Interaction of cortisol and epinephrine in the regulation of leucine kinetics in man

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Summary. To assess the interaction of the two major stress hormones epinephrine and cortisol in the regulation of leucine kinetics in man, epinephrine (50 ng/kg/min) was infused either alone or in combination with cortisol (2 µg/kg/min) into two groups of 6 postabsorptive normal male subjects during 180 min. Plasma leucine concentrations decreased by 28% ($p < 0.05$) from baseline during epinephrine treatment (plasma levels 515 pg/ml); this was due to a decrease of leucine appearance (determined by 1-^{13}C-leucine infusions) by 23% ($p < 0.025$); leucine oxidation decreased by 29% ($p < 0.05$). However, when plasma cortisol concentrations were elevated to supraphysiological levels (16.3 µmol/l) during epinephrine administration, the decreases of leucine plasma concentrations, appearance and oxidation were abolished. Plasma glucose and FFA concentrations were similarly elevated during both kinds of treatment. Since leucine appearance represents a measurement of total body protein breakdown and leucine disappearance into non-oxidative pathways reflects protein synthesis, the data indicate that plasma epinephrine concentrations during severe stress exert a protein anabolic effect in man which may counteract catabolic properties of elevated plasma cortisol.

Key words. Cortisol; epinephrine; leucine kinetics; leucine oxidation; branched chain amino acids; protein turnover; stress hormones; free fatty acids; glucose; somatostatin.

Critical illness and injury are associated with protein catabolism which contributes to morbidity and mortality of these conditions[2, 10]. Elevated concentrations of catecholamines, cortisol and glucagon have been reported during severe stress[6, 11, 15, 17]. The protein catabolic role of cortisol is generally recognized[23]. However, the influence of epinephrine on protein turnover and its interaction with cortisol is less clear. Studies in rat hemidiaphragms showed accelerated rates of branched chain amino acid oxidation during incubation with epinephrine[3]. Garber[5] and Li[14] reported a lowering effect of catecholamines on protein breakdown and on amino acid release from rat skeletal muscle. Studies in human subjects demonstrated decreased leucine flux during infusion of epinephrine[18]; however, counterregulatory insulin release during epinephrine infusion made interpretation of the findings difficult.

The present studies aimed to assess the interaction of elevated plasma epinephrine and cortisol concentrations in affecting whole body leucine kinetics as a parameter of whole body protein metabolism in man. The 1-^{13}C-leucine infusion technique was used to measure plasma leucine flux and oxidation[16, 26]. In order to diminish counterregulatory effects of epinephrine and cortisol on plasma insulin and glucagon concentrations, somatostatin was infused in all studies, combined with replacement infusions of insulin and glucagon. The data from the studies on epinephrine infusion alone will be reported elsewhere[13].

Materials and methods. Subjects. Written, informed consent was obtained from 12 healthy men aged 26 ± 2 years, weighing 74 ± 13 kg. Plasma glucose, hemoglobin, triglycerides, renal and hepatic function, ECG and blood pressure were normal prior to the study. None was performing vigorous physical exercise nor taking any medication. The experimental protocol was reviewed and approved by the Human Ethics Committee of the University Hospital, Basel.

Procedures. The subjects were admitted to the hospital after a 12-h overnight fast. At 7:30 a teflon cannula (19 G) was inserted into the right antecubital vein for infusions. Following a bolus injection of 2 µmol/kg 1-^{13}C-leucine (Cor Isotopes, Cambridge, Mass.) and of 0.15 mg/kg $NaH^{13}CO_3$ (Cor Isotopes, Cambridge, Mass.) at −120 min, a continuous infusion of 1-^{13}C-leucine was administered at 0.04 µmol/kg/min until the end of the study. After tracer equilibration of 120 min, blood samples were drawn from a heated hand vein[1] and from a deep forearm vein, cannulated in the retrograde manner, at 10- and 15-min intervals during a 30-min control period and during 180 min of epinephrine (Streuli, Uznach, Switzerland) infusion at 50 ng/kg/min. In the experiments with elevated plasma cortisol concentrations, hydrocortisone sodium succinate (Solucortef, Upjohn) was infused at a rate of 2 µg/kg/min from −120 min until the end of the study (+210 min). During epinephrine infusion, using both protocols, plasma insulin and glucagon were maintained by administration of somatostatin (Serono, Italy; 6.5 µg/kg/h) and replacement amounts of insulin (100 µU/kg/min) and glucagon (0.8 ng/kg/min). Forearm blood flow was determined at each time point using strain gauge plethysmography (Hokanson EC4, Issaquah, WA, USA). Aliquots of expired air were collected in a 10-l bag from which 100 ml sealed glass flasks were filled for later analysis of $^{13}CO_2$ using isotope ratio mass spectrometry (Finnigan MAT 251 spectrometer) and 2-l plastic bags were filled at 20, 90 150, and 210 min for determination of CO_2 production and O_2 consumption using a respirometer (Hewlett Packard) and a CO_2 and O_2 infrared gas analyzer (E. Jäger, Würzburg, Germany).

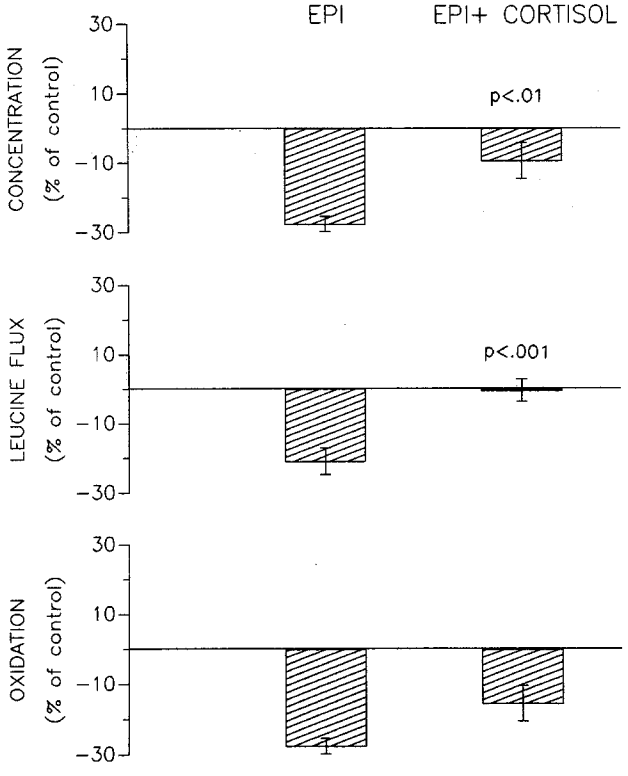
Analyses and calculations. The processing of blood samples and assay of plasma insulin, glucagon, catecholamines, cortisol, FFA and glucose has been described previously[4, 8]. Concentration and isotopic enrichment of plasma leucine and a-ketoisocaproate (KIC) were determined by gas-chromatography masspectrometry (Hewlett Packard, Mod. 5890), utilizing D10-leucine and D10-a-KIC as internal standards[21]. Flux and oxidation of leucine were calculated during the steady state of the basal period, and during that of the last 60 min of hormone infusions, using isotope dilution equations[26]. Steady state was assumed when leucine plasma concentration and ^{13}C enrichment did not change over time when tested by analysis of variance. Leucine oxidation was obtained using the enrichment of a-KIC in the calculation[22]. Net forearm leucine balance was calculated as the product of the arteriovenous concentration difference and the forearm blood flow. Results are means ± SEM. Statistical comparisons were performed using Student's t-test for paired and for unpaired data as appropriate.

Results. The table demonstrates that plasma epinephrine concentrations increased from basal values of 60 pg/ml to 851 ± 62 pg/ml during combined epinephrine and cortisol infusion, and to values which were somewhat below those observed during epinephrine infusion alone. Cortisol administration resulted in plasma cortisol concentrations of 16.3 µmol/l compared to 0.24 µmol/l during epinephrine infusion alone. Arterialized plasma leucine concentrations decreased during epinephrine infusion alone from 96 to 69 µmol/l ($p < 0.05$) compared to an insignificant decrease (−9 ± 5%) during cortisol infusion. The relative decreases of plasma leucine concentrations were significantly different (fig., $p < 0.01$). Leucine flux (fig.) decreased significantly

Effect of epinephrine infusion alone and of epinephrine combined with cortisol on plasma leucine kinetics, plasma hormones, FFA and glucose concentrations

		Epinephrine (n = 6)			Epinephrine + Cortisol (n = 6)		
		0–30 min	150–210 min	% Change	0–30 min	150–210 min	% Change
Leucine conc. art.	(μmol/l)	96	69*	−28	94 ± 5	85 ± 4	−9 ± 5^{++}
leucine conc. deep venous	(μmol/l	105	79*	−25	97 ± 4	85 ± 4*	−13 ± 4
Leucine flux	(nmol/kg/min)	1280	990*	−23	1033 ± 46	1029 ± 60	−1 ± 3^{+++}
Leucine oxidation	(nmol/kg/min)	670	430*	−29	293 ± 25	248 ± 27	−16 ± 5^{++}
Net leucine forearm balance	(nmol/min/100gr)	−32	−85*	−195	−6 ± 2	+18 ± 11*	+347 ± 247
FFA conc. art.	(μmol/l)	584	899*	+76	710 ± 81	1211 ± 223	+73 ± 26
Glucose conc. art.	(mmol/l)	5.3	12.9***	+143	5.1 ± 0.2	12.7 ± 0.8***	+147 ± 10
Epinephrine conc. art.	(pg/ml)	60	515***	+772	96 ± 7	851 ± 62***	+824 ± 110
Norepinephrine conc. art.	(pg/ml)	51	66	+50	120 ± 41	161 ± 63	+24 ± 35
Cortisol conc. art.	(μmol/l)	0.24	0.14**	−39	n.d.	16.2 ± 0.3	---
Insulin conc. art.	(μE/ml)	14	19	+52	14 ± 6	27 ± 3*	+190 ± 64^{+}
Glucagon conc. art.	(pg/ml)	100	136	+44	88 ± 15	92 ± 9	+7 ± 12

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0005$ vs baseline (0–30 min); $^{+}$ $p < 0.05$; $^{++}$ $p < 0.01$; $^{+++}$ $p < 0.001$ vs epinephrine alone; n.d. = not determined.



Changes of plasma leucine concentrations, leucine flux and leucine oxidation during infusion of epinephrine (50 ng/kg/min) alone (n = 6) and during combined infusion of epinephrine and cortisol (2 μg/kg/min; n = 6). Data are means ± SEM during the last 60 min of infusion; p values refer to differences between the two protocols.

during epinephrine infusion alone whereas hypercortisolemia abolished this decrease. The changes in leucine flux were significantly different in the two investigations ($p < 0.001$).

Leucine oxidation decreased by 29% during epinephrine alone, compared to a 16 ± 5% decrease during elevated cortisol ($p < 0.01$ vs epinephrine alone).

Net forearm leucine release increased by 195% during epinephrine infusion alone; in contrast leucine forearm balance reverted from a small but significant release to net uptake during hypercortisolemia (347 ± 247%); however, because of the large SEM the differences between the two sets of results were not statistically significant (table).

Plasma FFA concentrations increased during epinephrine alone by 76% ($p < 0.05$), and from 710 ± 81 to 1211 ± 223 μmol/l during cortisol. The peak mean FFA concentrations were higher during cortisol but statistically significant differences were not reached. Plasma glucose concentrations increased from 96 to 233 mg/dl during epinephrine administration and similarly during additional cortisol infusion.

Plasma insulin and glucagon concentrations were slightly raised during the replacement infusions. Plasma insulin concentrations were significantly higher during cortisol infusion ($p < 005$) than during infusion of epinephrine alone.

Discussion. Previous studies by others [18] and by us [13] demonstrated that acute elevation of plasma epinephrine concentrations in man resulted in decreased plasma leucine concentrations due to decreased leucine flux. These data suggested an inhibitory effect of epinephrine on total body proteolysis which may play a role in preventing excessive protein losses during severe stress when catabolic hormones such as cortisol are released [6]. To examine this hypothesis, the present study was designed to examine the interaction of elevated plasma cortisol and epinephrine concentrations in regulating leucine kinetics in man. The data demonstrated that the epinephrine-induced decreases of leucine concentration, flux and oxidation were abolished when plasma cortisol was elevated to supraphysiological concentrations.

Epinephrine administration resulted in a rapid increase in plasma FFA and glucose concentrations due to the known lipolytic and glycogenolytic effect of the catecholamines [12]. The increase in FFA availability may explain at least in part the epinephrine-induced decrease of leucine flux [24]. This effect did not explain, however, the stimulatory effect of cortisol on leucine flux. Acute elevation of plasma cortisol to similar concentrations as those observed in the present study resulted in increased leucine flux [23] in the absence of simultaneous epinephrine administration, and in elevation of most other plasma free amino acid concentrations [4]. When prolonged infusion of a mixture of epinephrine, cortisol and glucagon were administered to normal subjects there were net protein catabolic effects [7]. Glucocorticoids have been demonstrated to increase leucine oxidation in skeletal muscle in vitro [20], in agreement with the present findings in vivo. The simultaneous and parallel increases in flux and oxidation of leucine observed in the present study suggested that leucine incorporation into proteins, and thus protein synthesis, were not affected. In contrast, previous studies in vitro

demonstrated inhibitory effects of glucocorticoids on muscle protein synthesis [19]. The present finding that cortisol administration increased leucine flux and therefore protein breakdown is in accord with the previous observation that 3-methylhistidine excretion increased following glucocorticoid administration to rats [9].

In view of the potency of the pancreatic hormones insulin and glucagon in affecting leucine turnover [25] it appeared to be of importance to maintain plasma insulin and glucagon concentrations unchanged during stress hormone infusion. This was attempted by somatostatin adminstration combined with insulin and glucagon replacements. Surprisingly, plasma insulin concentrations were higher during cortisol administration than with epinephrine alone, suggesting either diminished metabolic clearance of insulin, or a breakdown of the somatostatin blockade during cortisol administration. The higher insulin concentrations argue in favor of the role of cortisol in increasing leucine flux since insulin would exert an opposite effect [25].

Thus, the interaction of epinephrine and cortisol in regulating leucine flux demonstrated in the present study suggests that acute hypercortisolemia exerts protein catabolic effects by increasing protein breakdown. These effects are blunted when there is a simultaneous increase in plasma epinephrine. Whether this interaction is equally operative in clinical conditions of severe stress has to be examined in further studies.

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Influence of ventilatory and circulatory changes on the pharmacokinetics of halothane and isoflurane

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Summary. In two groups of dogs, uptake and elimination of halothane and isoflurane were studied using a closed-loop anesthesia system which automatically controlled end-tidal halothane or isoflurane partial pressure at minimal alveolar concentration (MAC) equivalent levels. Hemodynamic and respiratory variables were recorded and the anesthetic partial pressure was measured in the inspired and expired air, as well as in the arterial, cerebrovenous and mixed venous blood. Data were recorded during wash-in, hyperventilation, hypercirculation, hypotension and wash-out. For halothane, the controller delivered a higher inspired partial pressure than for isoflurane to compensate for the higher blood/gas partition coefficient. This was especially pronounced during the wash-in and the hypercirculation periods. Smaller differences between halothane and isoflurane partial pressures occurred during hyperventilation, hypotension and the wash-out period and could be explained by the lower solubility of isoflurane. These results show that even under unstable ventilatory and hemodynamic conditions, the inspired concentration of isoflurane has to be adjusted less often and to a smaller degree than that of halothane if end-tidal concentrations are to be maintained constant.
Key words. Dog anesthesia; halothane; isoflurane; hemodynamic variables; respiratory variables.

The goal of inhalational anesthesia should be to obtain rapidly and safely an adequate partial pressure of the volatile anesthetic in the brain. Isoflurane, because of its reportedly lower partition coefficients (blood/gas and tissue/blood), is considered to have a more favorable pharmacokinetic profile than halothane [4, 7]. Although brain tissue partial pres-