Effects of epidermal growth factor on epinephrine-stimulated heart function in rodents

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Lorita, Jordi, Noèlia Escalona, Susanna Faraudo, Maria Soley, and Ignasi Ramírez. Effects of epidermal growth factor on epinephrine-stimulated heart function in rodents. Am J Physiol Heart Circ Physiol 283: H1887–H1895, 2002. First published July 18, 2002; 10.1152/ajpheart.00217.2002.—Epidermal growth factor (EGF) interferes with β-adrenergic receptor (β-AR) signaling in adipocytes and hepatocytes, which leads to decreased lipolytic and glycogenolytic responses, respectively. We studied the effect of EGF on the heart. EGF interfered with the cAMP signal generated by β-AR agonists in cardiac myocytes. In perfused hearts, EGF decreased inotropic and chronotropic responses to epinephrine but not to 8-(4-chlorophenylthio)adenosine 3’,5’-cyclic monophosphate. Sustained epinephrine infusion induced heart contracture, which resulted in altered heart function as demonstrated by decreased inotropy and increased heart rate variability. EGF prevented all these alterations. In the whole animal (anesthetized mice), EGF administration reduced the rise in heart rate induced by a single epinephrine dose and the occurrence of Bezold-Jarisch reflex episodes induced by repeated doses. Sialoadenectomy enhanced the response to epinephrine, and EGF administration restored normal response. All these results suggest that, by interfering with β-AR signaling, EGF protects the heart against the harmful effects of epinephrine.

THE EPIDERMAL GROWTH FACTOR (EGF) gene is expressed in several tissues (10). In rodents, the highest expression is found in submandibular salivary glands (SMG) (15), which accumulate a large amount of EGF protein. In male mice, EGF accounts for >0.5% of SMG protein (19). In target tissues, EGF is recognized by the EGF receptor (also known as ErbB1), which can dimerize with any member of the ErbB receptor family (62). The EGF receptor recognizes not only EGF but also other members of the EGF family (transforming growth factor-α, epiregulin, β-cellulin, amphiregulin, and heparin-binding EGF-like growth factor).

Catecholamines stimulate endocrine secretion of EGF from SMG in mice (9, 19). Accordingly, emotional stress (immobilization of the animal) causes a rapid and transient increase in plasma EGF concentration (14). Social stress (experience of defeat in intermale confrontation) induced a much higher and longer-lasting increase in plasma EGF concentration (50). Because catecholamine concentration in plasma remains high during the stress experience and for some time afterwards (52), tissues are exposed to combined stimulation by both catecholamines and EGF.

We reported previously (20, 56–58) that a high, but physiological, EGF concentration interferes with β-adrenergic receptor (β-AR) signaling and its metabolic consequences both in adipose tissue and in liver. Here we examined the heart because it is another target tissue of catecholamines, in which the most important effects (increase of both inotropy and chronotropy) are mediated by β-AR stimulation. However, β-AR hyperstimulation or overexpression may cause heart disease (49). The heart is also sensitive to EGF (37, 38, 63). In addition, some cardiovascular effects of EGF were described in several animal species (16, 26, 35). The results reported here support the view that, by interfering with β-AR signaling, EGF protects the heart against the harmful effects of epinephrine.

MATERIALS AND METHODS

All experimental procedures using rats and mice were approved by the Committee on Animal Care of the University of Barcelona and by the Autonomous Government of Catalonia.

Cardiac myocyte isolation and incubation. Calcium-tolerant myocytes were isolated from adult rat hearts by previously described methods (47) with minor modifications [collagenase B (Boehringer Mannheim) concentration in perfusion and digestion buffer was 1 mg/ml]. The Joklik medium (GIBCO) used in the preparation of different solutions was supplemented with 25 mM HEPES. Isolated myocytes retained their rod shape and high viability, as determined by the exclusion of Trypan blue dye. Only preparations in which >80% of the cells excluded the dye were incubated further. After isolation, cardiac myocytes were rinsed twice in Joklik’s minimal essential medium supplemented with 1.2 mM MgSO4, 1 mM L-carnitine, 1.5 mM CaCl2, and 1% fatty acid-free bovine serum albumin (Sigma-Aldrich). Myocytes were then incubated in a modified Joklik medium containing 1% fetal bovine serum (FBS; GIBCO) and 5% CO2 in air at 37°C.

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acid-free bovine serum albumin, diluted to 0.4–0.6 × 10^6 cells/ml, and immediately incubated in a rotating (60 cycles/min) water bath at 37°C under constant oxygenation with 95% O_2-5% CO_2. At indicated times, a sample was obtained and placed onto enough HClO_4 to give a final concentration of 3%. cAMP was determined from deproteinized samples as described previously (57).

**Heart perfusion.** Male Wistar rats (300–350 g) (Interfauna, Barcelona, Spain) were anesthetized (60 mg/kg pentobarbital; sodium), and a cannula (PE 10, Clay-Adams; filled with phosphate-buffered saline supplemented with 100 U heparin/ml) was introduced through the left carotid artery to the aortic arch and connected to an electronic pressure transducer (UF1, Pioden) maintaining a hydrostatic pressure of 60–80 mmHg. The heart was connected to an electronic tension transducer (UF1, Pioden) maintaining a diastolic tension of 1 g. After 15 min of basal recording, epinephrine or EGF was infused through the aortic cannula. The infusion rate was adjusted to obtain a final concentration (after dilution with the perfusion buffer) of 10 μM (epinephrine) or 10 nM (EGF). Some hearts were infused with EGF 5 min before, and then throughout, epinephrine infusion. From the continuous tension record, developed tension (DT) in each 20-s period was calculated (difference between mean maximal and mean minimal tension). The first derivative of the tension register was obtained, and the mean maximal value in 20-s periods was calculated (+dT/dt).

**Cardiovascular function in mice.** Male Swiss CD-1 mice (40 g; Interfauna) were anesthetized (60 mg/kg pentobarbital sodium), and a cannula (PE 10, Clay-Adams; filled with phosphate-buffered saline supplemented with 100 U heparin/ml) was introduced through the left carotid artery to the aortic arch and connected to an electronic pressure transducer (SensoNor 840). EGF and epinephrine doses were administered through a three-way valve connected to the cannula. From the continuous blood pressure recording, the mean arterial blood pressure (MABP) and heart rate (HR) were calculated for every 30-s period. In some experiments, mice were sialoadenectomized as described previously (19) 1 wk before the experiment. Sham-operated mice were used as control animals in these experiments. Plasma EGF was determined as described previously (19).

**RESULTS**

**Effect of EGF on cardiac myocytes and perfused hearts.** EGF increased cAMP concentration in rat cardiac myocytes in a dose-dependent manner (Fig. 1). The effect was moderate (38% increase) compared with the effect of a maximal dose of the β-AR agonist isoproterenol (240% increase). These results confirm a previous report by Nair et al. (36). In many cells in which EGF does not modify resting cAMP concentration, it is able, however, to interfere with the cAMP signal generated by several Gα-coupled receptors. This happens in some targets of catecholamine action, adiocytes (57) and hepatocytes (20). Here we show that at 0.5 μM isoproterenol (Fig. 1), the effects of EGF and isoproterenol on cAMP were not additive. At a higher isoproterenol concentration (10 μM), EGF actually decreased the cAMP signal.

In keeping with the moderate effect of EGF on cAMP in isolated myocytes, we observed that infusion of EGF into perfused rat hearts also had a moderate and transient effect on heart function (Fig. 2). EGF had a positive inotropic effect (14% over basal +dT/dt) and a negligible effect on chronotropy (2% over basal HR). The consequence of the increased inotropy was the increase in DT (23% over basal DT) and in heart work, as calculated by the tension-rate product (TRP; 19% over basal TRP). As expected, epinephrine infusion had stronger effects than EGF on both inotropy (98% increase) and chronotropy (42% increase). The consequence of both effects was the increase in DT (57% over basal) and TRP (107% over basal).

Figure 3 shows the contractile response to epinephrine of hearts infused with or without EGF. At the start of epinephrine infusion, all recorded parameters were identical in hearts infused with EGF and in control hearts. The maximal inotropic and chronotropic responses to epinephrine (achieved after 20–40 s) were reduced by EGF. Similar results were obtained when hearts were infused with isoproterenol (not shown). In these experiments we started EGF infusion 5 min before epinephrine because we had observed that simultaneous infusion of EGF and epinephrine resulted in a weaker, although still significant, effect on heart response (data not shown).

To determine whether the effect of EGF on the contractile response to epinephrine was due to interference with β-AR signaling, we studied the effect of EGF...
on the response of perfused hearts to the cAMP analog 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (CPT-cAMP) (Fig. 4). The rise in HR and +dT/dt was slower (maximal response after 80–100 s) than when hearts received epinephrine (see Fig. 2). EGF did not decrease the response to CPT-cAMP. The rise in HR actually increased significantly.

It is known that the sympathetic nervous system is involved in arrhythmogenesis associated with ischemic heart disease (18) and that a high dose of epinephrine may induce arrhythmias in nonischemic hearts (1). To study the effect of EGF on the arrhythmogenic effect of epinephrine, we ran new experiments in which epinephrine infusion lasted for 10 min. After 4 min of epinephrine infusion, the heart became progressively contractured, indicated by a rise in end-diastolic tension (Fig. 5). Simultaneously, there was a pronounced decrease in inotropy (+dT/dt dropped to 50% of initial value) and a sudden increase in HR variability (shown by the large increase in standard error of the mean value). EGF infusion reduced the magnitude of heart contracture and stopped all other alterations in contractility.

Effect of EGF on cardiovascular function in mice. We studied male mice to determine the physiological significance of such an interplay between catecholamines and EGF on the entire animal. Both the effect of exogenously administrated EGF and the function of endogenous EGF can be studied in male mice because they accumulate a huge amount of EGF in their SMG, which is released to both saliva and plasma on adrenergic stimulation (9).

In anesthetized mice, intravenous administration of EGF had a transient (disappeared in ~8 min) and moderate effect on MABP (increased by just 6–8 mmHg), but EGF had no effect on HR (Fig. 6). EGF administration did not alter the rise in MABP induced by epinephrine. Epinephrine increased MABP by 48 ± 4 and 44 ± 4 mmHg in control and EGF-treated mice, respectively (nonsignificant differences) but reduced the rise in HR by ~60% [73 ± 7 and 30 ± 6 beats/min (bpm) in control and EGF-injected mice, respectively (P < 0.001)].

In a further experiment, we administered EGF 20 (as above), 40 or 60 min before epinephrine. The rise in MABP induced by epinephrine (46 ± 6 mmHg) was not significantly affected by EGF administration 20 (33 ± 5 mmHg), 40 (48 ± 5 mmHg) or 60 (45 ± 3 mmHg) min before epinephrine. The rise in HR induced by epinephrine (66 ± 5 bpm) was significantly reduced by EGF administration either 20 or 40 min before epinephrine [28 ± 6 bpm (P < 0.001) and 41 ± 8 bpm (P < 0.05), respectively] but not 60 min before epinephrine [47 ± 9 bpm (nonsignificant difference)].

Strong inotropic stimulation of the heart may induce a powerful depressor reflex originating in the heart itself, known as the Bezold-Jarisch reflex, which is...
characterized by sudden hypotension and bradycardia
(31). We observed such a response after epinephrine
administration in some mice (Fig. 7B; compare with
the more common regular response shown in Fig. 7A).
In the next experiment, we administered repeated and
increasing doses of epinephrine to control and EGF-
treated mice (Fig. 7C). At the first dose, only one of
nine control mice had Bezold-Jarisch reflexes during
the 5-min period after epinephrine. This frequency
steadily increased with every new dose: after three
20-min-spaced administrations of 62.5 nmol/kg epinephrine,
50% of mice had Bezold-Jarisch reflexes. After
the fifth dose, this proportion increased to near 100%.
EGF administration 20 min before the first epinephrine
dose made mice more resistant to the induction of
Bezold-Jarisch reflex by epinephrine administration.
Thus only 15% of the animals had Bezold-Jarisch reflex
episodes after the third dose and <80% after the fifth
dose.

The consequence of having Bezold-Jarisch reflex ep-
isodes after epinephrine administration was a large
increase in HR variability (measured by standard de-
viation of the HR calculated every 5 s during the 1st
Fig. 4. Effect of EGF on the response of perfused rat hearts to
8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (CPT-
cAMP). Perfused rat hearts were maintained for 15 min to record
basal function parameters (referred to as 100%). EGF (40 pmol/min)
or KHB (control) was then infused. Five minutes later, CPT-cAMP
infusion (2.4 μmol/min) was started (defined as zero time). From the
continuous tension record, DT in each 20-s period was calculated.
The 1st derivative of the tension function was obtained, and the
mean of maximal +dT/dt in 20-s periods was calculated. The number
of beats in each 20-s period was used to calculate HR. Results are
means ± SE of % of basal (preinfusion) value (n = 4). Statistical
differences were determined by 2-way ANOVA, and the significance
of the treatment (±EGF) factor is shown.

Fig. 5. Effect of EGF on contractile dysfunction induced by sustained
infusion of epinephrine. Perfused rat hearts were maintained for 15
min to record basal function parameters (referred to as 100%). EGF
(40 pmol/min) or KHB (control) was then infused. Five minutes later
(zero time), epinephrine (Epi) infusion (40 nmol/min) was started. A:
end-diastolic tension (EDT) calculated as the mean of minimal ten-
sion values in each 30-s period. Results are means ± SE of % of basal
(preinfusion) values (n = 5). Statistical differences were determined
by 2-way ANOVA, with the significance of the treatment (±EGF)
factor shown. B: maximal value of +dT/dt (left) and HR (right) at the
indicated time point after epinephrine infusion. Values with and
without EGF at each time point were compared by Student's t-test
(*P < 0.05). The Levene test indicated no homogeneity of variance of
HR in hearts infused without EGF.

Fig. 6. Effect of EGF on cardiovascular function in anesthetized
mice. A cannula connected to a pressure transducer was introduced
into the left carotid artery of anesthetized mice, which received a
dose of EGF (12 nmol/kg) or saline (control) 20 min before the
administration of epinephrine (62.5 nmol/kg). Mean arterial blood
pressure (MABP; A) and HR (B; expressed as beats per min (bpm))
were calculated in every 30-s period. Results are means ± SE of 9
(control) or 8 (EGF) mice.
min after each epinephrine dose). Thus HR variability was 19 ± 5 bpm after the first dose (A) and increased to 36 ± 4 bpm after dose E in control mice. In EGF-injected mice, it was 11 ± 2 and 22 ± 3 bpm after doses A and E, respectively. HR variability before every dose ranged from 8 to 10 bpm in all animals.

In the last experiment we studied the response of the cardiovascular system to epinephrine in sialoadenectomized (Sialo) mice, with or without a previous dose of EGF, compared with control (sham operated) mice. The response to the first 62.5 nmol/kg dose of epinephrine is shown in Fig. 8A. Although the rise in MABP was similar in Sialo mice, with or without previous administration of EGF (12 nmol/kg) and in control mice, the increase in HR was enhanced in Sialo but not in Sialo+EGF mice. The percentage of mice having Bezold-Jarisch reflex episodes after repeated and increasing epinephrine doses is shown in Fig. 8B. We observed Bezold-Jarisch reflex episodes after the first epinephrine dose in 19% of Sialo mice, a proportion similar to that observed in control mice after the second dose. Administration of EGF to Sialo mice 20 min before the first epinephrine dose returned the percentage of animals that had Bezold-Jarisch reflex episodes to control values. Plasma EGF concentration in awake noncannulated mice was 0.11 ± 0.02 and 0.15 ± 0.02 nM in control and Sialo mice, respectively. At the end of the experiment (10 min after dose E), plasma EGF concentration was 5.64 ± 1.32 nM in control mice (P < 0.05 vs. corresponding awake value), 0.14 ± 0.04 nM in

![Fig. 7. Effect of EGF on epinephrine-induced Bezold-Jarisch reflex. Most animals in the experiment described in Fig. 6 responded to epinephrine with a regular pattern (A). The continuous arterial blood pressure (ABP) record and calculated HR are shown here. The break corresponds to the time of epinephrine administration. Some mice (B) had an irregular response to epinephrine, with brief and repeated periods of sudden hypotension and bradycardia (Bezold-Jarisch reflex). To study the effect of EGF on epinephrine-induced Bezold-Jarisch reflex, mice were treated with EGF (12 nmol/kg) or saline (control; C) (10 animals/group) (C). Twenty minutes later, mice received repeated and progressive doses of epinephrine (A–E) according to the procedure shown at bottom. Percentage of animals having Bezold-Jarisch reflex in the 5-min period after each dose is shown in C. Significance of the difference between control and EGF-treated mice was determined by 2-way ANOVA after appropriate transformation of the frequency value.

![Fig. 8. Effect of sialoadenectomy on the response of the cardiovascular system to epinephrine. One week after surgery, control (sham operated; C) and sialoadenectomized (Sialo; S) mice were anesthetized. A cannula connected to a pressure transducer was then introduced into the left carotid artery to obtain a continuous recording of MABP. Mice received repeated and progressive doses of epinephrine according to the procedure shown in Fig. 7C. A: maximal effect of the 1st epinephrine dose on MABP and HR. Results are means ± SE of 5–8 animals/group. After 1-way ANOVA, comparisons vs. control value were made by Tukey’s test; *P < 0.05. B: % of animals having Bezold-Jarisch reflex in the 5-min period after each dose. Significance of the differences were determined by 2-way ANOVA after appropriate transformation of the frequency value. ns, Not significant.

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Sialo mice (nonsignificant differences vs. corresponding awake value), and 4.54 ± 1.77 nM in Sialo+EGF mice (P < 0.05 vs. corresponding awake value).

DISCUSSION

Models. This paper combines studies in myocytes isolated from rat hearts to find the effect of EGF on cAMP, in Langendorff-perfused rat hearts to find the functional consequences of these effects, and in anesthetized mice to find the physiological relevance of the ex vivo findings. We observed previously (21, 46, 56, 57) that EGF has similar effects in both liver and adipose tissue of rats and mice. Here, we also observe close corroboration between results obtained from rat preparations and from whole mice. Thus EGF had a negligible effect on HR in Langendorff-perfused rat hearts, and we could not observe any effect on HR in whole mice. In addition, the most remarkable effect described here, the attenuation of the response to epinephrine, was clearly observed in all the systems studied.

The use of pentobarbital-anesthetized mice to study the in vivo physiological significance of the ex vivo findings requires a preliminary consideration. Pentobarbital anesthesia is known to depress heart function and, indeed, HR values in our system were lower than in urethane-anesthetized mice (43) or in conscious mice (24, 30). However, the heart was sensitive to adrenergic stimulation and retained Bezold-Jarisch reflex regulation. The retention of both Bezold-Jarisch and arterial baroreceptor reflexes in pentobarbital-anesthetized rats (33) and cats (25) has been reported. Indeed, to reach a more definitive conclusion on the physiological relevance of EGF in cardiovascular regulation, further studies on the acute response to stress in conscious animals are required.

Effects of EGF on otherwise nonstimulated cardiovascular system. Confirming the results published by Patel and coworkers (37, 38, 63), we found that EGF had a moderate effect on cAMP in cardiac myocytes and on contractility in perfused hearts. Later studies from Patel’s group showed that purified Gsα can directly associate to the juxtamembrane region of purified EGF receptor (54, 55) and that phosphorylation of Gsα in tyrosine residues by purified EGF receptor increases the ability to activate adenyl cyclase (44, 45). The effect of EGF on cAMP accumulation requires the expression of type V adenyl cyclase (11). Such a requirement may explain why the effect of EGF increasing cAMP is observed only in very few cell types.

We were unable to observe an increase in HR after EGF administration to anesthetized mice, which is in keeping with the results obtained in perfused rat hearts. However, Keiser and Ryan (26) observed that EGF infusion increased HR in conscious rats and monkeys. Several differences in the experimental procedure may explain the differences between our results and those of Keiser and Ryan concerning the effect of EGF on HR in the whole animal. First, although we used an EGF dose (12 nmol/kg, ~72 μg/kg) that is within the range used by Keiser and Ryan (30–300 μg/kg), we administered it in a bolus injection, whereas they infused it for 20 min. Second, and perhaps the most important difference, our experiments used pentobarbital-anesthetized mice and rats, not conscious animals.

In addition to the cAMP-mediated effect on heart function, EGF stimulates arterial contraction in vitro (6, 16, 35). Although there is some controversy concerning the mechanisms involved in the effect of EGF, no studies suggested the involvement of cAMP. We observed a transient and moderate hypertensor effect of EGF in anesthetized mice, which is in keeping with the in vitro studies mentioned above and with results obtained in conscious rats by Keiser and Ryan (26). These authors showed, however, that EGF induced a hypertensor response in conscious monkeys (26).

The physiological relevance of all these effects of EGF has yet to be established. They are observed when systems are stimulated with a much higher EGF concentration than that found in plasma of mice or any other mammal under normal conditions (15). Nevertheless, effects of EGF on the cardiovascular system will have to be considered when exploring any therapeutic use of this peptide.

Effects of EGF on catecholamine-stimulated cardiovascular system. In addition to the effect of EGF on an otherwise nonstimulated cardiovascular system, our results clearly indicate that this peptide decreases the response of both rat and mouse hearts to epinephrine. Several lines of evidence suggest that this effect of EGF is the consequence of the interference with β-adrenergic-induced rise of cAMP. First, EGF decreased the effect of a maximal dose of isoproterenol on cAMP in myocytes. Second, EGF decreased the chronotropic and inotropic responses of perfused hearts to epinephrine (or isoproterenol) but not to a cAMP analog. This demonstrates that the target of EGF action in perfused hearts is located upstream of protein kinase A. Very likely, the effect observed in perfused hearts is the consequence of the interference with the cAMP signal observed in isolated myocytes.

This effect of EGF on epinephrine-stimulated hearts is in apparent contradiction with that discussed above. However, it has been shown that association of purified Gsα-subunit with purified EGF receptor and phosphorylation of the former in tyrosine residues was decreased by activation of Gsα with guanosine 5′-O-(3-thiotriphosphate) (44, 54). Therefore, the stimulatory effect of EGF on Gs proteins can be expected to decrease when Gs proteins are activated by other receptors. It should be noted that we observed less interference with the cAMP signal when myocytes were stimulated with 0.5 μM than with 10 μM isoproterenol.

In addition, mechanisms involved in EGF action may be more complex in whole cells than in reconstituted systems. A variety of effects on cAMP signal generated by several hormones was described in whole cell systems. In A-431 human epidermoid carcinoma cells, in which EGF inhibits cAMP accumulation induced by bradykinin (27), phosphorylation of the Gsα-subunit in...
tyrosine residues by activated EGF receptor leads to reduced guanosine nucleotide exchange (27) and hence inactivation of Gs protein (i.e., the opposite of the effect in the reconstituted system). In other cells, such as gastric mucosa parietal cells, in which EGF inhibits glucagon-like peptide-1-induced acid production, the effect appears to involve the activation of a Gi protein (51). A somewhat different effect of EGF on Gi protein function was described by Tehar et al. (57, 58) in rat adipocytes. In this system EGF appeared to increase the sensitivity of Gs-stimulated adenylate cyclase to the inhibitory effect of Gi proteins.

Studies of mouse hepatocytes, although they do not rule out an effect of EGF at the Gi protein level, strongly suggest that a phosphodiesterase is the main target of EGF action in this cell type (20). Recent studies from Houslay's lab (3, 22, 29) demonstrate that extracellular signal-regulated kinase (ERK)2, which is phosphorylated by the EGF receptor signaling cascade, phosphorylates several isoforms of type 4 phosphodiesterase (PDE4). Furthermore, phosphorylation may increase (PDE4D3 or PDE4D5 isoforms) or decrease (PDE4D1 or PDE4D2 isoforms) phosphodiesterase activity. Thus, depending on the abundance of these isoforms, EGF may have the opposite effect on cAMP in different cell types.

Therefore, it is conceivable that stimulation of Gs proteins by β-AR (or other Gs-coupled receptors) abrogates the stimulatory effect of EGF on Gi protein and allows for other effects on Gs or Gi proteins and/or on phosphodiesterases. This hypothesis explains why EGF did not inhibit, but rather enhanced, some of the effects of CPT-cAMP in perfused hearts. Because CPT-cAMP directly activates protein kinase A, any effect of EGF on phosphodiesterases or on the signal transduction from β-AR to adenyl cyclase would not operate. Under these conditions, we would expect EGF actually to increase intracellular cAMP levels and so enhance the effect of CPT-cAMP.

The mechanisms involved in the cross talk between signaling systems have received much attention for more than a decade. cAMP-elevating agents interfere with EGF effects in a variety of cells including several fibroblast cell lines and smooth muscle cells (see Ref. 7 for review). In keeping with this, it was shown that purified catalytic subunit of protein kinase A phosphorylates EGF receptor and this is accompanied by decreased autophosphorylation and a diminished tyrosine kinase activity of the receptor (5). Furthermore, cAMP may interfere with the perhaps most important signaling cascade of the EGF receptor, the activation of ERK1 and ERK2 (12, 28, 60). However, in some cell systems cAMP-elevating agents do not interfere with EGF-induced activation of ERK1 and ERK2 (4, 32) or they can even enhance EGF-dependent activation of these mitogen-activated protein kinases (48, 61). As discussed above, recent studies that show that isoforms with different domain composition may even have an opposite response to certain stimulus illustrate how the cross talk between signaling systems can be cell type specific. This was reviewed recently by Houslay and Kolch (23).

We have not further explored the mechanisms involved in the effect of EGF on cAMP. Rather, we studied the physiological significance of such an effect. It should be noted that the effect of EGF was observed at 10^{-8} M. This concentration is within the physiological range of variation of plasma EGF in mice under several stress conditions (14, 39, 50) and similar to that obtained on adrenergic stimulation of SMG (9, 19).

We conclude that the effect of EGF on heart response to epinephrine is physiologically relevant in mice, not only because exogenous EGF administration decreased the effect of epinephrine on HR but also because the lack of endogenous secretion of EGF from SMG (due to sialoadenectomy) enhanced such an effect of epinephrine, which was normalized by previous administration of EGF. These studies were performed in anesthetized mice. New experiments will be required to determine whether the effect of EGF persists in conscious mice. Nevertheless, the results presented here may explain an apparent paradox: catecholamines, through α1-adrenergic receptors, stimulate the release of EGF to the bloodstream (9, 19), which in turn interferes with β-AR mediated responses in heart and other tissues (20, 57).

We suggest that the biological significance of such an interference is to protect the heart against harmful effects of intense adrenergic stimulation. We observed in perfused hearts that EGF reduced the contracture and the arrhythmogenic effect of sustained epinephrine infusion. In anesthetized mice, EGF prevented the induction of Bezold-Jarisch reflex by repeated epinephrine doses. Although the physiological significance of the Bezold-Jarisch reflex is not well understood, epinephrine-induced sudden hypotension and bradycardia is indeed a harmful effect of this hormone. In humans this has been linked to syncpe in several clinical situations (53).

Many studies have looked at physiological functions of EGF in adult animals, mostly rats or mice. Conclusions were obtained through the administration of EGF or arose from the analysis of the consequences of sialoadenectomy. Thus it was established that EGF accelerates wound repair (8), is involved in male reproductive function (59), and is also involved in female mammary gland development during gestation (40). EGF helps control liver regeneration (34) and protects gastric mucosa against ulcerogenic agents (42). These reports, and many others, tell us about quite long-term (several days) functions of circulating EGF. Therefore, none of them gives a satisfactory explanation of one the most important facts concerning accumulation of EGF in mice SMG, acute secretion on adrenergic stimulation, which results in a rapid and transient rise in plasma EGF concentration (19).

It can be argued that acute increase in plasma EGF concentration after adrenergic stimulation is not a general phenomenon. For instance, it does not occur in female mice (19) and male rats (41). However, the EGF receptor (ErbB1) binds other members of the EGF family (TGF-α, amphiregulin, HB-EGF), which act...
mainly through autocrine and paracrine mechanisms (62). Therefore, it cannot be excluded that the EGF receptor (not because of stimulation with plasma EGF but with other locally produced ligands) may be involved in heart protection in species in which SMG accumulate much less EGF and there is no rise in plasma EGF on adrenergic stimulation. In fact, some roles for HB-EGF in the heart have been reported (2, 17). Very recently, mice with ventricular restricted deletion of Erbb2 (a member of the Erbb gene family that can heterodimerize with ErbB1; Ref. 62) developed dilated cardiomyopathy (13). All these recent reports indicate that ErbB receptors and their ligands have a function in long-term protection of the heart. Our results indicate that one of these ligands can also protect the heart against the harmful effects of catecholamines in the short term. Therefore, it will be worth exploring the usefulness of EGF, or other Erb1 ligands, as protective agents against ischemia-induced heart disease, given that local release of catecholamines is involved in ischemia-induced arrhythmias and heart failure (18).

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