Adrenergic Influence on Glucocounterregulation in Man

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Summary. To investigate the adrenergic role in glucocounterregulatory mechanisms, single-blind randomised studies were performed in 7 normal males during severe insulin-induced hypoglycaemia with or without adrenergic blockade. Intravenous phentolamine administration (5 mg stat and 0.5 mg/min) did not interfere with the restoration of euglycaemia from hypoglycaemia. However, recovery of blood glucose in the presence of propranolol (3 mg/3 min and 0.8 mg/min) was retarded when compared with control studies (mean plasma glucose levels $\pm$ SEM, 50 ± 6 mg/dl versus 66 ± 4 mg/dl at 120 min after insulin administration) despite appropriate glucagon, epinephrine, cortisol, and growth hormone responses. Plasma norepinephrine response was unaffected by propranolol but augmented threefold by phentolamine. Increases in plasma lactate, pyruvate and non-esterified fatty acids were blunted with propranolol while rebound non-esterified fatty acid was observed with phentolamine infusion. These data suggest that complete recovery of blood glucose from severe hypoglycaemia requires full sympathetic nervous system activity despite the integrity of other counterregulatory mechanisms.

Key words: Hypoglycaemia, adrenergic blockade, catecholamines, glucagon, cortisol, growth hormone, lactate, pyruvate, non-esterified fatty acids.

Glucose homeostasis under normal circumstances is mediated largely by insulin but, during hypoglycaemia, counterregulatory mechanisms become important. These include the release of catecholamines, glucagon, growth hormone, and cortisol, apparently triggered by a critical blood glucose level rather than the rate of fall in glucose concentration [1, 2]. Many investigations have been focussed on the role of the sympathetic nervous system in glucose homeostasis, but there is no general agreement as to whether adrenergic mechanisms participate in the restoration of blood glucose in the face of glucopenia.

Propranolol, a β -adrenergic blocker, has been shown to retard glucose recovery from insulin-induced hypoglycaemia in man [3–5]. Lack of glucocounterregulation during 2-deoxy-D-glucose-induced intracellular glucopenia in adrenalectomised subjects [6] and in subjects where the sympathetic outflow is interrupted [7], supports the importance of adrenergic mechanisms in glucose homeostasis. However, doubt is cast on the essential role of adrenergic mechanisms in glucose recovery from insulin-induced hypoglycaemia by the finding that restoration of euglycaemia is unaffected in adrenalectomised subjects [8–11] or by adrenergic blocking agents [12–14] in the presence of other counterregulatory hormonal responses.

The present study was conducted to examine the influence of adrenergic mechanisms on the recovery from severe insulin-induced hypoglycaemia and on substrate mobilisation.

Materials and Methods

Seven healthy male subjects, aged 19–30 years, within 10% of ideal body weight (Metropolitan Life Insurance Company Tables 1959), 60–82 kg, participated in the study after giving informed consent, with the right to withdraw from the study. None were suffering from any illness or taking any medication during the study. The design of the study consisted of six experiments administering the following treatments: 1) insulin (bolus of 0.15 U/kg IV) and saline (0.154 mol/l) infusion ($n = 7$); 2) insulin and propranolol (3 mg IV over 3 min then 0.8 mg/min) ($n = 7$); 3) insulin and phentolamine (5 mg IV stat then 0.5 mg/min) ($n = 5$); 4) saline and propranolol ($n = 5$); 5) saline and phentolamine ($n = 4$); 6) saline alone ($n = 4$). All experiments were conducted in a single blind, randomised fashion at 1 week intervals after overnight fasting. All subjects were instructed to consume 300 g carbohydrate daily for 3 days prior to each experiment. At 0800 h indwelling 19 G butterfly needles, kept patent with 0.154 mol/l saline, were inserted via both antecubital veins. Subjects remained supine for at least 45 min before blood samples were obtained. After baseline sampling at -10 and 0 min, crystalline insulin or sham injection with saline was given as an IV bolus via a contralateral indwelling needle. Where appropriate, propranolol or phentolamine infusion was commenced immediately before insulin administration. Sham infusion with saline was given in a similar fashion, and the volume of all solutions was kept constant. Blood pressure, pulse rate and subsequent blood samples were obtained

Table 1. Comparison of plasma substrate and hormone responses (mean $\pm$ SEM) to insulin-induced hypoglycaemia with or without adrenergic blockade in normal subjects

Treatment	Time	Glucose mg/dl	Lactate mmol/l	Pyruvate mmol/l	NEFA μ mol/l	Epi- nephrine pg/ml	Norepi- nephrine pg/ml	Immuno- reactive glucagon pg/ml	Cortisol μ g/ml	Growth hormone ng/ml
Insulin- alone (n = 7)	0 min	91 $\pm$ 3	1.14 $\pm$ 0.1	0.068 $\pm$ 0.004	378 $\pm$ 76	30 $\pm$ 8	172 $\pm$ 30	81 $\pm$ 8	12.3 $\pm$ 1.6	1.2 $\pm$ 0.05
	peak/nadir	25 $\pm$ 3	2.62 $\pm$ 0.2	0.135 $\pm$ 0.011	224 $\pm$ 31	945 $\pm$ 125	331 $\pm$ 49	258 $\pm$ 43	27.9 $\pm$ 0.8	48.8 $\pm$ 7.0
	120 min	66 $\pm$ 4	1.73 $\pm$ 0.2	0.102 $\pm$ 0.009	345 $\pm$ 32	76 $\pm$ 13	242 $\pm$ 29	105 $\pm$ 18	26.6 $\pm$ 1.1	38.5 $\pm$ 16.7
Insulin- propranolol (n = 7)	0 min	91 $\pm$ 4	1.09 $\pm$ 0.1	0.068 $\pm$ 0.011	379 $\pm$ 54	26 $\pm$ 9	151 $\pm$ 29	83 $\pm$ 9	13.2 $\pm$ 1.4	1.5 $\pm$ 0.1
	peak/nadir	24 $\pm$ 1	1.52 $\pm$ 0.1 ^d	0.105 $\pm$ 0.012	174 $\pm$ 15	1905 $\pm$ 149 ^d	282 $\pm$ 32	260 $\pm$ 58	30.9 $\pm$ 2.2	68.5 $\pm$ 8.8 ^c
	120 min	50 $\pm$ 6 ^a	1.18 $\pm$ 0.1 ^b	0.072 $\pm$ 0.009	182 $\pm$ 29 ^c	356 $\pm$ 114 ^b	190 $\pm$ 30	177 $\pm$ 30 ^c	28.3 $\pm$ 2.5	62.8 $\pm$ 9.8 ^c
Insulin- phenolamine (n = 5)	0 min	93 $\pm$ 2	0.98 $\pm$ 0.1	0.067 $\pm$ 0.008	338 $\pm$ 28	25 $\pm$ 13	161 $\pm$ 40	72 $\pm$ 8	12.9 $\pm$ 2.3	1.4 $\pm$ 0.3
	peak/nadir	26 $\pm$ 3	2.67 $\pm$ 0.4	0.150 $\pm$ 0.011	193 $\pm$ 27	1073 $\pm$ 249	913 $\pm$ 188 ^c	203 $\pm$ 38	28.1 $\pm$ 0.8	36.5 $\pm$ 5.7
	120 min	71 $\pm$ 3	1.82 $\pm$ 0.4	0.099 $\pm$ 0.066	575 $\pm$ 105 ^a	149 $\pm$ 38	800 $\pm$ 139 ^c	103 $\pm$ 18	23.4 $\pm$ 1.8	21.0 $\pm$ 3.8 ^b

^a $p < 0.05$; ^b $p < 0.025$; ^c $p < 0.01$; ^d $p < 0.005$. P values represent significance of difference from studies with insulin alone by paired t-test

at 10 min intervals for the first hour and at 15 min intervals for the second hour. Additional blood samples (0.3 ml) were obtained at 5-min intervals during the first hour for glucose determinations with a Beckman glucose analyser.

Blood for catecholamines was collected in chilled heparinised tubes containing reduced glutathione and EGTA, for glucagon in tubes containing 500 KIU of trasylol (FBA Pharmaceuticals, Leverkusen-Bayerwerk, West Germany) and EDTA (1.5 mg/ml of blood) and for growth hormone and cortisol in heparinised tubes. Plasma was separated after refrigerated centrifugation and kept frozen until analysed. Catecholamine levels were determined by radioenzymatic assay [15] with modification [2]. The intra- and interassay coefficients of variation were less than 10% at the range of concentration reported in the Result Section, and the sensitivity of the assay based on twice the blank count varied between 5–10 pg/ml. Plasma glucagon levels were determined on acetone extracts of plasma [12] by radioimmunoassay using 30K antiserum [16]. The intra- and interassay coefficients of variation were within 5% at the level of 300 pg/ml. The sensitivity of the assay was 50 pg/ml. Measurement of growth hormone was performed by radioimmunoassay kit from Kallestad Laboratories (Chaska MN), and a radioimmunoassay kit from Diagnostic Products (Los Angeles CA) was used for plasma cortisol assay. Plasma was stored at -20°C for non-esterified fatty acid (NEFA) [17, 18] and lactate [19] determination. Aliquots of plasma were immediately deproteinised with 3 mol/l perchloric acid and supernatant was frozen at -70°C until assayed for pyruvate [20], of which there was no detectable degradation within 3 months of storage.

The results were expressed as mean $\pm$ SEM and significance of difference between the means was determined by Student's paired t-test. Significance of overall difference in each variable between experiments was determined by twoway Anovar with the interaction of two trial factors, treatment and time, using P2V program, BMDP-1977, University of California.

Results

Following IV insulin alone, systolic blood pressure increased significantly from 103 ± 4 mmHg to 122 ± 6 mmHg, while diastolic blood pressure declined from 73 ± 4 mmHg to 59 ± 4 mmHg, with a rise in pulse rate from 58 ± 2 beats/min to 80 ± 6 beats/min at 30 min ($p < 0.005$). Following insulin-propranolol, there were increases in both systolic and diastolic blood pressure from 104 ± 5 mmHg to 116

± 5 mmHg, and 75 ± 3 mmHg to 84 ± 4 mmHg at 30 min respectively ($p < 0.01$), but pulse rate remained at 54 ± 2 beats/min. Insulin-phenolamine similarly raised systolic blood pressure to 115 ± 9 mmHg but markedly decreased diastolic blood pressure from 71 ± 2 mmHg to 48 ± 2 mmHg at 30 min ($p < 0.01$), and pulse rate rose from 58 ± 3 beats/min to 106 ± 10 beats/min ($p < 0.01$). The comparison of the metabolic substrate and hormonal responses with or without adrenergic blockade is summarised in Table 1.

Insulin Alone

After insulin alone (Fig. 1), plasma glucose level declined from 91 ± 3 mg/dl to a nadir of 25 ± 3 mg/dl at 25 min; it then rose abruptly by 40 min and subsequently reached 66 ± 4 mg/dl at 120 min. Baseline plasma epinephrine concentration was 30 ± 8 pg/ml; it had risen significantly by 30 min ($p < 0.01$) and reached a peak level of 945 ± 125 pg/ml ($p < 0.0005$) at 40 min. Plasma norepinephrine was 172 ± 30 pg/ml initially, rising significantly to levels of 225 ± 39 pg/ml by 20 min ($p < 0.01$), and achieving peak levels of 331 ± 49 pg/ml at 40 min. Its level remained elevated through the whole 120 minutes of study. Plasma glucagon concentration increased by 30 min ($p < 0.005$) from the baseline level of 81 ± 8 pg/ml and reached 258 ± 43 pg/ml ($p < 0.0025$) at 40 min. Plasma cortisol rose by 40 min ($p < 0.01$) from 12.3 ± 1.6 μ g/dl and attained the level of 27.6 ± 1.5 μ g/dl at 60 min ($p < 0.0005$). Plasma growth hormone increased from baseline level of 1.2 ± 0.05 ng/ml to 14.9 ± 5.2 ng/ml and 48.8 ± 7.0 ng/ml at 40 min ($p < 0.05$) and 60 min ($p < 0.001$) respectively, and remained elevated throughout 120 min.

Figure 2 illustrates changes of plasma lactate, pyruvate, and NEFA following IV insulin with and without β -adrenergic blockade. After insulin alone,

Fig. 1. Plasma glucose, epinephrine, norepinephrine, glucagon, cortisol, and growth hormone levels (mean $\pm$ SEM) following insulin 0.15 U/kg IV with continuous saline (---○---) or propranolol (3 mg/3 min, 0.8 mg/min; —●—) infusion in 7 normal male subjects. * $p < 0.05$; ** $p < 0.025$; † $p < 0.01$; ‡ $p < 0.005$. (p values represent significance of differences between saline vs propranolol by paired t-test)

plasma lactate concentration rose from 1.14 ± 0.1 mmol/l to 1.48 ± 0.11 mmol/l by 20 min ($p < 0.005$) and 2.62 ± 0.2 mmol/l at 60 min ($p < 0.0025$). Plasma pyruvate increased from the baseline level of 0.068 ± 0.004 mmol/l to 0.135 ± 0.01 mmol/l at 60 min ($p < 0.0025$), while the lactate/pyruvate ratio did not significantly change. Plasma NEFA levels declined from the baseline level of 378 ± 76 μ mol/l to 224 ± 31 μ mol/l by 20 min ($p < 0.025$) then subsequently rose towards baseline values.

Insulin and Propranolol

During insulin-propranolol (Fig. 1), plasma glucose declined to a nadir comparable to that found with insulin alone. Recovery of plasma glucose levels from 75 to 120 min was, however, retarded, being only 50

± 6 mg/dl at 120 min ($p < 0.05$, by paired t-test). Plasma glucose response from 60 min to 120 min was significantly different from the control ($F = 6.52$, $p < 0.001$). During the first 20 min following administration of propranolol, plasma epinephrine was unaffected, but the subsequent response from 40 to 120 min was approximately 2- to 3-fold of control. The overall epinephrine responses between control and propranolol were significantly different ($F = 5.62$, $p < 0.0001$). Plasma norepinephrine responses were, in contrast, not significantly affected. The increment in plasma glucagon was unaffected but the recovery of glucagon level was significantly delayed ($p < 0.01$). Plasma cortisol responses were virtually unaffected by propranolol but growth hormone response was augmented significantly. During insulin-propranolol, plasma lactate response was markedly decreased (Fig. 2). Pyruvate response was also

Fig. 2. Plasma lactate, pyruvate and NEFA levels (mean $\pm$ SEM) following insulin 0.15 U/kg IV with continuous saline (---○---) or propranolol (3 mg/min, 0.8 mg/3 min; —●—) infusion in 7 normal male subjects. * $p < 0.05$; ** $p < 0.025$; † $p < 0.01$; ‡ $p < 0.005$. (p values represent significance of differences between saline vs propranolol by paired t-test)

significantly less than control ($F = 2.33$, $p < 0.03$), but the difference was significant by paired t-test only at 75 min. Plasma NEFA levels declined following insulin administration and remained suppressed with propranolol infusion compared to saline alone ($F = 4.19$, $p < 0.0007$).

Insulin and Phentolamine

Comparison of plasma glucose, epinephrine, norepinephrine, glucagon, cortisol, and growth hormone responses following IV insulin with and without α -adrenergic blockade are shown in Figure 3. Plasma glucose levels fell to similar values with and without α -blockade and there was no significant difference in the rate of recovery ($F = 1.3$, $p > 0.3$). There was no difference in the magnitude of the epinephrine response, but the recovery of epinephrine was significantly retarded with phentolamine treatment. In contrast, sustained elevation of norepinephrine level was observed throughout the phentolamine infusion period ($F = 15.77$, $p < 0.0001$). Plasma cortisol and glucagon responses

were not statistically altered by α -blockade, while the growth hormone response was significantly smaller only at 120 min ($p < 0.025$). The responses of plasma lactate, pyruvate and NEFA levels are shown in Figure 4. The lactate and pyruvate responses were unchanged between the experiments. However, with phentolamine, NEFA levels promptly recovered by 40 min as compared to saline, and maintained this level throughout the 120 min ($F = 5.14$, $p < 0.004$).

Saline, Propranolol, and Phentolamine Alone

Saline infusion alone for 2 hours did not significantly affect the levels of any metabolic substrates and hormones measured. Either blockade alone did not affect plasma glucose, lactate, pyruvate, NEFA, glucagon, cortisol, growth hormone and epinephrine levels, with the exception that after propranolol administration, growth hormone rose to 6.6 ± 1.9 ng/ml ($p < 0.06$) at 120 min. Following propranolol infusion there was transient and modest increase in norepinephrine concentration from 50 to 90 min when compared to baseline levels, but the overall responses were not significantly different from saline alone ($F = 1.56$, $p > 0.1$). After phentolamine infusion, plasma norepinephrine rose significantly by 10 min to a level of 255 ± 65 pg/ml ($p < 0.05$) and continued to increase to a level of 932 ± 354 pg/ml at 120 min. Its overall measurements were significantly different from saline alone ($F = 4.97$, $p < 0.0005$).

Discussion

The present study shows that blood glucose recovery from severe insulin-induced hypoglycaemia is retarded by β -adrenergic blockade with propranolol despite appropriate counterregulatory hormonal responses. This finding, in agreement with previous reports, both in animals [21, 22] and in man [3–5, 23, 24] supports the concept that β -adrenergic mechanisms play an important role in the acute restoration of euglycaemia following hypoglycaemia. It is, however, important to recognise that propranolol does not completely prevent a compensatory rise in plasma glucose levels, possibly due to the availability of glucagon. Furthermore, as shown in the present study, the return of glucagon levels toward baseline during the recovery period was delayed, perhaps responding to the sluggish increase in plasma glucose toward baseline. Without such a compensatory response, glucose recovery could have been even further impaired. Other investigations have shown that somatostatin inhibition of glucagon secretion during hypoglycaemia decreased glucose production

Fig. 3. Plasma glucose, epinephrine, norepinephrine, glucagon, cortisol, and growth hormone levels (mean $\pm$ SEM) following insulin 0.15 U/kg IV with continuous saline (---○---) or phentolamine (5 mg stat, 0.5 mg/min; —●—) infusion in 5 normal male subjects. * $p < 0.05$; ** $p < 0.025$; † $p < 0.01$. (p values represent significance of differences between saline vs phentolamine by paired t-test)

and subsequently led to a delayed rise in glucose [14, 25]. The reason that other groups have failed to show an effect of adrenergic blockers, either β -adrenergic alone [12, 13], or combined β - and α -adrenergic blockers [14], on plasma glucose recovery remains unclear. It is possible that in some of these latter studies, giving smaller doses of insulin, there was less profound hypoglycaemia, and the glucagon response alone was sufficient to normalise blood glucose. However, where more severe hypoglycaemia is produced, combined homeostatic mechanisms are necessary for the restoration of euglycaemia. In fact, impairment of glucose recovery was greater when combined adrenergic blockers were administered in addition to somatostatin inhibition of glucagon release than during somatostatin infusion alone [14]. Hypoglycaemia in the adrenalectomised subjects, whose glucagon release was suppressed by somatos-

tatin, was also more profound when compared to that in the normal individuals receiving a similar treatment [25]. Therefore, these findings suggest that adrenergic mechanisms may indeed play a contributory role in immediate glucose counterregulation.

Enhancement of (stimulatory) epinephrine levels, but not of norepinephrine, associated with hypoglycaemia during propranolol treatment, is similar to the effect noted in the hypertensive subjects receiving metoprolol [26], while phentolamine infusion resulted predominantly in an increase in norepinephrine concentration. Thus, combined α - and β -adrenergic blockade during hypoglycaemia could account for increases observed in both epinephrine and norepinephrine [14] levels. The possible mechanism for increase in epinephrine levels during β -adrenergic blockade may be due to decreased epinephrine uptake, as propranolol has been shown to augment

Fig. 4. Plasma lactate, pyruvate and NEFA levels (mean $\pm$ SEM) following insulin 0.15 U/kg IV with continuous saline (---○---) or phentolamine (5 mg stat, 0.5 mg/min; —●—) infusion in 5 normal male subjects. * $p < 0.05$; ** $p < 0.025$; † $p < 0.01$. (p values represent significance of differences between saline vs phentolamine by paired t-test)

the rise in plasma epinephrine concentration during exogenous epinephrine infusion [27]. However, the effect of propranolol on non-stimulatory epinephrine levels was not evident in our study.

Our data, in agreement with others [3, 4, 28], suggest that mobilisation of lactate, pyruvate, and lipolysis are mediated by β -adrenergic receptors under enhanced sympathetic nervous system activity. Marked suppression of lactate, pyruvate, and NEFA mobilisation was observed in the presence of higher circulating epinephrine level. Although glucose turnover studies were not performed, lack of lactate and pyruvate substrate availability may decrease hepatic gluconeogenesis [29, 30], thereby impairing the restoration of blood glucose from hypoglycaemia. Since not all hepatic glucose output from gluconeogenesis is derived from lactate [31], impaired glucose recovery from shortage of this fuel can only be partial. It has also been suggested that NEFA can promote gluconeogenesis via the generation of acetyl CoA [32]. However, the higher NEFA level observed during hypoglycaemia accompanying α -adrenergic blockade was not significantly associated with more

rapid glucose recovery, suggesting that the latter pathway is less important in this event. Alternatively, hepatic NEFA uptake is in some way interfered with during α -receptor blockade.

While insulin is by and large known to dominate the control of substrate economy, evidence both in vitro [33] and in vivo [34, 35] exists that growth hormone can promote lipolysis. Our data, showing a diversified response of growth hormone and NEFA following hypoglycaemia with adrenergic blockade, suggest that the mechanism for NEFA mobilisation under this circumstance is predominantly mediated by adrenergic activity. It has also been shown that growth hormone [36] and cortisol [37] can increase hepatic glucose output, but it is unlikely that such hormonal milieu are involved in the immediate counterregulatory function. Inhibition of growth hormone [14, 25, 38] and cortisol [38, 39] responses during hypoglycaemia did not affect the restoration of blood glucose. Conversely, marked growth hormone and cortisol responses in sympathectomised subjects were not associated with substrate mobilisation during glucopenia [6, 7].

During counterregulation, the relationships of catecholamine response and glucose flux have been emphasised [40], but more recent studies show that the glucagon response precedes that of any other counterregulatory hormones [14]. It is noteworthy, however, that using a similar protocol, significant increments in norepinephrine preceding those of other hormonal responses were observed in our study. It has been thought that exogenous intravenous insulin itself may be responsible for such norepinephrine changes [2, 41]. Finally, hypoglycaemia by itself can initiate hepatic compensatory response, as increase in glucose output has been shown to occur independent of other regulatory mechanisms [42, 43].

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