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Physiology

Hemodynamic effects of epidermal growth factor in conscious rats and monkeys

(vasoactive hormones/hypotension)

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ABSTRACT **The therapeutic application of growth factors to human disease has become closer to reality with the advent of faster means of synthesizing these molecules and novel drug delivery strategies. Epidermal growth factor (EGF) belongs to a large family of molecules with the ability to modulate growth. Purified extracts of EGF have been used clinically to modulate gastrointestinal secretion of hormones and accelerate healing. EGF is also reported to have both vascular smooth muscle contractile and relaxing activity. Cardiovascular studies were performed with the bioactive 48-amino acid fragment of human EGF in rodents and primates to determine the effects of EGF on blood pressure and heart rate in conscious animals. Intravenous infusion of EGF induced an initial pressor response in rats followed by a prolonged decrease in blood pressure. In contrast, in monkeys, EGF had dose-related blood pressure-lowering effects only; significant hypotension was observed at doses ranging from 3 to 300 μg/kg i.v. Hypotension was associated with modest tachycardia in both species. To our knowledge, this is the first report of hemodynamic effects of EGF in primates, and it clearly documents that the mitogenic role of growth factors such as EGF is but one aspect of their physiology.**

In addition to the well-recognized mitogenic activity of growth factors, there is an increasing understanding of their vasoactive effects. Epidermal growth factor (EGF) belongs to a large family of molecules with the ability to modulate growth (1, 2). EGF is also reported to have both vascular smooth muscle contractile and relaxing activity *in vitro* (3–6). Berk *et al.* (4) reported that EGF produced contractions in isolated rat aorta that were roughly 40% of the maximal response to angiotensin; time to peak tension was relatively short (2–3 min). In contrast, Namiki and Akatsuka (7) reported that EGF relaxed isolated rat aortic strips; Muramatsu *et al.* (6) reported similar findings in isolated canine mesenteric arteries. Divergent results have also been obtained from *in vivo* studies. Gan *et al.* (8) reported that EGF had no effect on regional hemodynamics in anesthetized rats; however, regional bed vasodilatation was observed in the anesthetized dog (5). Thus, there is remarkable species heterogeneity in the vasoactive properties of EGF.

A number of growth factors are receiving increased attention as potential therapeutic agents. EGF, hepatocyte growth factor, and insulin-like growth factor are reported to protect against or ameliorate renal ischemic injury in a variety of models (9–13), although these effects show some species variation (14). EGF is also reported to accelerate gastric ulcer healing and inhibit gastric acid secretion (15–17). The potential therapeutic effects of EGF have been of interest for a number of years. Partially purified EGF preparations extracted from human urine (referred to as urogastrone) have been administered to humans as early as the mid-1970s to modulate gastric acid secretion (18–21). Using gene transfer techniques to deliver a gene encoding the secreted form of vascular endothelial cell growth factor, the first human study attempting to accelerate new blood vessel growth is ongoing (22), and studies exploring the therapeutic benefit of genes for basic fibroblast growth factor in animal models are underway (23).

In light of the growing interest in the use of growth factors to treat disease and the vasoactive properties of these agents, we examined the hemodynamic effects of i.v. administered EGF. Cardiovascular studies were performed with the bioactive 48-amino acid fragment of human EGF in rodents and primates to determine the effects of EGF on blood pressure and heart rate in conscious animals.

MATERIALS AND METHODS

All procedures and protocols involving the use of animals were approved by the Parke–Davis Animal Care and Use Committee and adhered to American Association of Laboratory Animal Care and federal guidelines for the humane care and treatment of animals.

Chemicals. Recombinant human EGF-(1–48) (M_r, 5443), a bioactive 48-amino acid fragment of human EGF, was prepared by expression of the human EGF-(1–53) gene in *Escherichia coli*. The peptide was subsequently purified and enzymatically cleaved to the 1–48 moiety with trypsin. Final purity was subsequently determined to be >98%. The functional effects of this compound in rats and monkeys have been described (15, 24).

Hemodynamic Activity in Conscious Normotensive Rats. Normotensive male CD rats (12–13 weeks old; Charles River Breeding Laboratories) were prepared with indwelling aortic catheters for continuous monitoring of arterial blood pressure and heart rate. A catheter was implanted in the abdominal vena cava for drug administration. Each animal was fitted with a harness swivel apparatus that allowed the animal free range of motion and served to route catheters out the top of the cage where the catheters were fixed to a Gould–Statham pressure transducer (Spectramed, Oxnard, CA). Animals were given food and water ad libitum and allowed 24 hr for postsurgical recovery before experimentation.

EGF was dissolved in distilled water and infused i.v. over 20-min intervals in ascending doses of 30, 100, and 300 μg/kg (n = 8) in a dose volume of 1 ml/kg. Animals were given a minimum 4-hr washout between each dose. The drug was prepared immediately before dosing. Blood pressure and heart rate were monitored with a computer-based data acquisition system (model MA-16; PO-NE-MAH, Storrs, CT) for up to 2 hr after each rising dose of EGF. Measurements from individual animals were excluded from group means when the arterial pulse pressure was <20 mmHg. Data were collected as 1-min averages and means were calculated over 10-min intervals during drug administration and for the first hour after dosing; subsequent data points were averaged over 30-min

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Abbreviations: EGF, epidermal growth factor; MABP, mean arterial blood pressure.

intervals. Predrug control blood pressure and heart rate were obtained by averaging the 60-min interval just before dosing.

Hemodynamic Activity in Conscious Normotensive Monkeys. Studies were conducted in male normotensive cynomolgus monkeys (*Macaca fascicularis*, 5.5–9.8 kg; Charles River Primates, Port Washington, NY) from a colony instrumented with vascular access ports (Access Technologies, Skokie, IL) to measure arterial blood pressure and to infuse drugs i.v. Monkeys had been previously trained to sit quietly in a primate restrainer (Primate Products, Woodside, CA). Arterial blood pressure was measured directly using the vascular access port connected to a Gould–Statham pressure transducer. Approximately 2 hr after a 6 a.m. feeding, monkeys were placed in a primate chair for measurement of blood pressure and heart rate. After a 1-hr acclimation, animals were dosed with EGF by i.v. infusion. In the first series of experiments, EGF was given in total doses of 0.3, 3, and 300 μg/kg i.v. over 20 min. Experiments were conducted on separate days with a minimum 2-week washout between doses; not all animals received all doses. A second set of experiments used total doses of 3 and 20 μg/kg infused over 4 hr with an 8-day washout between doses. EGF was prepared in distilled water 30 min before dosing. The concentration was adjusted so that the dose was given in a total volume of 0.3 and 4 ml/kg using the 20-min and 4-hr infusion protocols, respectively.

Computer base data acquisition (model HD-4; PO-NE-MAH) was used to acquire and summarize arterial blood pressure and heart rate data at 1-min intervals. These data points were further averaged at 5- and 15-min intervals in the 20-min and 4-hr infusion protocols, respectively.

Statistics. All data are expressed as mean ± SE. Linear regression analysis were used to generate IC_{50} or EC_{50} values. An ANOVA (Statview; Abacus Concepts, Berkeley, CA) with a Bonferroni test (adjusted for multiple comparisons) was used to compare treatment groups or to make within group comparisons to a baseline, respectively. Statistical significance was defined as $P < 0.05$.

RESULTS

Hemodynamic Activity in Conscious Normotensive Rats. Mean arterial blood pressure (MABP) and heart rate in these conscious rats averaged ≈100–105 mmHg and 350–390 beats per min, respectively ($n = 8$). Intravenous administration of EGF did not significantly affect blood pressure in these animals during the 20-min infusion of either 30 or 100 μg/kg EGF, although pressure tended to rise during the infusion and fall in the postdose period (Table 1). At the end of the 20-min infusion protocol, MABP averaged 6%, 7%, and 12% higher than control values in individual animals from the 30, 100, or 300 μg/kg EGF groups, respectively. At the 300 μg/kg dose, infusion of EGF significantly elevated blood pressure compared with the predose control blood pressure. During the 2-hr postdose period, blood pressure decreased significantly compared with the increased pressures attained during the 300 μg/kg EGF infusion. The maximum decrease in blood pressure was 8% at 40–50 min after the 100 μg/kg EGF infusion and 10% ($P < 0.05$) at 60 min after the 300 μg/kg EGF infusion.

EGF administration tended to produce dose-related increases in heart rate in conscious rats (Table 2), although this effect was statistically significant only in the 100 and 300 μg/kg dose groups. Heart rate was increased 8%, 11% ($P < 0.05$), and 22% ($P < 0.05$) at the end of 20-min infusions of 30, 100, and 300 μg/kg EGF, respectively. A sustained tachycardia was observed in the 300 μg/kg dose group during the postdose period. Within 60 min of terminating the EGF infusion, heart rate had returned to predose levels within animals.

Hemodynamic Activity in Conscious Normotensive Monkeys. *The 20-min infusion protocol.* The effects of a 20-min infusion of EGF (0.3, 3.0, and 300 μg/kg i.v.) on blood pressure and heart rate in conscious monkeys are summarized in Figs. 1 and 2, respectively. Baseline MABP ranged from 88 to 96 mmHg, and baseline heart rate ranged from 145 to 168 beats per min in the three dose groups. The lowest dose of EGF (0.3 μg/kg, $n = 5$) had no significant effects on blood pressure or heart rate.

EGF infused at 3 μg/kg ($n = 6$) produced a significant drop in arterial blood pressure and a related tachycardia. MABP began to decrease immediately after starting the infusion and plateaued at 14% below control ($P < 0.05$) during the infusion period; 30 min after the infusion was stopped, MABP was further reduced to 20% below baseline ($P < 0.05$). MABP returned to baseline within 2 hr. Heart rate significantly increased (18%) during the infusion in parallel with the change in MABP and returned to baseline within 1 hr after the infusion was stopped.

An infusion of 300 μg/kg EGF ($n = 6$) produced a greater decrease in MABP; within the first 10 min of initiating an infusion, MABP was 27% below baseline ($P < 0.05$) and 32% below baseline when the infusion was stopped. MABP remained significantly decreased for 2 hr postdose.

Appetite was suppressed in all six monkeys treated with the 300 μg/kg EGF for ≈1 week postdose. During the 20-min infusion protocol, emesis was noted in four of six monkeys at both 3 and 300 μg/kg; no emesis was observed at the lowest EGF dose (0.3 μg/kg).

The 4-hr infusion protocol. The effects of 4-hr i.v. infusion of vehicle (saline; $n = 6$) and 3 ($n = 6$) and 20 ($n = 5$) μg/kg EGF on MABP and heart rate are depicted in Figs. 3 and 4, respectively. Baseline MABP was between 94 and 100 mmHg in the three groups; baseline heart rate was between 155 and 184 beats per min. Vehicle alone had no significant effects on blood pressure during a 4-hr infusion protocol. Heart rate tended to decline in the vehicle group; however, the change was not statistically significant. During the 4-hr infusion of 3 μg/kg EGF, MABP gradually decreased, with an 11% reduction ($P < 0.05$) noted at the end of the infusion protocol. Blood pressure remained significantly decreased throughout the 1-hr postdose observation period. Heart rate significantly increased by 20% during the first hour and remained elevated for the duration of the study.

Infusion of 20 μg/kg over 4 hr also resulted in a gradual but significant reduction in blood pressure; MABP was 23% below

Table 1. Effects of EGF on MABP in conscious rats

EGF 1-48		Time, min									
		EGF infusion		Postdose							
μg/kg i.v.	Control	10	20	10	20	30	40	50	60	90	120
30	103 ± 2	104 ± 2	109 ± 2	110 ± 3	107 ± 3	101 ± 2	101 ± 2	99 ± 2	98 ± 2	101 ± 2	102 ± 2
100	104 ± 3	105 ± 3	111 ± 2	106 ± 3	102 ± 3	97 ± 3	96 ± 4	96 ± 3	97 ± 2	99 ± 2	99 ± 3
300	101 ± 3	109 ± 2	113 ± 2*	111 ± 2	103 ± 2	101 ± 3†	97 ± 2†	96 ± 2†	91 ± 2†	94 ± 2†	104 ± 2

Data are expressed as mean ± SE, $n = 8$.
*Significantly different ($P < 0.05$) from control value within group.
†Significantly different ($P < 0.05$) from response (20 min) during EGF infusion.

Table 2. Effects of EGF on heart rate in conscious rats

EGF 1-48 μg/kg	Control	Time, min: EGF infusion 10	EGF infusion 20	Postdose 10	20	30	40	50	60	90	120
30	371 ± 6	361 ± 8	399 ± 5	424 ± 12	403 ± 9	400 ± 7	407 ± 8	384 ± 4	386 ± 8	381 ± 9	372 ± 8
100	388 ± 6	385 ± 11	430 ± 13*	421 ± 10	438 ± 9*	426 ± 7	433 ± 10*	427 ± 12	415 ± 12	405 ± 11	372 ± 8
300	353 ± 6	379 ± 9	432 ± 15*	429 ± 11*	431 ± 12*	427 ± 13*	408 ± 11*	420 ± 9*	394 ± 9	383 ± 7	396 ± 4

Data are expressed as mean ± SE, $n = 8$.
*Significantly different ($P < 0.05$) from control value within group.

baseline at the end of the infusion protocol. Heart rate maximally increased (19%; $P < 0.05$) during the first hour of the infusion and remained elevated for the duration of the infusion period. At the end of the postdose observation period, heart rates had returned to baseline levels. When the 4-hr infusion protocol was used, no emesis was observed at total doses up to 20 μg/kg.

DISCUSSION

EGF and several other growth factors have received increased attention as potential therapeutic agents. With the use of recombinant production technologies, the potential therapeutic application of peptides in disease states has grown exponentially. In both animal and human studies, EGF treatment accelerates healing of skin wounds (17), healing of gastric ulcers (16, 17, 21), and recovery from renal tubular damage (9–13). Several investigators have described vascular effects of EGF *in vitro* (3, 4, 6, 8) and *in vivo* (5, 25). In the studies presented here, we demonstrate that EGF has significant dose-related systemic hemodynamic activity in both rats and monkeys.

The vasoactive effects of EGF in isolated vascular beds and in isolated tissue preparations shows marked vascular bed and species variation. Gan *et al.* (8) reported that EGF (30 mg/kg i.p.) had no significant effects on hindlimb resistance in anesthetized rats; however, these same authors and others have reported increased tone (vasoconstriction) in arterial strips from rats treated with EGF (3, 4, 26). EGF is also reported to induce vasodilatation in a number of vascular beds (femoral, mesenteric, coronary, and renal) in anesthetized dogs and sheep (5, 25, 27). In clinical studies, partially purifed EGF extracted from human urine (urogastrone) was administered to modulate gastric acid secretion; no significant changes in cardiovascular parameters were described (18–21). In addition to the vasoactive properties of EGF, a number of other nonmitogenic responses have been described, such as effects on plasma calcium concentrations (28, 29) and inhibition of renin secretion (30).

To our knowledge, our studies detail the first characterization of the dose-related effects of EGF in conscious, chronically instrumented animals. Although the hemodynamic effects of EGF in rats were consistent and significant, the magnitude of the response in rodents was modest. The highest dose of EGF administered to conscious rats (300 μg/kg) produced a 12% decrease in MABP. In contrast, monkeys were much more sensitive to the blood pressure-lowering effects of EGF; 3 μg/kg EGF induced a 20% fall in MABP, and a 300 μg/kg dose decreased MABP by 33%. These differences in the potency of EGF in rats and monkeys are not completely attributable to differing biological activity of EGF in the two species. Guglietta and Lesch (15) have shown that EGF-(1–48) has a similar ED_{50} value (1 nmol/kg) for inhibition of gastric acid secretion in rats and monkeys; however, the maximal effect of EGF in monkeys was observed at a 10-fold lower dose. Whether there are marked species differences in agonist binding at EGF receptors or receptor subtypes within species remains unclear (31). Interestingly, a recent report describes a toxicologic evaluation of EGF in rats and cynomolgus monkeys (32). Doses of EGF ranging from 0.9 to 3 mg/kg per day were lethal in monkeys, but not in rats. No specific cardiovascular effects of EGF were reported in these monkeys; however, EGF was administered as a single subcu-

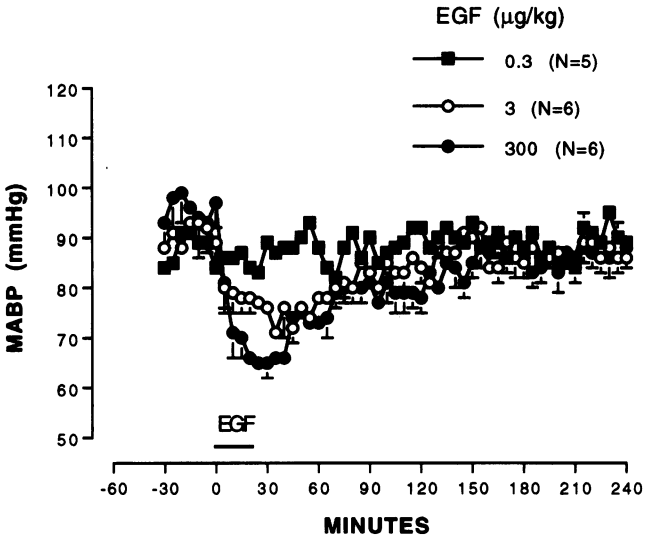

FIG. 1. Effect of a 20-min infusion of EGF on MABP in conscious monkeys. EGF (0.3, 3, or 300 μg/kg) was i.v. infused into conscious, chair-restrained cynomolgus monkeys for 20 min; animals were monitored for 2 hr postdose. Data for each point represent a 10-min average in individual animals; data are expressed as group mean ± SE.

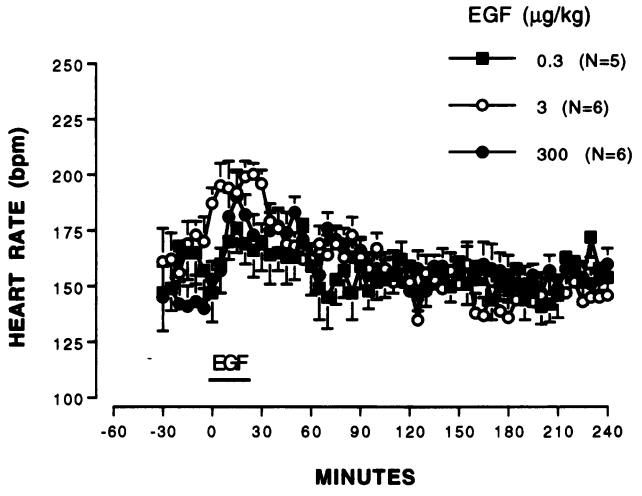

FIG. 2. Effect of a 20-min infusion of EGF on heart rate in conscious monkeys. EGF (0.3, 3, or 300 μg/kg) was i.v. infused into conscious, chair-restrained cynomolgus monkeys for 20 min; animals were monitored for 2 hr postdose. bpm, Beats per min. Data for each point represent a 10-min average in individual animals; data are expressed as group mean ± SE.

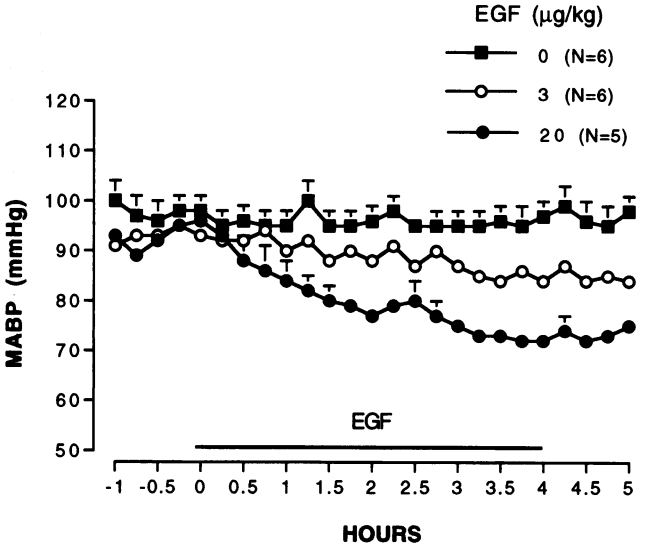

FIG. 3. Effect of a 4-hr infusion of vehicle or EGF on MABP in conscious monkeys. EGF (3 or 20 μg/kg) was i.v. infused into conscious, chair-restrained cynomolgus monkeys for 4 hr; animals were monitored for 1 hr postdose. Data for each point represent a 10-min average in individual animals; data are expressed as group mean ± SE.

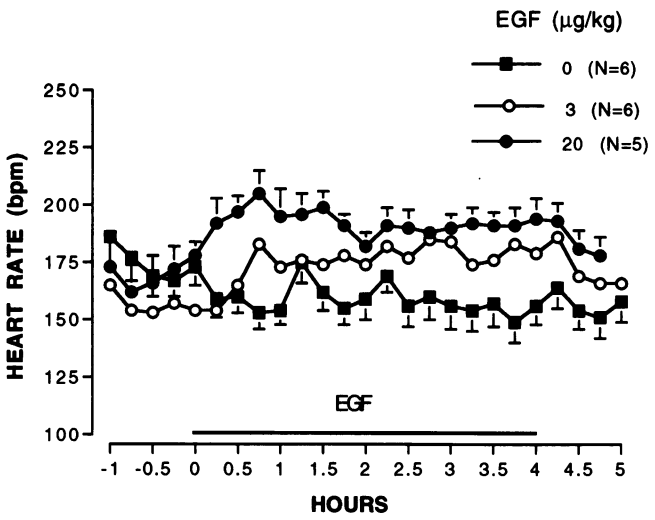

FIG. 4. Effect of a 20-min infusion of vehicle or EGF on heart rate in conscious monkeys. EGF (3 or 20 μg/kg) was i.v. infused into conscious, chair-restrained cynomolgus monkeys for 4 hr; animals were monitored for 1 hr postdose. Data for each point represent a 10-min average in individual animals; data are expressed as group mean ± SE.

taneous bolus once daily, making dose comparison with our studies difficult. EGF-induced hypotension has been briefly described in both male and female cynomolgus monkeys (33).

Elder *et al.* (18) administered EGF (urogastrone) to normal human volunteers at doses ranging from 0.125 to 0.5 μg/kg per hr for 1 hr; no changes in heart rate or systemic blood pressure were observed. In addition, the doses of urogastrone used in Elder's study (18) significantly inhibited gastric acid secretion. Similarly, urogastrone was administered to patients with Zollinger–Ellison syndrome (19) or duodenal ulcers (20) at 0.25 μg/kg per hr for 1 hr, and, again, no cardiovascular effects were noted. The hemodynamically active doses of EGF used in our monkey studies ranged from 3 to 300 μg/kg total dose. Thus, our doses are higher than those described in previous human studies with urogastrone; therefore, our results may be attributable to the higher dosing paradigms. However, it should be noted that in our 4-hr infusion studies doses as low as 0.75 μg/kg per hr were hemodynamically active; this dose is only 1.5- to 3-fold higher than doses previously administered to humans. A recent report by Itoh and Matsuo (21) describes a clinical trial of 6 μg human EGF administered twice weekly to gastric ulcer patients. Interestingly, the beneficial effects of EGF on measures of ulcer healing rate were not apparent after 4 weeks of therapy but proved significant at 8 weeks. However, there were no significant effects of EGF on measures of pain relief compared with the control group. Thus it is possible that lower doses of EGF may prove efficacious and avoid potential cardiovascular side effects.

The hemodynamic profile of EGF in conscious rats was biphasic, with both pressor and dilator responses observed. In contrast, despite the numerous reports of EGF-induced vasoconstriction in isolated tissue preparations, there was no evidence of increased blood pressure in monkeys. Rather, significant blood pressure-lowering activity was observed with short EGF infusions in conscious monkeys.

Gan *et al.* (8) described tachyphylaxis to the vasodilator effects of EGF in anesthetized dogs. In contrast, when we infused EGF over longer time periods in monkeys, blood pressure continued to decrease throughout the duration of the infusion protocol (4 hr), suggesting that over prolonged periods, there is little or no tachyphylaxis to the blood pressure-lowering effects of EGF in conscious primates. Our prolonged infusion studies also suggest that extending the delivery time at a given total dose of EGF does not completely abrogate its hemodynamic activity. Administration of a total dose of 3 μg/kg over 20 min or 4 hr produced maximal decreases in MABP of 20% and 11%, respectively.

These studies did not attempt to define the mechanism of action or signal transduction pathways involved in EGF-induced changes in blood pressure. EGF produced emesis in conscious monkeys after i.v. administration; thus we cannot rule out a possible centrally mediated effect of EGF in these monkeys. The tachycardia observed in conjunction with the hypotension is consistent with peripheral vasodilatation rather than direct effects of cardiac pump function. Laniyonu *et al.* (34) have demonstrated a link between G protein-linked vascular agonists and tyrosine kinase activation in some vascular preparations. Tyrosine kinase activation and phosphorylation events are well-described pathways in response to growth factor receptor activation. Saifeddine *et al.* (35) have suggested that tyrosine kinase pathways may play a potential role in control of smooth muscle cell tension.

In the studies reported here we have used human recombinant EGF-(1–48), with 5-amino acid residues deleted from the C-terminal end of the molecule. Early studies with truncated EGF analogues produced by protease digestion of the 1–53 parent moiety suggested that EGF-(1–48) had considerably reduced biological activity (36). However, subsequent reports documented that these same proteolytic digestions could generate 1–45, 1–46, and 1–47 fragments with considerably reduced activity (37). Proton NMR studies have shown that EGF-(1–48) and EGF-(1–53) have similar conformations (38). Guglietta and Lesch (15) observed similar potencies when directly comparing recombinant EGF-(1-53) and -(1–48) *in vivo*.

In summary, the growth-promoting agent EGF has significant, dose-related hemodynamic activity in conscious rats and monkeys. Primates appear more sensitive to the blood pressure-lowering effects of EGF. The potential to induce hypotension or alter regional blood flow should be considered when using EGF and other growth factors therapeutically.

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