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Synergistic effect of counterregulatory hormones during insulin-induced hypoglycemia in rats: participation of lipolysis and gluconeogenesis to hyperglycemia[1]

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KEY WORDS fasting; insulin; blood glucose; liver glycogen; glucagon; hydrocortisone; adrenergic beta-agonists; gluconeogenesis; nonesterified fatty acids; lipolysis

AIM: To study the synergistic effect of G (glucagon, 0.02 mg·kg^{-1}), H (hydrocortisone, 20 mg·kg^{-1}) and E (phenylephrine + isoproterenol, both 1 mg·kg^{-1}) during insulin-induced hypoglycemia (IIH) in rats with 6 h of food deprivation (F_6 group). **METHODS**: I (insulin, 1 U·kg^{-1}) was injected ip and 30 min later saline (F_6 + I group), H, G and E individually or combined (G+H, G+E, H+E and G+H+E) were all injected ip and all experiments started 1 h after I injection. **RESULTS**: The rise in glycemia with H+G+E was greater than the sum of the responses to ip H, G and E individually or in double combination plus any single hormone. This effect was reproduced by G + H + Iso (isoproterenol, 1 mg·kg^{-1}), G+H+Iso+Met (metoprolol, 1 mg·kg^{-1}) and G + H + Sal (salbutamol, 1 mg·kg^{-1}). A clear relationship was shown between glycemia and free fatty acids levels. Liver gluconeogenesis from glycerol (2 mmol·L^{-1}) was higher in the group which received G+H+β-adrenergic agonist *vs* control rats (F_6 or F_6 + I groups). **CONCLUSION**: (a) Acute hyperglycemia is obtained from a condition of IIH by combined ip of G+H+β-adrenergic agonists; (b) This effect cannot be ascribed to a single hormone, but is a consequence of the combined effects of these substances; (c) Blood insulin levels and liver glycogen have no participation; (d) Lipolysis mediated by a β-adrenergic mechanism and gluconeogenesis from glycerol contribute to the hyperglycemia.

The anti-insulin hormones, glucagon (G), hydrocortisone (H) and epinephrine (Ep) interact synergistically in normoglycemic rats[1], dogs[2] and humans[3]. Except our papers[1,4] synergism of counterregulatory hormones from insulin-induced hypoglycemia (IIH) was never reported by others.

Hypoglycemia induces release[5] of G, H, and Ep, which increase the gluconeogenesis. G and Ep accelerate glycogenolysis. Ep produces elevation in plasma free fatty acids (FFA) levels and decreases the glucose clearance. In hyperinsulinemia the simultaneous increase of these hormones can not blunt insulin action and restore glycemia[5,6].

The perspective of G administration has been the focus of interest[6,7] but little attention[1,4] has been given to the impact of administration of G combined with H and Ep during hypoglycemia.

The purpose of this work was to determine the participation of lipolysis, liver glycogen, and gluconeogenesis[8] to the hyperglycemia promoted by combined injection of counterregulatory hormones during IIH, and to verify the role of insulinemia and advance our understanding of the adrenergic participation.

MATERIALS AND METHODS

Albino ♂ Wistar rats weighing 150 - 250 g were deprived of food from 08:00 to 14:00.

At 14:00 insulin 1 U·kg^{-1} (I) was injected ip and 30 min later saline (control group), G (0.02 mg·kg^{-1}), H (20 mg·kg^{-1}) and/or E* were injected either alone (G, H, E) or combined (G+H, G+E, H+E, G+H+E) One hour after I injection, rats were decapitated and blood was collected

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for serum determinations of glucose[9], insulin[10], and FFA[11]. The liver was taken into 30 % KOH, and glycogen content of powdered liver was measured[12].

For measurement of gluconeogenesis rats were anesthetized (with pentobarbital sodium) and livers were perfused *in situ* through portal vein using Krebs/Henseleit-bicarbonate buffer, pH 7.4, gassed with 95 % O_2 + 5 % CO_2, without recirculation of the perfusate. Glycerol 2 mmol $\cdot L^{-1}$ was infused during the period between 10 and 30 min of perfusion. The gluconeogenic activity was evaluated as the difference between rates of glucose released during glycerol infusion and the corresponding rates before glycerol infusion.

During the time between I injection and the experiments food was not available to the rats.

Rats with 6 h of food deprivation (F_6 group) and rats F_6 which received I and were killed 1 h later (F_6 + I group) were used as controls. In some experiments, fed *ad lib* rats (Fed group) and 24-h fasting rats (F_{24} group) were used as controls. The experimental groups consisted of a F_6 + I which received single or combined injection of counterregulatory hormones 30 min after ip I.

(−) Isoproterenol hydrochloride (Iso) and *L*-phenylephrine hydrochloride (Phe) were purchased from Sigma, USA. Crystaline beef glucagon was from Eli Lilly of Brazil LTDA (São Paulo, Brazil) and regular insulin (Neosulin) was from Biobras (Montes Claros, MG, Brazil).

All drugs above described were injected ip.

Glucagon was based in the dose used as a treatment of hypoglycemia (glucagon from pancreatic extraction, 1 mg, im). Dihydrocortisone was based in the dose used in emergency situations (1000 mg, iv). The dose of adrenergic agonists was based in the dose which promoted synergic effect (combined with glucagon and dihydrocortisone) in normoglycemic rats (Souza *et al*. Arq Biol Tecnol 1994; **37**: 737 − 44).

Statistical analyses were done by ANOVA using a computer program (Primer Bioestatistics: The Program).

RESULTS

Because hypoglycemia was well established 30 min after ip I[4], this time was selected for ip counterregulatory hormones. The decision to wait 30 min after the ip of these hormones to start the experiments was based on that these hormones caused hyperglycemia 30 min (not 90 min), after ip from normoglycemia or hypoglycemia[1]. Based on this design[1,4], we found a synergistic effect of G+H+E, causing acute hyperglycemia from a condition of IIH (Fig 1). The basal value for glucose concentration was 400 mg $\cdot L^{-1}$ resulted in an increase of 250 % in glycemia with ip G+H+E. The rise in plasma glucose produced by G+H+E was greater ($P<0.05$) than the sum of the effect of each hormone ip individually or the sum of the responses to each combination of 2 counter-regulatory hormones and the respective complementing single-hormone ip.

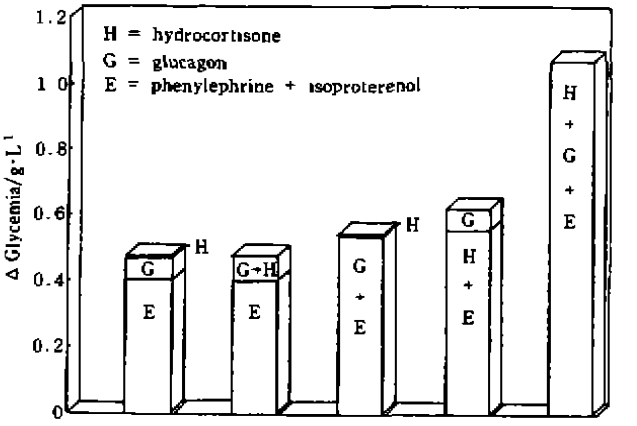

Fig 1. Changes in glycemia obtained by isolated or combined ip of G (0.02 mg $\cdot kg^{-1}$), H (20 mg $\cdot kg^{-1}$) and E. To compare the participation of α- and β-adrenergic mechanisms, epinephrine was replaced by phenylephrine + isoproterenol (both 1 mg $\cdot kg^{-1}$).

A clear relationship was found between glycemia and FFA levels. Hyperglycemia and increased FFA were obtained with G+H+Iso. In contrast, ip Phe combined with G + H did not promote glucose recovery (Fig 2).

I ip resulted in a hyperinsulinemia. I levels increased about 6 folds in the groups which received I ip (120 mU $\cdot L^{-1}$) *vs* F_6 group (20 mU $\cdot L^{-1}$).

Hepatic glycogen was higher ($P<0.05$) in the Fed group. In addition, 6 h of food deprivation (F_6 group) caused a decrease ($P<0.05$) in the hepatic glycogen, which was not affected by ip I (F_6 + I group) or anti-insulin hormones (F_6 + I + G + H + Iso group). Hepatic glycogen levels during normoglycemia (F_6 group), hypoglycemia (F_6 + I group) and hyperglycemia (F_6 + I + G + H + Iso group) were similar (Tab 1).

* To study the participation of α and β adrenergic mechanisms, epinephrine was replaced by: Phe + Iso (both 1 mg $\cdot kg^{-1}$); Phe (1 mg $\cdot kg^{-1}$); Iso (1 mg $\cdot kg^{-1}$); Iso + metoprolol (both 1 mg $\cdot kg^{-1}$) or salbutamol (1 mg $\cdot kg^{-1}$).

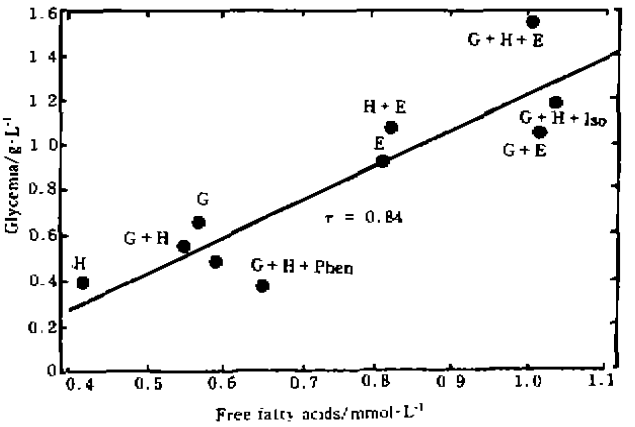

Fig 2. Relationship between glycemia and FFA in rats injected ip insulin 1 U·kg^{-1} and 30 min later: saline (I group), G, H, E, G+H, G+E, H+E, G+H+E, G+H+Iso or G+H+Phen injection. G (glucagon, 0.02 mg·kg^{-1}), H (hydrocortisone, 20 mg·kg^{-1}), E (phenylephrine + isoproterenol, both 1 mg·kg^{-1}), Phe (phenylephrine, 1 mg·kg^{-1}), Iso (isoproterenol, 1 mg·kg^{-1}).

Tab 1. Liver glycogen and glycemia of 6-h-fast rats (F_6) injected ip: a) saline (F_6 group); b) insulin, 1 U·kg^{-1}(F_6 + I, group); c) insulin and 30 min later a combined injection of hydrocortisone 20 mg·kg^{-1}(H) + glucagon 0.02 mg·kg^{-1}(G) + isoproterenol 1 mg·kg^{-1}(Iso) (F_6) + I + H + G + Iso group). Fed (Fed group) and 24-h fast rats (F_{24} group) were included as controls. $\bar{x} \pm s$.
$^aP>0.05$, $^bP<0.05$ *vs* F_6 + I.

Rats	*n*	Liver glycogen/ mg·g^{-1}	Glycemia/ mg·L^{-1}
Fed	11	37.8±2.9^b	1 129±102^b
F_6	16	14.7±2.6^a	1 123±43^b
F_6 + I	10	18.5±2.2	525±79
F_6 + I + (G+H+Iso)	6	23.9±5.8^a	1 746±370^b
F_{24}	8	1.6±0.2^b	730±49^b

F_6 rats submitted to IIH which received ip Iso (combined with G + H) were compared with Iso + metoprolol (combined with G + H) or Sal (combined with G + H). The results showed similar increase of glucose and FFA for all groups (Tab 2).

Glucose production from glycerol (2 mmol·L^{-1}) in perfused liver *in situ* showed similar results in F_6 + I + G + H + Sal group and fasting rats (F_{24} group) (Fig 3). The basal glucose production was lower in F_{24} group. This difference corresponds to the glucose released from glycogen, which was lower in F_{24} rats (Tab 1).

Tab 2. Percent of increase in free fatty acids (FFA) and glycemia from 6-h-fast rats (F_6) injected ip insulin (I) 1 U·kg^{-1} and 30 min later saline (F_6 + I group) or a combined ip of glucagon 0.02 mg·kg^{-1}(G) + hydrocortisone 20 mg·kg^{-1}(H) + isoproterenol 1 mg·kg^{-1}(Iso) (F_6 + I + G + H + Iso group), G + H + Iso + metoprolol 1 mg·kg^{-1}(Met) (F_6 + I + G + H + Iso + Met group) or G + H + salbutamol 1 mg·kg^{-1}(Sal) (F_6 + I + G + H + Sal group). The results were expressed as percent of increase from F_6 + I rats. $\bar{x} \pm s$. $^aP>0.05$ *vs* F_6 + I + G + H + Iso group.

Rats	*n*	FFA	Glycemia
F_6 + I + (G+H+Iso)	6	47±12	156±28
F_6 + I + (G+H+Iso+Met)	7	41±10^a	181±19^a
F_6 + I + (G+H+Sal)	6	40±9^a	153±25^a

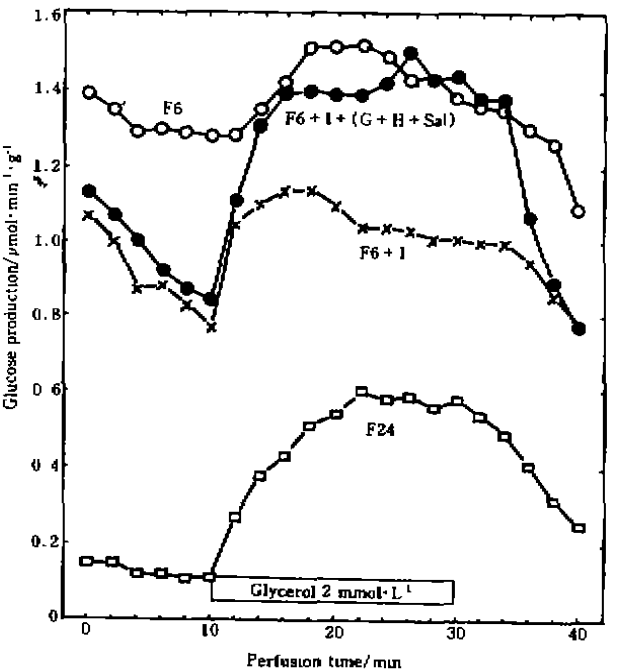

Fig 3. Glucose production from glycerol in perfused liver *in situ*. The effluent perfusate was sampled in 2-min intervals. *n* = 6 - 8 livers. F_{24} group: 24-h-fast, F_6 group: 6-h-fast, F_6 + I group: F_6 rats ip insulin 1 h before perfusion, F_6 + I + (G + H + Sal) group: F_6 rats ip a combined injection of glucagon (G) + hydrocortisone (H) + salbutamol (Sal) 30 min after insulin and were submitted to liver perfusion 30 min later.

DISCUSSION

A role for insulinemia, glycogenolysis, gluconeogenesis, adrenergic mechanisms, and lipolysis to glucose recovery after IIH was well-known[5,8,13]. But these investigations did not use

administration of anti-insulin hormones.

Experiments using pharmacological doses of anti-insulin hormones during hypoglycemia usually employed G[6,7]. This rat model[1,4] involved not only G but also H and adrenergic agonists during IIH. Our results demonstrated that G injected simultaneously with H and Iso promoted hyperglycemia from hypoglycemia.

To understand better how hyperglycemia can be obtained, a further study on the role of hepatic glycogen, insulin levels, adrenergic mechanisms, lipolysis, and gluconeogenesis was made.

Acute hyperglycemia was obtained with simultaneous administration of H + G + E during IIH (Fig 1). The influence of intestinal absorption of glucose can not be considered because we employed 6-h-fast rats (F_6 group), in which glycogen was markedly depleted ($P<0.05$) *vs* Fed group (Tab 1). But 6-h-fast (F_6 group) represented a short fasting because gluconeogenesis during this time was not significantly increased (Fig 3).

An unexpected observation was that liver glycogen was not depleted in the group which received combined injection of G + H + Iso during IIH. Liver glycogen was basically the same (Tab 1) in conditions of normoglycemia (F_6 group), hypoglycemia (F_6 + I group), and hyperglycemia (F_6 + I + G + H + Iso group). Because insulin promoted phosphorylase inactivation and activates glycogen synthetase, the hyperinsulinemia in these groups could provide a possible explanation for this result. Alternatively, the absence of a decrease in liver glycogen did not exclude increased glycogenolysis.

The increased glycemia and FFA after ip of G + H + Iso, G + H + Iso + Met or G + H + Sal suggested that glucose counterregulation during IIH depends on lipolysis mediated by a β-adrenergic mechanism (probably β_2). This suggestion is consistent with the study[14] which showed that lipolysis and β-adrenergic mechanisms play a central role in defense against hypoglycemia.

The concept of glucose/fatty acid cycle was introduced to explain the reciprocal relationship between the rates of oxidation of glucose and fatty acids[15]. When fatty acids are mobilized from adipose tissue, their rate of oxidation by muscle increases, which, in turn, decreases the rate of glucose utilization. Based on this concept, we might speculate that raised FFA levels (Fig 2) could produce a "Randle effect" and prevent a decrease in glycemia. In addition, increased formation of reducing equivalents by β-oxidation in liver can give favorable conditions to liver gluconeogenesis.

Despite high insulin levels, gluconeogenesis from glycerol was similar (Fig 3) in 24-h-fast rats (F_{24} group) and the rats which received simultaneous injection of H + G + Sal during IIH. Since glycerol probably is enhanced in this group (Fig 2), the possibility of an increased gluconeogenesis should be considered. It is interesting to consider that glycerol, (in contrast to lactate, pyruvate and alanine) enters in the gluconeogenic pathway after the mitochondrial step and can bypass the inhibition of PEPCK promoted by insulin.

Finally, it must be stressed that our recent results[1,4] and the considerations herein discussed support the suggestion that lipolysis mediated by a β-adrenergic mechanism and gluconeogenesis contributed to the powerful glucose counterregulation during IIH when G was injected simultaneously with H and β-adrenergic agonists.

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在大鼠胰岛素引发的低血糖过程中对抗调节激素间的协同作用：脂肪分解与糖元异生参与高血糖

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关键词 禁食；胰岛素；血糖；肝糖元；高血糖素；氢化可的松；肾上腺素β激动剂；糖元异生；非酯类脂肪酸类；脂肪分解

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