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Epidermal Growth Factor Secreted from Submandibular Salivary Glands Interferes with the Lipolytic Effect of Adrenaline in Mice*

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ABSTRACT

We had described that epidermal growth factor (EGF) interfered with the lipolytic effect of catecholamines in isolated adipocytes. Since catecholamines stimulate the release of EGF from submandibular salivary glands to blood plasma in male mice, we studied whether EGF affected also the lipolytic response to adrenaline in whole animals. We studied the effect of adrenaline in sialoadenectomized and sham-operated mice receiving or not a high dose of EGF following adrenaline injection. There was no difference in plasma EGF concentration between sham-operated and sialoadenectomized animals receiving saline. After adrenaline administration plasma EGF increased by 20-fold in sham-operated but did not increase in sialoadenectomized mice. Indeed, the increase was much higher (more than 100-fold) in mice receiving exogenous EGF. The effect of adrenaline on plasma concentration of both glycerol and nonesterified fatty acids was higher as lower was plasma EGF concentration. Isolated adipocytes obtained from sham-operated or sialoadenectomized mice had identical lipolytic response to adrenaline. The lipolytic response of adipocytes to isoproterenol was decreased by addition of EGF. To study whether the interference with the *in vivo* lipolytic effect of adrenaline had further metabolic consequences, we measured plasma β-hydroxybutyrate concentration in plasma. There was no difference in the response to adrenaline between sham-operated and sialoadenectomized mice in spite of the difference in plasma nonsterified fatty acid concentration. Studies in isolated hepatocytes indicated that ketogenesis run at near maximal rate in this range of substrate concentration. These results suggest that EGF in the physiological range decreases the lipolytic effect of adrenaline but does not compromise further metabolic events like the enhancement of ketogenesis. (*Endocrinology* **141:** 876–882, 2000)

EPIDERMAL GROWTH FACTOR (EGF) is a mitogen for many cells in culture, adipocyte precursors among them. In these cells, EGF stimulates proliferation and blocks differentiation (1, 2). The physiological relevance of this effect of EGF in adipose tissue development was established by Serrero and Mills (3). They found that daily sc administration of EGF to newborn rats for 10 days resulted in a substantial decrease of fat pad weight, but also in an increase of adipocyte precursor cell number.

Besides of the mitogenic effect, EGF is able to affect the function of nonproliferating cells too. A remarkable example is mature adipocytes. In these cells EGF has opposed effects to those in their precursors: it stimulates adipogenesis (4). In keeping with this function, it has been reported that EGF stimulates metabolic pathways leading to lipid accumulation in differentiated adipocytes. Thus, EGF stimulates acetyl-CoA carboxylase activity (5) and increases conversion of ^{14}C-glucose into fatty acids (6). EGF stimulates also the release of lipoprotein lipase from adipocytes (6) and inhibits both glucagon- and isoproterenol-stimulated lipolysis (7).

Metabolic studies in mature adipocytes generate questions at both the molecular and physiological levels. Regarding molecular mechanisms involved in the effect of EGF, progress has been slow because the magnitude of effects in mature adipocytes is small. Although effects of EGF resemble those of insulin, it becomes clear that the mechanisms involved are different. This was shown for the effect on lipolysis. Both EGF and insulin modulate the cAMP signal generated by lipolytic hormones like glucagon or catecholamines (6). While the target of insulin action is the cyclic GMP-inhibited phosphodiesterase (cGI-PDE) (8), through a phosphatidylinositol 3-kinase-dependent mechanism (9), the target of EGF action is the interaction between Gs and Gi proteins in the control of adenylate cyclase (7). This effect does not involve wortmannin-sensitive substrates (6), which is in keeping with the recent observation that EGF does not stimulate phosphatidylinositol 3-kinase in 3T3-L1 adipocytes (10).

Concerning the physiological relevance of the effects observed in differentiated adipocytes, different approaches raised opposite conclusions. Thus, surgical removal of the major production site of EGF in mice, submandibular salivary glands, impaired adipose tissue weight gain induced by ovariectomy (11), or by aging (12) in mice. On the contrary, systemic administration of EGF reduced fat mass in rats (13). Because all these studies involved long-term experiments, some indirect effects could explain the differences in these results. Adipose tissue mass depends on the balance between adipogenic and lipolytic hormones. In mice, among other effects, catecholamines stimulate the secretion of EGF from

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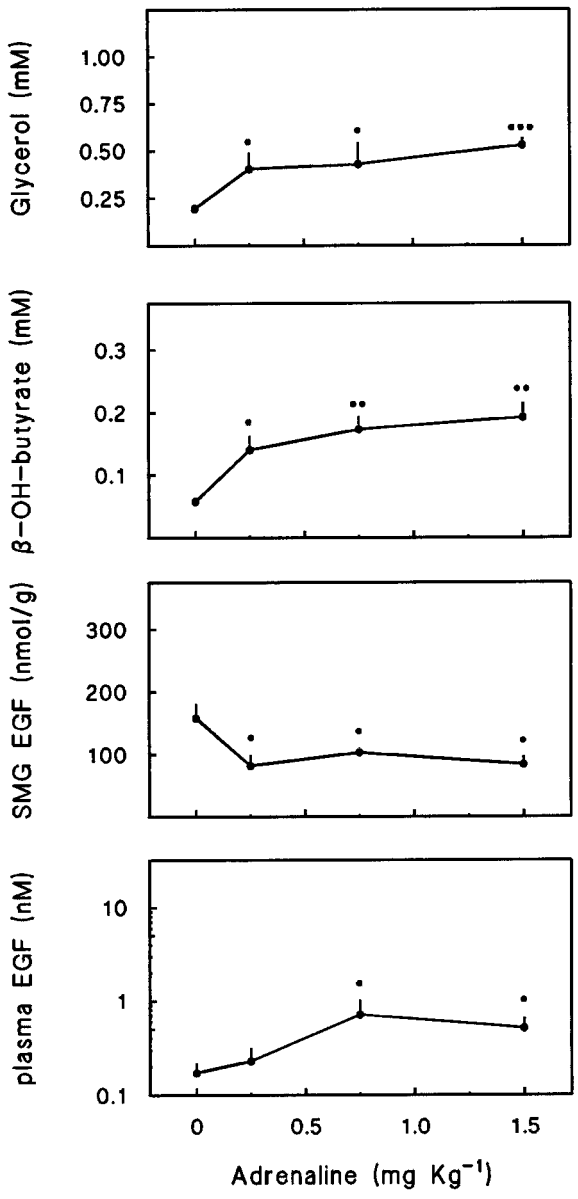

FIG. 1. Dose-dependent effect of adrenaline on lipolysis and EGF in plasma. Anaesthetized mice were injected with 0.37 mg kg^{-1} of adrenaline (iv) and the indicated dose ip. Controls received iv and ip volumes of saline. After 10-min samples were obtained to measure plasma glycerol, and β-hydroxybutyrate, and plasma and submandibular gland (SMG) EGF concentration. Results are the mean ± SE of five animals per group. Statistical comparisons *vs.* control value were made by Student's *t* test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

submandibular salivary glands to saliva and also to blood plasma (14). We took advantage of this model to determine not only if exogenous EGF could interfere with the lipolytic effect of adrenaline, as we had found in isolated cells (6, 7), but also whether endogenous EGF secreted upon adrenaline stimulation could do so.

Materials and Methods

Animals

Adult Swiss-CD1 mice were obtained from Interfauna (Barcelona, Spain). All animals were male, fed *ad libitum*, and maintained under a constant 12-h light, 12-h dark cycle (lights on at 0800 h) and controlled conditions of humidity (45–55%) and temperature (22 ± 1 C). All experimental procedures were approved by the Committee on Animal Care of the University of Barcelona.

Sialoadenectomy

In diethyl ether-anaesthetized mice, a small incision was made to expose the submandibular salivary glands, which were then ligated and excised. In control (sham-operated) animals, the glands were exposed, and a ligature was passed, but not tied. The wound was stitched and disinfected. Sham-operated and sialoadenectomized animals were kept fasted for the next 24 h. We have observed that postsurgery fasting allows similar recovery and growth pattern of both groups. Two weeks later, mice had recovered completely and were used for experiments.

Experiments in whole animals

Mice were anaesthetized (sodium pentobarbital 60 mg kg^{-1}) before receiving an iv (0.37 mg kg^{-1}) plus an ip (1.25 mg kg^{-1}) injection of adrenaline (Sigma, St. Louis, MO). In some experiments, animals were immediately injected with EGF (Roche Molecular Biochemicals, Mannheim, Germany) (0.25 mg kg^{-1}, iv). Control animals received identical volumes of saline. At indicated time, blood was collected into heparinized syringes from the inferior vena cava. Blood plasma was obtained by centrifugation. A sample was deproteinized and neutralized as indicated in (15) and used for glucose (16), glycerol (17), nonesterified fatty acid (NEFA) (NEFA C ACS-ACOD method, Wako Pure Chemical Co., Neuss, Germany), and β-hydroxybutyrate (18) quantification. Another sample was processed as indicated in (19) for EGF quantification. Immediately after bleeding, submandibular salivary glands were excised and homogenized in 10 ml PBS. After centrifugation (100,000 × *g* for 60 min at 4 C) the supernatant was stored at −40 C for EGF quantification (19).

To study the effect of immobilization stress, mice (under a very light ether anesthesia) were fixed with adhesive tape to a table in a supine position. In less than 1 min, the effect of ether completely disappeared and acute stress symptoms were observed. After 20 min, animals were killed and processed as indicated above. Liver glycogen and plasma glucose were determined as indicated elsewhere (15).

Experiments in isolated adipocytes

Adipocytes were isolated from the epididymis by the method of Rodbell (20) with minor modifications. One gram of adipose tissue (combining the mass obtained from several mice) was digested in plastic vials with 3 ml of Krebs-Henseleit buffer containing 1 mM $CaCl_2$, 5 mM glucose, 3% BSA (buffer A), and 0.5 mg of collagenase/ml. Digestion was carried out at 37 C for 40 min and under constant shaking (120 cycles/min). Dispersed cells were filtered through a double-layered nylon mesh and washed four times with buffer B (as buffer A but with 0.5 mM $CaCl_2$). After counting in a Neubauer hemocytometer, the cells were suspended in buffer B. Lipolysis was determined in isolated adipocytes as the amount of glycerol produced by the cells during an incubation period. After isolation, adipocytes (1.75×10^5 cells/ml) were placed in plastic vials (containing the indicated additions) at 37 C, under constant shaking and oxygenation with O_2/CO_2 (95%:5%). Both at zero time and 30 min afterwards, a sample (1 ml) was withdrawn and immediately placed into enough perchloric acid to give a final concentration of 3%. Neutralized supernatants were used to determine glycerol (17). In every experiment, incubations were performed in triplicate. Preliminary experiments showed that the rate of lipolysis was proportional to cell density between 0.5×10^5 and 4×10^5 adipocytes/ml, and linear with incubation time for at least 60 min, even in the presence of lipolytic agents.

Experiments in isolated hepatocytes

Hepatocytes were isolated from the liver of adult male mice as previously described (15). Initial cell viability measured by the trypan blue exclusion test was over 90% and decreased less than 10% during the incubations (up to 60 min). Isolated hepatocytes were then incubated (2×10^6 cells/ml, final volume 2 ml) in a 20 mM-HEPES (pH 7.4)-containing buffer supplemented with 1%-albumin (21), but without

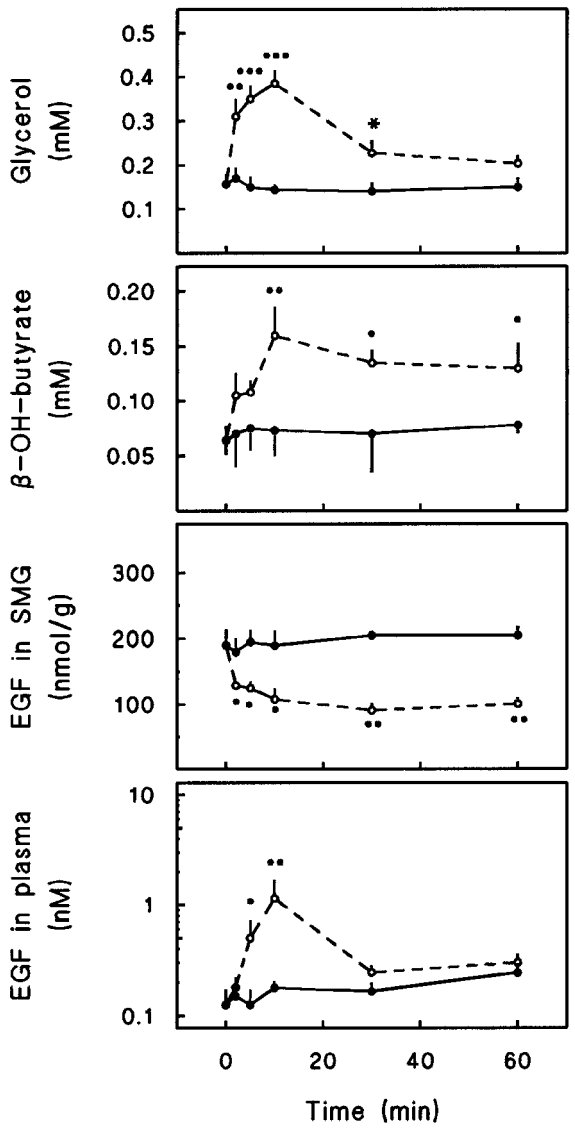

FIG. 2. Time-course of the effect of adrenaline on lipolysis and EGF in plasma. Anaesthetized mice were injected with adrenaline (0.37 mg kg^{-1} iv and 1.25 mg kg^{-1} ip). Controls received iv and ip volumes of saline. At indicated times, animals were killed and samples were obtained to measure plasma glycerol, and β-hydroxybutyrate, and plasma and submandibular gland (SMG) EGF concentration. Results are the mean ± SE of five animals per group. Statistical significance of differences *vs.* corresponding control value are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

glucose. Oleate-albumin complex was prepared as in (22) immediately before use. At the end of the incubation a sample of the suspension was placed into enough ice-cold $HClO_4$ to give a final concentration of 3%. After neutralization, β-hydroxybutyrate and acetoacetate concentrations were determined (18).

Results

In a preliminary experiment we studied the effect of different ip doses of adrenaline primed by an iv dose of 0.37 mg kg^{-1} (Fig. 1). All three doses raised plasma glycerol concentration up to similar level. β-Hidroxybutyrate, a product of fatty acid metabolization in liver, showed a similar pattern. Plasma EGF concentration increased only after the middle (0.75 mg kg^{-1}) and the highest (1.5 mg kg^{-1}) doses. The increase of glycerol concentration was significant since the second min after adrenaline injection (1.25 mg kg^{-1}), reached maximal value at 10 min and decreased thereafter (Fig. 2). β-Hidroxybutyrate concentration reached also maximal value at 10 min, but remained higher than control values for at least 60 min. EGF content in submandibular glands decreased to a half in about 10 min after adrenaline injection and remained low for at least 60 min. EGF in plasma progressively increased during the first 10 min, and decreased thereafter. The kinetics of EGF secretion upon adrenaline injection was similar to that obtained after phenylephrine injection (19).

To determine whether the effects of the adrenaline dose chosen for next experiments (priming dose of 0.37 mg kg^{-1} iv, followed by 1.25 mg kg^{-1} ip) were similar to those of a physiological stress situation, we compared the effect of adrenaline in anaesthetized mice to that of immobilization of conscious mice. In both, adrenaline injected and immobilized mice liver glycogen was decreased and plasma glucose concentration was increased (Table 1). The raise of plasma glucose concentration (2.0-fold) and the decrease of liver glycogen content (40%) was higher in adrenaline injected than in immobilized mice (1.7-fold increase and 28% decrease for plasma glucose and liver glycogen, respectively). In both models, submandibular gland EGF content decreased and plasma EGF concentration increased in a similar extent.

Because we wanted to study the effect of a stress hormone in surgically manipulated animals, we had to allow them to recover from surgical stress before the experiment. We let the animals to stand for 2 weeks before adrenaline administration (time required for complete wound healing); therefore, we had to compare first both groups: sham-operated and sialoadenectomized mice. Results are shown in Table 2.

TABLE 1. Effect of adrenaline administration to anaesthetized mice or immobilization of conscious mice on glycogenolysis and plasma EGF

Time (min) . . .	IMMO		ADR	
	0	20	0	20
Liver glycogen (mg/g)	45.3 ± 1.9	32.7 ± 2.3[a]	45.4 ± 5.0	27.3 ± 4.0[b]
Plasma glucose (mM)	11.9 ± 0.2	18.2 ± 0.7[a]	10.6 ± 0.5	24.2 ± 0.6[a]
EGF in SMG (nmol/g)	142 ± 20	68 ± 11[b]	190 ± 25	90 ± 10[b]
EGF in plasma[d] (nM)	0.19 ± 0.03	1.24 ± 0.74[c]	0.13 ± 0.05	1.14 ± 0.54[c]

Mice were either anaesthetized and injected with 0.37 mg kg^{-1} (iv) and 1.25 mg kg^{-1} (ip) of adrenaline (ADR), or subjected to immobilization stress (IMMO). At the indicated time (0 or 20 min) animals were killed to obtain samples. Results are the mean ± SE of 10–15 animals per group. Statistical significance of differences *vs.* corresponding control value are indicated by: [a] $P < 0.001$; [b] $P < 0.01$; [c] $P < 0.05$. SMG, Submandibular glands. ([d] Since there were no homogeneity of variances, statistical analysis was performed on logarithmic transformation of values).

TABLE 2. Characteristics of sham-operated and sialoadenectomized mice

	Sham-operated	Sialoadenectomized
Whole animal parameters		
Body weight (g)	47.3 ± 0.7	47.2 ± 0.9
Food intake (g/day)	6.7 ± 0.2	7.0 ± 0.5
Water intake (ml/day)	8.9 ± 1.0	6.7 ± 0.8
Tissue weight (g)		
EAT	0.48 ± 0.07	0.51 ± 0.02
Liver	2.25 ± 0.11	2.18 ± 0.04
Plasma biochemistry		
Glucose (mM)	9.8 ± 0.3	9.8 ± 0.4
Glycerol (mM)	0.12 ± 0.01	0.12 ± 0.01
NEFA (mM)	0.53 ± 0.03	0.49 ± 0.04
β-hydroxybutyrate (mM)	0.05 ± 0.01	0.06 ± 0.01
EGF (nM)	0.11 ± 0.02	0.15 ± 0.02

Animals were operated under ether anesthesia. They were kept fasted for the next 24 h and then allowed to have free access to food. This table compares both groups of animals 2 weeks after surgery. Results are mean ± SE of six animals. Food and water intake were measured through the recovery period, and the results shown corresponds to the whole period mean value. EAT, Epididymal adipose tissue (both depots of each animal). Statistical comparisons were made by Student's *t* test. Differences were not significant.

There were no differences in the appearance of sialoadenectomized and sham-operated mice. Both groups had similar body, epididymal adipose tissue, and liver weight. Both had similar daily food intake. Sialoadenectomized mice had somehow lower water intake, but the differences were not significant. They also had similar blood plasma glucose, glycerol, and nonesterified fatty acids concentration. Finally, although sialoadenectomized mice had no submandibular glands, plasma EGF concentration was not decreased.

Next we studied the effect of adrenaline injection, with or without an additional supplementation of EGF, in sham-operated and sialoadenectomized mice. The results are shown in Fig. 3. Plasma EGF concentration did not increase in sialoadenectomized mice upon adrenaline administration, but increased near 20-fold in sham-operated animals. As expected, plasma EGF concentration at the time the mice were killed was even higher in both groups of mice receiving an exogenous bolus of EGF. Adrenaline injection to sham-operated mice decreased EGF content in submandibular salivary glands from 207 ± 12 nmol/g (saline-injected mice value) to 112 ± 9 nmol/g in adrenaline injected mice, and to 93 ± 12 nmol/g in adrenaline + EGF injected mice (differences are significantly different to control value, $P < 0.001$).

Adrenaline administration increased glycerol and NEFA concentration in plasma of sham-operated animals, the increase was significantly higher in sialoadenectomized mice. Exogenous EGF reduced the effect of adrenaline on glycerol concentration and almost abolished that on NEFA concentration in both groups of animals. To determine whether the alteration in the lipolytic response to adrenaline had consequences on ketogenesis we determined β-hydroxybutyrate concentration in plasma. Adrenaline administration increased β-hydroxybutyrate concentration in sham-operated and sialoadenectomized mice up to similar values. Exogenous supplementation of EGF reduced the response to adrenaline in both groups of animals.

In Fig. 4 we show the relationship between plasma EGF concentration and the effect of adrenaline (adrenaline minus saline values) on plasma glycerol and NEFA concentration. There was a significant negative correlation for both glycerol and NEFA. There was, however, no significant correlation between the effect of adrenaline on plasma β-hydroxybutyrate concentration and plasma EGF (not shown).

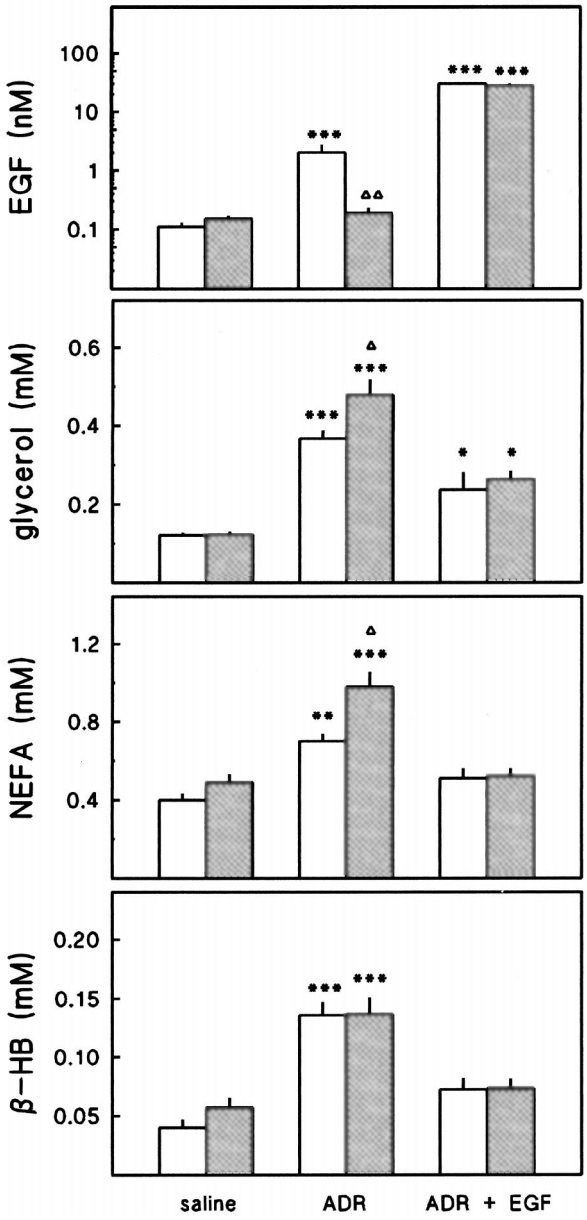

FIG. 3. Effect of EGF on the lipolytic effect of adrenaline. Two weeks after surgery sham-operated (*white bars*) and sialoadenectomized (*dotted bars*) mice were anesthetized and injected with adrenaline (0.37 mg kg^{-1} iv + 1.25 mg kg^{-1} ip) (ADR) or saline. Some mice received EGF (0.25 mg kg^{-1} iv) together with the adrenaline injection (ADR+EGF). Ten min afterward blood was obtained for assays in plasma. Results are mean ± SE of 12 to 20 animals per group. Statistical significance of the differences *vs.* corresponding saline (*) or sham-operated (△) value are indicated by: *, △ $P < 0.05$; **, △△ $P < 0.01$; *** $P < 0.001$.

To rule out that the enhanced lipolytic response to adrenaline in sialoadenectomized mice was due to differences in the sensitivity of adipose tissue cells to catecholamines, we

studied the effect of adrenaline in isolated adipocytes from sham-operated and sialoadenectomized animals. The results are shown in Fig. 5A. From the four experiments done, we obtained a basal (nonstimulated) lipolysis that was similar in cells from sham-operated and sialoadenectomized mice (0.13 ± 0.03 and 0.12 ± 0.01 μmol glycerol released/30 min × 10^6 adipocytes, respectively; differences were not significant). There were no differences either in ED_{50} value (0.22 ± 0.01 and 0.21 ± 0.01 μM for sham-operated and sialoadenectomized mice, respectively), or in maximally stimulated lipolysis (2.22 ± 0.25 and 2.06 ± 0.08 μmol glycerol released/30 min × 10^6 adipocytes for sham-operated and sialoadenectomized mice, respectively).

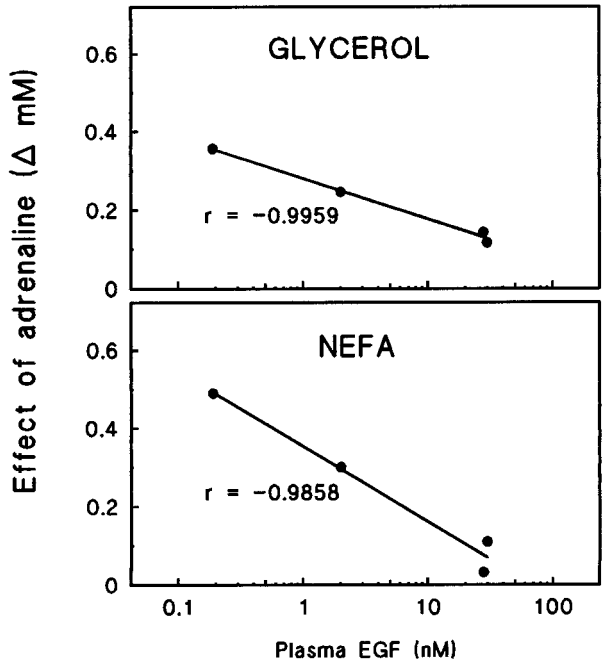

FIG. 4. Relationship between plasma EGF concentration and the lipolytic effect of adrenaline. Data in Fig. 3 was used to plot the relationship between mean plasma EGF and the effect of adrenaline on glycerol or NEFA concentration. The effect of adrenaline was calculated as the difference between mean adrenaline (with or without EGF injection) and its corresponding mean saline value.

Next, we tested the direct effect of EGF on lipolysis in isolated mice adipocytes (Fig. 5B). We had previously observed in rat cells (6, 7) that EGF interfered the lipolytic effect of catecholamines. Our purpose was, therefore, to demonstrate that it happens also in mouse cells. EGF did not affect basal glycerol release. In the presence of the β-adrenergic agonist isoproterenol, lipolysis increased to 0.55 ± 0.01 μmol glycerol/30 min × 10^6 cells, but only to 0.34 ± 0.02 μmol glycerol/30 min × 10^6 cells in cells incubated with isoproterenol and EGF. Very similar results were obtained if cells were incubated in the presence of adrenaline instead of isoproterenol (data not shown).

Finally, to understand the results concerning plasma β-hydroxybutyrate concentration we studied the effect of EGF on ketogenesis in isolated hepatocytes. Results are shown in Fig. 6. In the absence of EGF, increasing concentrations of oleate resulted in an enhanced rate of ketogenesis with an estimated maximal rate of 646 ± 78 nmol KB/10^6 cells × 60 min and $S_{0.5}$ (substrate concentration that allows a half-maximal rate of ketogenesis) of 0.29 ± 0.04 mM. EGF did not affect maximal rate of ketogenesis (709 ± 107 nmol KB/10^6 cells × 60 min, nonsignificant differences) but increased moderately $S_{0.5}$ value (0.45 ± 0.05 mM, $P < 0.05$).

Discussion

Adrenaline stimulates the secretion of EGF from submandibular salivary glands to both saliva and plasma in mice (14, 19). We have found that EGF interferes with the lipolytic response of isolated adipocytes to catecholamines (7). Our purpose in this paper was to find out whether this happens also in the whole animal. To this end we took advantage of the male mice model in which sialoadenectomy abolishes the increase in plasma EGF upon catecholamine administration.

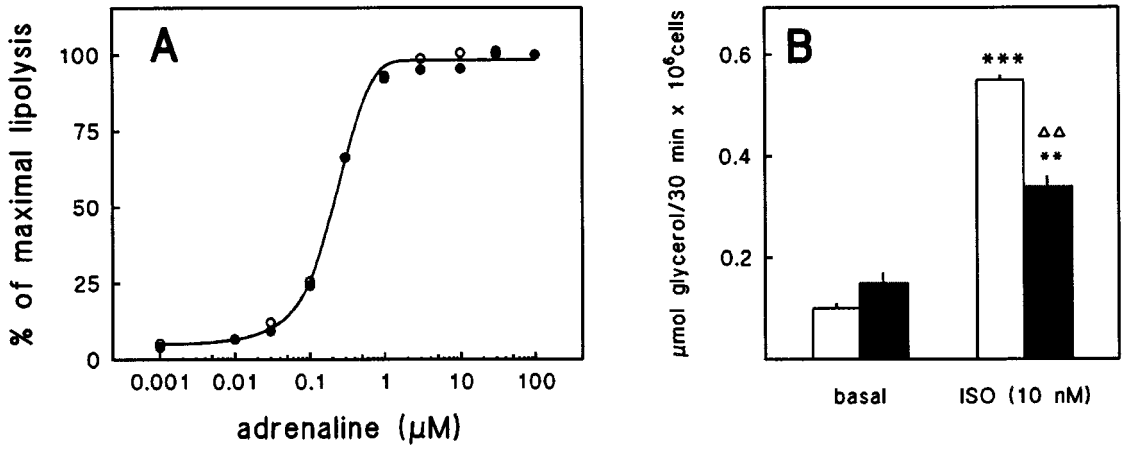

FIG. 5. Lipolysis in isolated adipocytes. A, Adipocytes were isolated simultaneously from combined epididymal adipose tissue of four sham-operated (●) or sialoadenectomized (○) mice in each experiment. After isolation, cells were incubated in triplicate with the indicated concentration of adrenaline at 37 C. After 30 min of incubation, lipolysis was measured as the amount of glycerol released to the incubation medium. This figure shows the results of a representative experiment (out of four identical experiments made with different cell preparations). Each point corresponds to the mean value of the triplicate. B, Adipocytes were isolated from nonoperated mice and incubated in the absence of EGF (*white bars*) or in the presence of 100 nM EGF (*black bars*), and with or without 10 nM isoproterenol (ISO). Results are the mean ± SE of four experiments. Statistical significance of the differences *vs.* corresponding basal (*) or no EGF (△) value are indicated by: **, △△ $P < 0.01$.

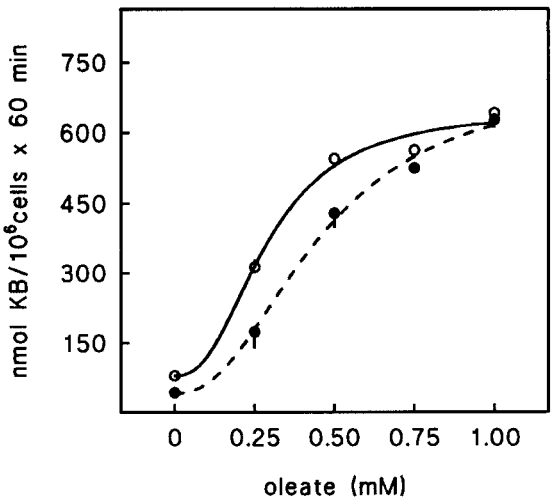

FIG. 6. Effect of EGF on ketogenesis in isolated hepatocytes. Hepatocytes were isolated from nonoperated mice. After isolation cells were incubated with the indicated concentration of oleate either without (○) or with (●) EGF (100 nM). After 60 min, incubation medium was obtained to determine ketone bodies (KB). Each experimental condition was made in triplicate. The results are the mean ± SE of four identical experiments made with different cell preparations.

We could therefore test whether endogenous EGF had any effect on adipose tissue response to catecholamines.

Based on preliminary experiments, we chose a dosage procedure that induced somehow stronger effect than immobilization stress on liver glycogen and plasma glucose concentration. It was described that plasma glucose concentration is sensitive to stress intensity (23); it was thus a good parameter to compare both models. In spite of this small difference, the effect on EGF in submandibular glands and plasma was identical.

Although sialoadenectomized mice had not submandibular glands, plasma EGF concentration was not decreased. This was observed by many investigators before (14, 19, 24, 25) and was attributed to the fact that EGF is synthesized in other cell types, some of which can overexpress the gene as a consequence of sialoadenectomy (26). What surgical removal of submandibular glands does is to abolish the increase of plasma EGF concentration upon adrenergic stimulation (14, 19). This explains why both groups have similar plasma EGF concentration when maintained in nonstressful conditions.

Our *in vivo* experimental design allowed to study the effect of both endogenous and exogenous EGF on the response of adipose tissue to adrenaline. The results demonstrate that EGF interferes with the lipolytic effect of adrenaline in the whole animal system: as lower is plasma EGF concentration, stronger is the effect of adrenaline on both glycerol and NEFA concentrations. The difference in the lipolytic response to adrenaline between sham-operated and sialoadenectomzed mice cannot be attributed to differences in adipose tissue mass or to adipocyte sensitivity to catecholamines. Rather, it may be the consequence of the interference with stimulation of lipolysis in adipocytes. Indeed, exogenous EGF decreased the effect of adrenaline, and the differences between sham-operated and sialoadenectomized mice disappeared. This is in keeping with the observation that plasma EGF concentration was similar in both groups animals.

Concerning the mechanisms involved in the effect of EGF on catecholamine-stimulated lipolysis, we had observed that it was the result of the interference with the cAMP signal induced by catecholamines in these cells (7). It is known that besides of the effect on cellular cAMP, the action on regional blood flow contributes also to the *in vivo* lipolytic effect of adrenaline (27). Whether the effect of EGF is attributable only to the interference with the intracellular signal, or it involves also an interference with the regional hemodynamic effect of adrenaline, as it happens with some antilipolytic hormones like vasopressin (28), will require further investigations. Although it is known that EGF has hemodynamic effects *in vivo* (29), and produces vasoconstriction in isolated aortic preparations (30, 31), nothing is known about the interaction between EGF and adrenaline on adipose tissue vasculature.

The liver is one of the main destinations of fatty acids derived from adipose tissue. In this organ, fatty acids may be incorporated into complex lipids (triglycerides and phospholipids) and finally into secreted lipoproteins, but they are also fuels that provide most of the energy required by the cells. Ketogenesis is tightly coupled to β-oxidation, which in turn depends on substrate availability. Our results illustrate the close relationship between plasma fatty acid concentration and ketogenesis. We obtained an $S_{0.5}$ for ketogenesis from oleate of 0.29 mM, a value close to plasma NEFA concentration in control animals. This indicates that ketogenesis is indeed sensitive to variation of plasma NEFA concentration.

Looking at the relationship between oleate concentration and ketogenesis in hepatocytes, the decreased β-hydroxybutyrate concentration found in EGF(+adrenaline) injected mice compared with values obtained in adrenaline injected animals can be attributed in part to a decreased substrate availability (in these animals plasma NEFA concentration dropped from 0.7–1.0 mM to 0.5 mM). In addition to substrate availability, mitochondrial fatty acid oxidation, and hence ketogenesis, depends on the control of carnitine palmitoyltransferase I (32). This enzyme is directly regulated by malonyl-CoA (33), the product of the acetyl-CoA carboxylase reaction. Both enzymes are thus, reciprocally regulated. EGF activates acetyl-CoA carboxylase in adipocytes (5). In hepatocytes, Holland and Hardie (34) found that EGF induced phosphorylation of this enzyme, which was in keeping with an enhanced lipogenesis. In keeping with these reported effects of EGF, we observed that this peptide increased oleate $S_{0.5}$ required for half-maximal ketogenesis in hepatocytes. Therefore, besides of the decreased substrate availability, the reduced ketogenic sensitivity of the liver may explain the low β-hydroxybutyrate concentration found in EGF injected mice.

In a more physiological context, the raise of plasma EGF concentration (the difference between sialoadenectomized and sham-operated animals receiving adrenaline), although decreased the lipolytic response to adrenaline, had no effect on plasma β-hydroxybutyrate. This is understandable because plasma NEFA concentration decreased from 1.0 mM (sialoadenectomized animals) to 0.7 mM (sham-operated animals with higher EGF concentration in plasma), and we

observed in isolated hepatocytes that within this range of oleate concentrations ketogenesis is near saturation and thus, unsensitive to such a small decrease in substrate availability. Therefore, we may conclude that, under physiological conditions, one of the metabolic consequences of adipose tissue lipolysis (the increased ketogenesis in liver) is not compromised by the effect of endogenously secreted EGF on lipolysis. If the interference with the lipolytic action of adrenaline has no further metabolic consequences, we could conclude also that such an interference is meaningful only for adipose tissue itself: EGF would reduce the intensity of one of the major lipolytic stimulus affecting thus, the balance between adipogenesis and lipolysis. This would favor triglyceride accumulation, which is in keeping with other effects of EGF described in adipocytes (4–6), and in whole animals (11, 12).

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