiserum preadsorbed to A-431 membranes that contain a large number of EGF receptors (Fabricant et al. 1977) was used, only a very slight reaction product was detected. As a positive control, a section of skin known to contain immunoreactive EGF receptors was included in each experiment.

Results of the EGF-R localization in human smooth muscle tissues are shown in Fig. 2A and C. The immunoperoxidase method showed that immunoreactive EGF-R were present in all of the SMC of the stomach (Fig. 2A). Intense staining was seen in the inner muscularis mucosae and the outer tunica muscularis layers of the stomach as well as the tunica media of arteries and veins within the wall of the stomach. Note that the staining of endothelial cells in the artery is less intense. Little staining is observed in the adventitia (Fig. 2A, B). In addition to staining of SMC sites of the stomach, other cells stain as well. The mucosal cells of gastric glands stains intensely, especially around the lumen.

When immunoreactive EGF-R were localized in human axillary skin, 3 different forms of smooth muscle had specific reaction product, indicative of EGF-R. Myoepithelial cells which surround the secretory tubules of apocrine sweat glands were intensely stained (Fig. 2C), as were the SMC in small dermal arteries (Nanney et al. 1984a) and SMC of arrector pili structures (Nanney et al. 1984a, c). Immunoreactive EGF-R was also be detected in SMC in dermal arteries (Nanney et al. 1984a), gastric arteries and veins (Fig. 2A), and placental arteries (Magid et al. 1986) and veins (data not shown). EGF-R was also localized autoradiographically by ^{125}I-EGF-binding in coronary arteries (Fig. 1G and H) and dermal arteries (Fig. 1E and F). The presence of EGF-R on SMC in the cardiovascular system was a consistent finding using either the autoradiographic or immunohistochemical methods. However, EGF-R levels are not equal in SMC throughout the cardiovascular system; e.g., adult human aorta has very low levels of EGF-R (data not shown). Either EGF-R are not present in human aorta (5 specimens) or the receptor numbers are too low to be detected with the 3-step immunoperoxidase method.

Biochemical characterization of ^{125}I-EGF-binding

In these studies, ^{125}I-EGF-binding was measured in confluent cultures of rat aortic SMC, and the dose-response

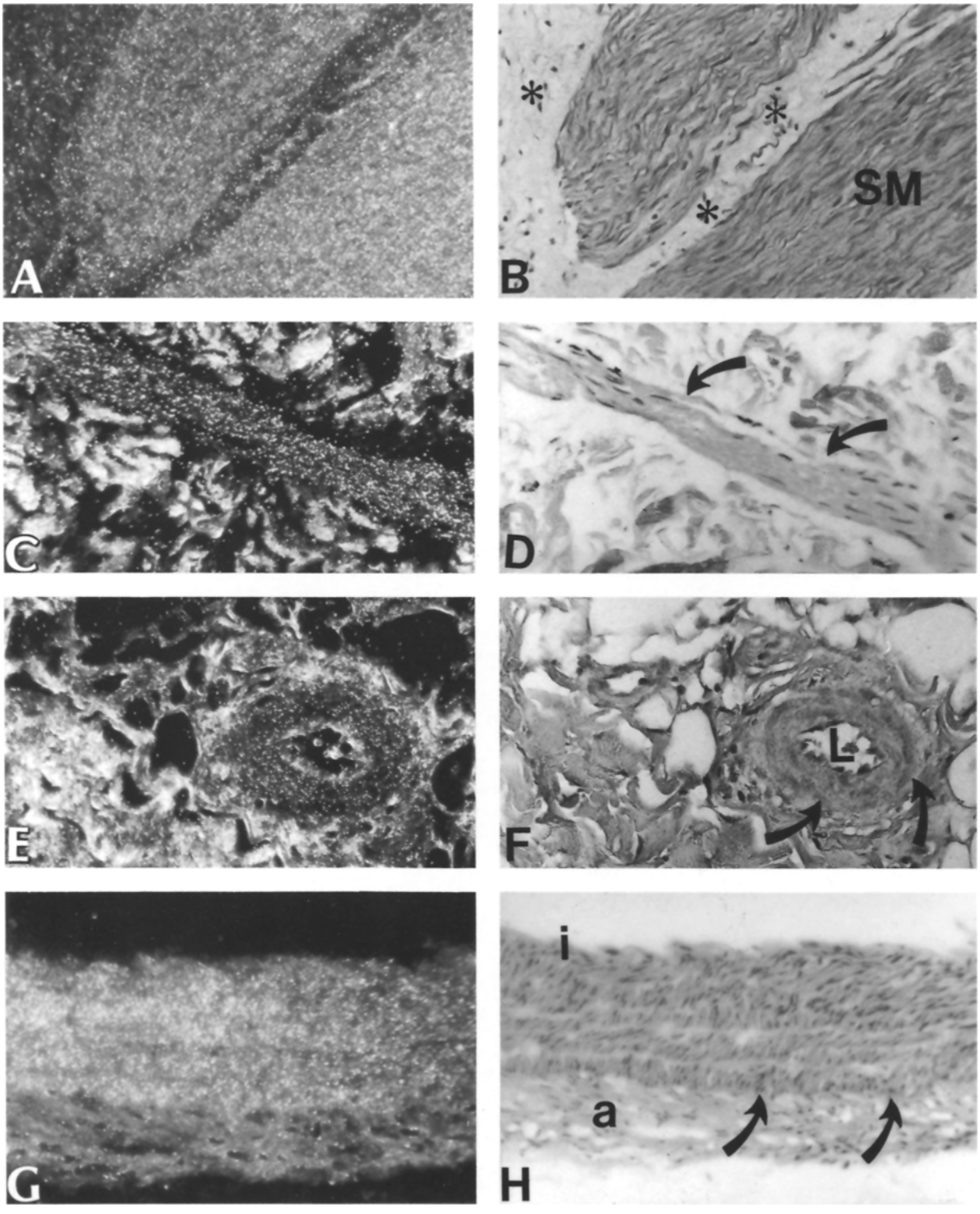

Fig. 1 A–H. Autoradiography of various smooth muscle tissues. Tissues incubated in ^{125}I-EGF and processed for autoradiography, as described in Materials and methods

Human urinary bladder (**A** dark field, **B** bright field) has silver grains indicative of EGF binding over circular smooth muscle (*SM*) with minimal density over intervening connective tissue (*). × 160

Human arrector pili muscle (**C** dark field, **D** bright field) has silver grains over longitudinal smooth muscle (*arrows*) and not over adjacent dermal connective tissue. × 100

Small, muscular artery from human skin (**E** dark field, **F** bright field). In this cross-sectional view, silver grains are dense over smooth muscle (*arrows*) and sparse over lumen (*L*) and surrounding tissue. × 250

Porcine coronary artery (**G** dark field, **H** bright field) shows silver grains are dense over smooth muscle of tunica media (*arrows*) and less dense over intimal (*i*) and adventitial (*a*) regions. × 150

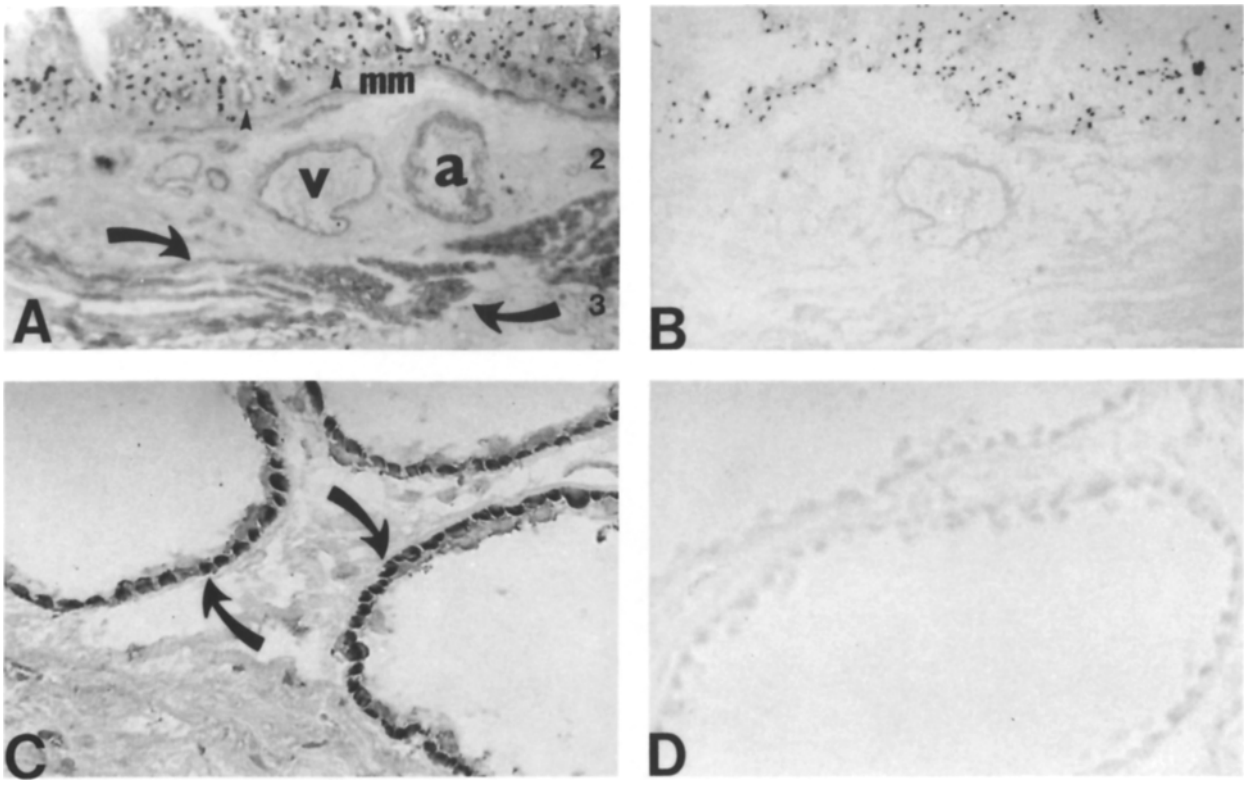

Fig. 2. Immunoperoxidase staining of human muscle tissues. Formalin-fixed, paraffin-embedded tissues sectioned, treated with anti-EGF-R antibodies, incubated with either secondary or tertiary antibodies labeled with peroxidase, and treated with chromagen (see Materials and methods). **A** In human stomach, reaction product indicative of immunoreactive EGF-R is seen over smooth muscle in tunica muscularis (*large arrows*), muscularis mucosae (*mm*), and smooth muscle of artery (*a*) and vein (*v*) in submucosa (*2*). Due to autolysis of autopsy material, presence of EGF-R in mucosal cells (*1*) cannot be determined. However, gastric glands (*small arrows*) are stained above background levels. Bacteria (black specks) are seen in mucosa of both experimental and control sections. Little staining is observed in adventitia (*3*). ×315. **B** Matching IgG control section shows faint reaction product over all regions except occasional mucosal cell that has endogenous peroxidase activity. ×315. **C** Human apocrine sweat gland shows immunoreactive EGF-R present on myoepithelial cells (*arrows*). Faint reaction product present over severely disrupted secretory epithelial cells. ×500. **D** Matching IgG control; no DAB precipitate is seen in myoepithelial cells, secretory epithelium, or adjacent dermis. ×500

curve of ^{125}I-EGF-binding to these rat aortic SMC is shown in Fig. 3. Saturation of EGF binding to the receptor was observed at an EGF concentration of 10 to 20 ng/ml, depending on time of incubation. The level of EGF required for saturation at 4 h was somewhat lower than that required at 30 min. The optimal dosage appeared to be 10 ng/ml of EGF.

In rat aortic SMC, ^{125}I-EGF-binding rapidly reached a maximum at 30 min, after which it declined, presumably due to down-regulation (Fig. 4). Adding chloroquine to the media inhibited the decline of cellular-bound ^{125}I-EGF, implying that the lysosomal compartment may be involved in EGF-R down-regulation, as it is in fibroblasts and A-431 cells (Carpenter and Cohen 1976).

A Scatchard plot of ^{125}I-EGF-binding to rat aortic SMC indicates that cultured SMC bind ^{125}I-EGF in a concentration-dependent fashion with a single affinity site (Fig. 5), and individual muscle cells have approximately 15000 receptors on the cell surface. As previously observed in cultures, these rat aortic SMC are stimulated to proliferate by EGF. A two-fold increase in cell numbers (as measured by a Coulter Counter) was observed in 3 days when 10 ng/ml EGF was added to these rat aortic SMC growing in Dulbecco's modified Eagle's medium containing 10% calf serum.

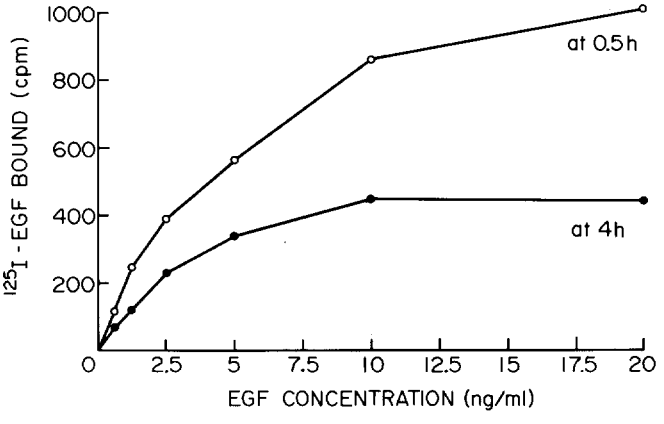

Fig. 3. Dose response curve of ^{125}I-EGF-binding to cultured smooth muscle cells of rat aorta grown to confluence and incubated in ^{125}I-EGF. Binding measured as described in Materials and methods

EGF-stimulated phosphorylation of smooth muscle cells

To show that the immunoreactive EGF receptor and the ^{125}I-EGF-binding activity in mammalian smooth muscle cells is similar to that observed in other tissues, the EGF-

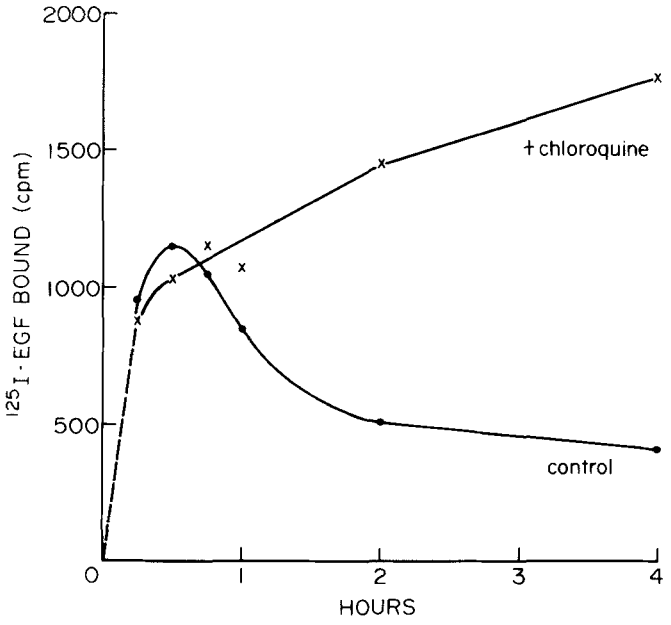

Fig. 4. Time course of ^{125}I-EGF-binding to cultured smooth muscle cells. Rat aortic smooth muscle cultured to confluence, incubated in ^{125}I-EGF and binding measured as described in Materials and methods. Rat smooth muscle cells rapidly bind ^{125}I-EGF, with binding peak at 30 min, after which down-regulation occurs. Down-regulation is inhibited by chloroquine

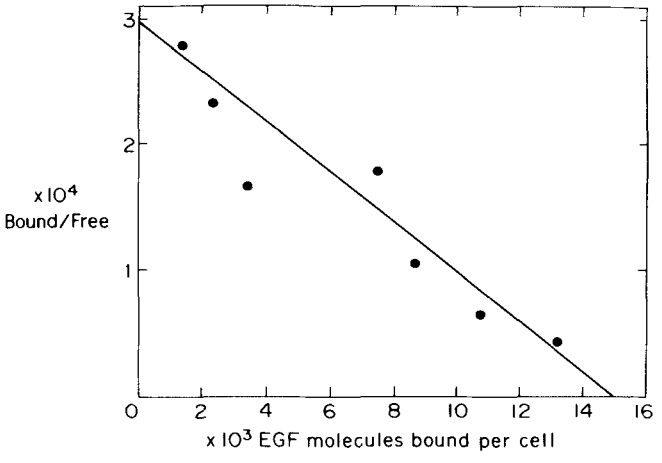

Fig. 5. Scatchard plot of ^{125}I-EGF-binding to rat aortic smooth muscle cells cultured to confluence, and incubated in ^{125}I-EGF. Binding performed as described in Materials and methods. X *axis* shows number of molecules of EGF bound per cell. Y *axis* shows product of the number of molecules bound over those free in medium

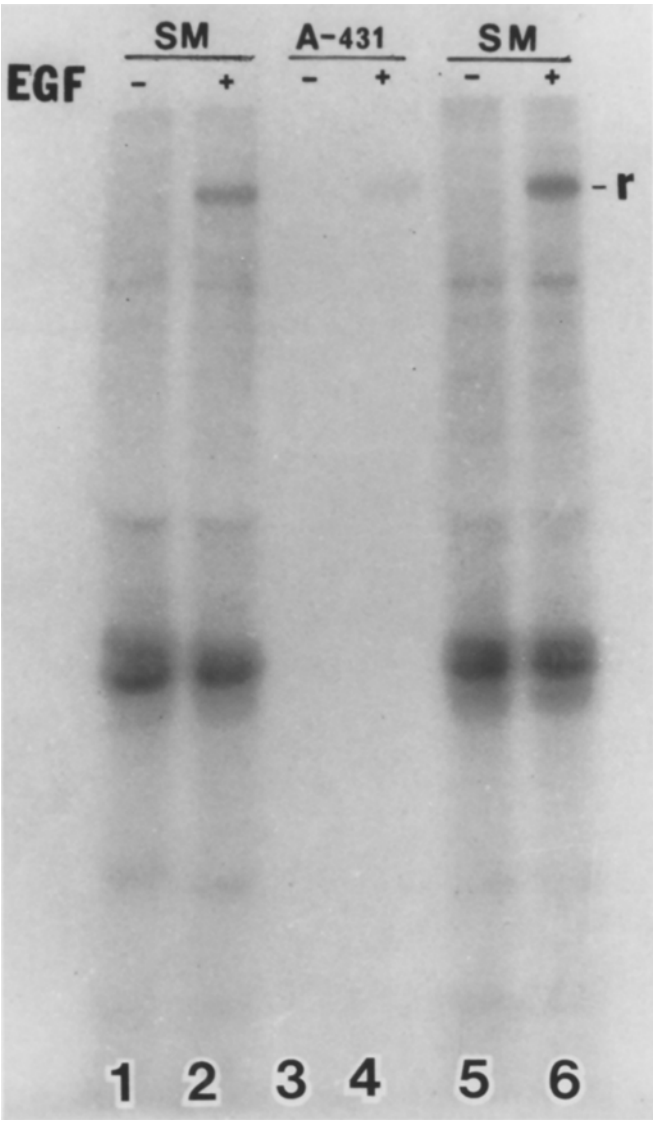

Fig. 6. EGF stimulation of smooth muscle protein phosphorylation. In-vitro phosphorylation experiment performed on rat aortic smooth muscle cell membranes (*SM*) either in the presence (+) or absence (−) of EGF. A-431 cell membranes (*lanes 3 and 4*) served as positive controls. Smooth muscle membranes (*SM*) in lines *5 and 6* contained 0.15% Triton X-100 to determine whether membrane solubilization altered distribution of phosphorylated proteins. EGF increased phosphorylation of 170 K band designated (*r*) in lanes *2 and 6* which co-migrated with phosphorylated EGF receptor material from A-431 cells (*lane 4*)

stimulated autophosphorylating activity of SMC membranes (66 μg) and A-431 cells (1 μg) was measured (Fig. 6). After phosphorylation with [γ-^{32}P]ATP in the presence or absence of EGF, the membranes were solubilized, subjected to electrophoresis on a polyacrylamide gel as previously described (King et al. 1980). In rat aortic SMC as in other tissues (Fernandez-Pol 1981; King et al. 1980; Stoscheck and King 1986b), the major phosphorylated band in membranes incubated with EGF had a Mr of 170000 daltons. The well-characterized EGF-R in A-431 membranes (King et al. 1980; Stoscheck and King 1986b), when phosphorylated under the same conditions, migrated with the same Mr as the phosphorylated protein in rat aortic SMC membranes (data not shown). Under the conditions used, EGF did not affect the phosphorylation of other membrane proteins in the extracts from normal rat aortic SMC (Fig. 6).

Discussion

The present study indicates that in-vivo mammalian smooth muscle tissues commonly express EGF receptors. This conclusion is based on the data from 2 entirely different mor-

phological approaches. These data are supported by in-vitro biochemical studies of EGF and EGF-R in rat aortic SMC. Using light-microscopic autoradiography, ^{125}I-EGF-binding to unoccupied, accessible EGF receptors was localized in smooth muscle tissue sites from several different body regions. In immunoreactive studies, EGF receptors were detected with an anti-EGF receptor antibody which recognizes both occupied and unoccupied receptors, precursor and degradation forms of the receptor (Stoscheck and Carpenter 1983a, b), thereby measuring the entire pool of extracellular and intracellular receptors. These 2 entirely different techniques were useful to determine if the localizations observed were valid or due to technical artefacts.

In cultured rat aortic SMC the biochemical data indicate that functional EGF receptors are present in SMC in-vitro. Binding data indicate that ^{125}I-EGF specifically binds to the rat aortic SMC membrane receptors and, like fibroblasts, the metabolism of rat aortic SMC EGF-R is regulated by the addition of EGF. Although EGF receptors are present in the rat aortic SMC, the Scatchard plot indicates that there are only approximately 14000 per cell in vitro. This is considerably lower than the numbers of EGF-R observed in hepatocytes (600000) (Dunn et al. 1986), fibroblasts (40000–100000) (Carpenter and Cohen 1976) and A-431 cells (1–4 million) (Wrann and Fox 1979). Interestingly, we did not observe either high levels of ^{125}I-EGF binding or immunoreactive EGF-R from fibroblasts (Nanney et al. 1984a, 1986) or glial cells (data not shown) in vivo. Thus, in vitro growth of these cells may induce a change in number of EGF-R expressed in vivo.

Whether these ^{125}I-EGF-binding sites and immunoreactive EGF receptors, detected in adult mammalian SMC in the present study, represent biologically active EGF receptor with its associated tyrosyl kinase activity is not known. Although indirect, the finding that EGF stimulated the phosphorylation of a 170000 MW protein in rat aortic SMC grown in-vitro indicates that active EGF-R are present in SMC. There appear to be no detectable differences between the EGF-R in these rat aortic SMC and those detected in other cell types (Buhrow et al. 1982; Cohen et al. 1982; Dunn et al. 1986; Fernandez-Pol 1981).

While it is unclear what roles EGF plays in SMC in-vivo, the presence of EGF-R in SMC from several body sites suggests that EGF-R are functionally important. Since EGF stimulates cell division of SMC in-vitro (Bhargava et al. 1979; Clemmons 1984; Gospodarowicz et al. 1981; Owen 1985), why these SMC with EGF-R do not divide rapidly in-vivo is unclear. One possibility is that EGF is normally not accessible to these cells. However, during wound repair, EGF may be released from circulating activated or aggregating platelets (Oka and Orth 1983) and stimulate SMC to divide. Indeed, the proliferative- and/or cholesterol-accumulating capabilities of the SMC have been implicated in the pathogenesis of atherosclerosis (Buhrow et al. 1982; Chamley-Campbell et al. 1981; Ross and Kariya 1980; Kruth 1985).

EGF may play a role in muscle contraction. EGF stimulates tyrosine phosphorylation of myosin light-chain in smooth muscle in-vitro (Gallis et al. 1983). Although it is not clear whether EGF induces myosin phosphorylation in-vivo, EGF causes morphological changes such as ruffling in cultured cells (Chinkers et al. 1981). Modulation of $Na^+/K^+/Cl^-$ and Na^+/H^+ co-transport (Owen 1984, 1985) by EGF may also affect contraction. A direct stimulation of smooth muscle contraction was observed in rat aortic strips (Berk et al. 1985); however, it is not clear why extremely high levels (144 mg/ml) were necessary to obtain a response. EGF released during wounding may play a role in vasoconstriction, since EGF has been shown to cause the contraction of smooth muscle cells (Berk et al. 1985; Muramatsu et al. 1985). EGF could also have an insulin-like function in SMC. EGF has been shown previously to stimulate glucose-transport and lactic acid production in other cell types (Barns and Colowick 1976; Diamond et al. 1978). Thus metabolically active cells might be expected to have high levels of EGF-R.

In summary, studies using light-microscopic autoradiography with ^{125}I-EGF and immunohistochemistry with anti-EGF receptor antibody showed EGF-R in SMC from most tissues. ^{125}I-EGF binding and autophosphorylation studies indicate that EGF-R in cultured SMC have the same characteristics as observed in other cell types. Since EGF-R are detectable on SMC in-vivo, the question of the physiological roles of EGF and EGF-R other than their mitogenic effects is intriguing, but unanswered.

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