Counterregulation to acute and recurrent hypoglycemia in rats
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Chapter 2

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Insulin-induced hypoglycemia elicits a number of counterregulatory responses, including activation of the autonomic nervous system and secretion of hormones. It has been suggested that these responses depend not only on glucose levels but also on insulin levels. The nutritional state of the body may also play a role. In the present study, the hormonal responses to hypoglycemia were studied at different insulin levels and in different nutritional states. Hypoglycemia was induced in normally fed and 48h food deprived rats by intravenous infusion of different insulin doses, ranging from 5.5 to 120 (fed rats) and 0.44 to 22 mU.kg⁻¹.min⁻¹ (fasted rats). Blood samples were frequently withdrawn for determination of glucose, insulin, glucagon, adrenaline, noradrenaline and corticosterone. In normally fed rats receiving insulin, the reduction in glucose levels was similar for almost all insulin doses. In contrast, the counterregulatory responses were different between the insulin doses. Low doses of insulin increased only glucagon, higher insulin loads led to dose-dependent increases in both glucagon and adrenaline levels, and the highest insulin dose was accompanied by increases in glucagon, adrenaline as well as corticosterone. In 48h fasted rats, a similar pattern was observed. Different doses of insulin resulted in similar glucose nadir levels and dose-dependent responses in glucagon, adrenaline, noradrenaline and corticosterone. However, when compared with normally fed rats, the nadir for glucose was lower and the magnitude of the counterregulatory responses was higher in the fasted rats. Together, these data provide evidence for a tight control of glucose levels during hypoglycemia, and a strong and complex coordination of the different hormonal counterregulatory responses, partly dependent on the ambient insulin levels and the nutritional state.

Introduction

Hypoglycemia is a common complication in insulin-treated diabetes. It is counteracted by counterregulatory responses to restore euglycemia. The counterregulatory responses to insulin-induced hypoglycemia have been described in humans (5, 7, 8, 19) and in animals such as rats (2, 20, 39) and dogs (13, 15). Several types of counterregulatory responses can be identified: local responses (most notably the immediate changes in glucose production by hepatic autoregulation), endocrine responses (changes in secretion of a range of hormones), and behavioral responses (initiation of food intake). Hierarchical relations have been proposed for the hormonal responses, generally posing that hypoglycemia is initially counteracted by an increase in glucagon secretion, then by activation of the adrenal medulla leading to adrenaline secretion, and finally followed by the release of other counterregulatory hormones such as glucocorticoids and growth hormone (4, 19, 21, 25).

There are several factors that may influence the counterregulatory response to hypoglycemia. It may be obvious that the circulating glucose level (i.e. the depth of hypoglycemia) is the primary factor that determines the magnitude of the counterregulatory responses (6, 27). But insulin by itself also seems to have an effect on the counterregulatory responses both in normal subjects (9) and in type 1 diabetes patients (10, 24), although there are some conflicting data (12). Less is known about the influence of the nutritional state of the body on the counterregulatory responses to hypoglycemia. Especially fasting can be of importance, since fasting is associated with many metabolic changes all aiming to spare
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glucose for the brain (23, 33). Insulin levels go down, insulin sensitivity increases, and glucose levels decrease. Hepatic glycogen stores will become depleted and gluconeogenesis will become increasingly important in the maintenance of blood glucose levels. These changes may significantly aggravate the threat induced by insulin and at the same time impede the increased endogenous glucose production needed to restore normoglycemia. It might also increase the importance of the level of insulinemia for the counterregulatory responses.

The current study was designed to investigate the importance of insulin and of fasting in the counterregulatory responses to hypoglycemia. Therefore, we studied the onset, duration, and magnitude of different counterregulatory responses to insulin-induced hypoglycemia in rats by varying the ambient insulin levels as well as the nutritional state.

Methods

Animals and surgery

Male Wistar rats were used, weighing 300-330 grams at the beginning of the experiments. They were individually housed in 25*25*30 cm cages with wood shavings bedding. Room temperature was 21 ± 1 °C and the lights were on from 08:00 until 20:00. Food (standard RMH chow, Hope Farms, Woerden, The Netherlands) and water were available ad lib unless otherwise stated. The animals were frequently handled and weighed.

Under halothane/N2O inhalation anesthesia, all rats were fitted with two permanent silicone catheters (Medica BV, Den Bosch, The Netherlands), one for i.v. infusions and the other for stress-free blood sampling. Both catheters were inserted via the jugular vein, according to the principle described by Steffens (31, 37), with the catheter tips ending in the superior vena cava just before the right atrium. In one group of rats, only the catheter for infusions was inserted via the jugular vein; the blood sampling catheter was inserted into the hepatic portal vein, according to the method described by Strubbe et al. (32). The catheter tip ends just downstream of the junction with the portal vein, so that glucagon secretion dynamics can be studied without the confounding effects of hepatic extraction.

The animals were allowed two weeks to recover after the surgery. During the recovery period, they were habituated to the experimental setup conditions (attachment of sampling and infusion tubes, etc.), so that the experiments could be performed with undisturbed freely-moving animals.

The experimental procedures were approved by the Animal Experiments Committee of the University of Groningen.

Experimental design

Experiment 1 was designed to study the effects of insulin on the counterregulatory responses in the fed state. In Experiment 1a, hypoglycemia was induced by a 90-minute intravenous infusion of insulin, at doses of 0, 5.5, 11, 22, 44, 88 or 120 mU.kg⁻¹.min⁻¹ (n=5-8 per group). Blood samples were withdrawn from the jugular vein catheter to determine glucose, insulin, glucagon, adrenaline, noradrenaline, and corticosterone levels. Experiment
1b was similar to Experiment 1a, but the blood samples were now withdrawn from the portal vein to determine glucose, insulin, and glucagon levels. In this experiment only two doses of insulin (11 and 22 mU.kg⁻¹.min⁻¹) were used (n=7 in each group).

Experiment 2 was designed to study the effects of insulin on the counterregulatory responses in the fasted state. In this experiment, rats were fasted for 48 hours prior to being subjected to hypoglycemia. Hypoglycemia was induced by a 90-minute intravenous infusion of insulin, at doses of 0.44, 2.2, 5.5, 11 or 22 mU.kg⁻¹.min⁻¹ (n=4-6 per group). Again, blood samples were withdrawn from the jugular vein catheter to determine glucose, insulin, glucagon, adrenaline, noradrenaline, and corticosterone levels.

All experiments were performed between 10:00 and 14:00. Food was removed 2 hours (fed state, Experiment 1) or 48 hours (fasted state, Experiment 2) earlier. The rats’ sampling and infusion catheters were connected to polyethylene tubings at least one hour prior to the experiment, to minimize adverse effects due to handling stress.

Two blood samples were then taken with a 10-minute interval, to serve as baseline values. At time point t=0, the 90-minute infusion of insulin (Velosulin, Novo Nordisk Farma, Alphen a/d Rijn, The Netherlands) or vehicle (0.9% NaCl) was started through the jugular infusion catheter, at an infusion speed of 3.2 ml.kg⁻¹.min⁻¹. During the infusion, blood was sampled at time points 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, and 90 minutes. Immediately after the t=90 sample the infusion was stopped, and a last blood sample was taken another 30 minutes later. Loss of blood volume was compensated by transfusing the same amount of heparinized blood from a donor rat after each blood sample.

Analysis

Blood samples were kept chilled at 0 °C during the experiment, in tubes with EDTA and aprotinin (Trasylol). Afterwards 50 μl blood was removed for glucose determination (Hoffmann's ferricyanide method (22)), the rest was centrifuged for 15 minutes at 2600 G and 5 °C. Plasma portions were stored at -80 °C for determination of glucagon (Glucagon RIA Kit, Linco Research Inc, St. Charles, MO, USA) and catecholamines (HPLC with electrochemical detection (30)), and at -20 °C for determination of insulin (Rat Insulin RIA Kit, Linco Research Inc) and corticosterone (HPLC with UV detection (11)).

Results are reported as average ± SEM (standard error of the mean). Statistical differences were determined with ANOVA or t-test (paired where relevant). The significance level was set to p<0.05.

Results

Experiment 1 – Insulin-induced hypoglycemia in fed rats

Figure 1 depicts the blood components that were measured before, during and after infusion of various doses of insulin in normally fed rats.

Infusion of different doses of insulin led to a dose-dependent elevation of plasma insulin during the whole infusion period (t=90 levels p<0.0001). Blood glucose levels decreased in all insulin-infused groups during the infusion, resulting in a nadir level of 4.2 ±
0.1 mM for the lowest insulin dose, but similar nadir levels of 3.1-3.4 mM for all other insulin doses (at t=90, there were only significant differences between the 0 dose vs all other doses, and between the lowest insulin dose vs all others). Plasma glucagon responded rapidly and in a dose-dependent manner to the insulin dose, especially in the first 20 minutes where the rise in glucagon levels was dose-dependent for the insulin dose (t=20 peak response p<0.0001). During the second part of the infusion period, the glucagon levels were still elevated over baseline, but to the same extent for most insulin doses (t=90 average levels were 96-109 pg/ml, p=0.733), again except the lowest insulin dose (77 ± 6 pg/ml).

Figure 1. Circulating insulin, glucose, glucagon, adrenaline, noradrenaline, and corticosterone levels during 90-minute infusion of insulin in normally fed rats (Experiment 1). Insulin doses were 0 (vehicle, ○), 5.5 (●), 11 (□), 22 (■), 44 (Δ), 88 (▲) and 120 (▼) mU.kg⁻¹.min⁻¹.
Plasma adrenaline did not change significantly during the insulin infusion period in the rats receiving the two lowest insulin doses (5.5 and 11 mU.kg\(^{-1}\).min\(^{-1}\)). After t=60, the two highest insulin administrations (88 and 120 mU.kg\(^{-1}\).min\(^{-1}\)) resulted in significant adrenaline responses (p=0.013 and 0.029 compared to baseline). At t=120 these responses reached their highest levels, and here the two medium insulin infusions (22 and 44 mU.kg\(^{-1}\).min\(^{-1}\)) also showed an adrenaline response. Plasma noradrenaline levels increased slightly with all insulin doses except the lowest, with t=90 levels being significantly elevated over baseline (p values <0.05 for the 11-120 mU.kg\(^{-1}\).min\(^{-1}\) doses). Corticosterone levels increased significantly only after administration of higher insulin doses (p<0.05 for 22, 88 and 120 mU.kg\(^{-1}\).min\(^{-1}\)).

To study glucagon dynamics more accurately, a group of animals with portal vein catheters had been infused with 11 or 22 mU.kg\(^{-1}\).min\(^{-1}\) insulin (doses which as mentioned above did not display a peak in the glucagon response in the general circulation). Glucose, insulin and glucagon measurements in blood samples from the hepatic portal vein during the first 30 minutes of the insulin infusion are depicted in Figure 2, together with the corresponding measurements from the samples from the jugular vein catheter.

Insulin and glucose levels in the portal vein were similar to those in the general circulation, but glucagon levels were significantly higher in the portal vein, both in the baseline situation (average baseline level in the general circulation 60 ± 3 vs in the portal vein 89 ± 6 pg/ml, p<0.0001) and during the responses (for the two doses used, t=20 levels in the portal vein were 203 ± 18 and 231 ± 18 pg/ml, whilst in the general circulation 83 ± 6 and 97 ± 8 pg/ml).

Glucagon secretion, as indicated by the portal vein samples, started increasing as soon as 5-10 minutes after the start of the insulin infusion (in both doses, t=7.5 was the first time point to be significantly higher than baseline), and not after 10-15 minutes as suggested by the data sampled from the jugular vein catheter (for these measurements, the first time points to be significantly different from baseline were t=15 for 11 mU.kg\(^{-1}\).min\(^{-1}\), and t=10 for 22 mU.kg\(^{-1}\).min\(^{-1}\)). This rise within 7.5 minutes in portal glucagon levels occurred when glucose levels were still in the normal range (at t=7.5 still between 5.4 ± 0.2 and 5.6 ± 0.2 mM, both in the general circulation and in the portal vein).

Furthermore, the portal venous glucagon response did indeed show a peak response at t=20, followed by a decline; similarly to the response shape seen in the general circulation with the higher insulin doses.
Figure 2. Insulin, glucose, and glucagon levels in the general circulation (squares □) and in the portal vein (triangles △▲) during 90-minute intravenous infusion of insulin in normally fed rats (Experiment 1). Insulin doses were 11 (open symbols □Δ) and 22 (solid symbols ■▲) mU.kg⁻¹.min⁻¹. Portal vein samples were taken up until t=30.

In summary, different doses of insulin resulted in similar decreases in glucose levels. The lowest two doses of insulin only affected glucagon release. Higher doses of insulin increased both glucagon and adrenaline levels, and the highest two doses led to increases in glucagon and adrenaline as well as corticosterone.
Experiment 2 – Insulin-induced hypoglycemia in fasted rats

*Figure 3* depicts the levels of the blood components that were measured before, during and after infusion of various doses of insulin in 48h food deprived rats.

Baseline levels of glucose and insulin were significantly lower than in the normally fed rats (glucose: fed rats overall average 6.14 ± 0.04 mM, fasted rats 4.43 ± 0.09 mM, p<0.0001; insulin: fed rats overall average 2.90 ± 0.20 ng/ml, fasted rats 0.21 ± 0.05 ng/ml, p<0.0001).

After the start of the insulin infusions at t=0, plasma insulin levels with the lowest insulin dose (0.44 mU.kg⁻¹.min⁻¹) did not change from baseline. In the other groups, plasma insulin reached significantly different steady state levels (p<0.0001) related to the infused amount of insulin. At the two doses which were used in both fed and fasted rats (5.5 and 11 mU.kg⁻¹.min⁻¹), the reached plasma insulin levels were comparable to those in the fed state (*Figure 1*). Blood glucose levels decreased with all insulin doses except the lowest (0.44 mU.kg⁻¹.min⁻¹). In the 2.2 mU.kg⁻¹.min⁻¹ dose, the response in glucose levels was smaller compared to the other three groups (with 2.2 mU.kg⁻¹.min⁻¹ the t=90 nadir level was 3.2 ± 0.2 mM, the nadir levels of the three higher doses were 2.6, 2.3 and 2.8 mM). No detectable plasma glucagon responses occurred with the lowest doses, but there were strong increases in the other groups (at t=30 p=0.047 and 0.010 for 11 and 22 mU.kg⁻¹.min⁻¹) which were positively correlated to the insulin dose, throughout the whole infusion period. The same effect was observed for plasma adrenaline, which did not change with the lowest insulin doses, but already after 30 minutes strongly responded in a dose-dependent manner with the higher doses (t=90 p=0.023 and 0.004 for 11 and 22 mU.kg⁻¹.min⁻¹). Plasma noradrenaline levels were also elevated over baseline in the highest insulin doses (t=90 p=0.012 and 0.001 for 11 and 22 mU.kg⁻¹.min⁻¹). Corticosterone showed high responses starting after 30 minutes in the three highest insulin administrations (t=90 p=0.008, 0.0004 and 0.001 for 5.5, 11 and 22 mU.kg⁻¹.min⁻¹).

In summary, in fasting rats different doses of insulin resulted in similar decreases in glucose levels. The nadir for blood glucose was much lower than in Experiment 1 (the fed animals). The two lowest doses of insulin did not result in statistically significant counterregulatory responses. The higher three doses of insulin resulted in strong responses in glucagon, corticosterone, adrenaline, and noradrenaline. When compared to the fed animals, similar insulin doses in the fasted rats led to faster onset as well as a greater magnitude of the counterregulation.
Discussion

The current study was undertaken to study the importance of insulin and of fasting in the counterregulatory responses to insulin-induced hypoglycemia. It was shown that low blood glucose resulted in counterregulatory responses, that blood glucose was maintained at the same level across the different insulin levels by differential action of counterregulatory
responses, and that a fasted state resulted in higher counterregulatory responses for similar insulin and glucose levels. Therefore, glucose levels, insulin levels, and the nutritional state each are important determinants of the counterregulatory responses to insulin-induced hypoglycemia in rats.

**Glucose and insulin are independent moderators of the counterregulatory responses**

One aim of this study was to investigate the counterregulatory responses to insulin-induced hypoglycemia in rats at different insulin levels. This was done by infusing groups of rats with different amounts of insulin while monitoring their blood glucose levels and counterregulatory responses. A more than ten-fold difference in the dose of administered insulin (between the 11 and 120 mU.kg\(^{-1}.\)min\(^{-1}\) administrations in the normally fed rats, with a resulting more than 30-fold difference in circulating insulin levels) did not result in differences in glucose levels. Instead there were different magnitudes in counterregulatory responses, with the highest counterregulatory responses at the higher doses of insulin.

The seemingly most obvious explanation – that insulin had reached a maximal effect already at the 11 mU.kg\(^{-1}.\)min\(^{-1}\) dose – is unlikely, for several reasons. Insulin is well able to reduce glucose levels to below 2 mM in non-fasted rats, for example as injections or rapid infusions – even when plasma insulin peak levels are comparable (26). Similarly, the initial drop in glucose levels did show dose-dependency, also when plasma insulin levels were already near steady-state levels (at t=15, see Figure 4). Most importantly, this hypothesis cannot explain the counterregulatory responses reacting differently in the different insulin dose groups.

**Figure 4. Blood glucose levels during infusion of insulin in normally fed rats.** Insulin doses were 0 (vehicle, ○), 5.5 (●), 11 (▲), 22 (■), 44 (Δ), 88 (▲) and 120 (△) mU.kg\(^{-1}.\)min\(^{-1}\). This figure represents time frame t=0-30 minutes from Figure 1.

Hence, the observation that the counterregulatory responses did differ between the different insulin doses implies that higher insulin doses indeed did have stronger effects on glucose disposal, but that the animals used stronger counterregulatory responses to
compensate, resulting in similar glucose nadir levels independent of insulin dose. This is also suggested by the fact that the drop in glucose levels is generally stronger with bolus administrations than with infusions (where animals have more time to produce and adjust counterregulatory responses).

If this hypothesis is true, it should result in some specific findings. Glucose levels should be maintained at or above a certain level, and the counterregulatory responses should be stronger when more insulin is administered and glucose levels hence are under a stronger pressure.

It may be clear from the data presented in Figure 1 that these conditions are indeed met. Although declining slowly, glucose levels were remarkably similar between the different insulin doses. The counterregulatory responses were not only related to the glucose levels but also to the insulin dose: the more insulin, the more and stronger counterregulatory responses, with adrenaline as clear example, and glucagon as well (especially when keeping in mind that the glucagon dose-responsivity was underestimated when measured in the general circulation, because of the buffering effect of hepatic extraction; as demonstrated in Experiment 1b; see Figure 2). Adrenaline did not respond at all at the lower insulin doses, even though blood glucose went down to the same level as where adrenaline started responding in the animals receiving the highest insulin doses.

The insulin dose cannot be the sole determinant of the counterregulatory responses; otherwise it would be impossible to explain why the adrenaline response did not occur until after 60-75 minutes, while plasma insulin levels were already in steady state from around t=15-30. The adrenaline response is therefore determined by at least both blood glucose level and insulin dose, for example by insulin affecting the glucose threshold level for adrenaline secretion.

This principle of the counterregulatory responses depending both on glucose and on insulin is further illustrated in Figure 5 where the response levels of glucose, insulin, and counterregulatory responses at the end of the infusion period (t=90) are plotted against the administered insulin dose (on the x-axis). It can clearly be seen that the doses of 11 and 22 mU.kg⁻¹.min⁻¹ already reached 90% of the maximal effect on glucose levels (reducing them to 3.4 mM), with the maximal effect (3.1 mM) equally seen at 44, 88 and 120 mU.kg⁻¹.min⁻¹ insulin. In contrast to these similar glucose levels, the t=90 response levels of adrenaline and corticosterone kept increasing with increasing insulin doses.

This dual effect of both glucose level and insulin dose results in blood glucose levels being maintained at a similar level, independent of the administered insulin dose. Furthermore, an increase in insulin dose seems to enhance the counterregulatory response magnitude by the appropriate amount to compensate for the increased glucose-lowering effect, thus maintaining blood glucose at the same level regardless of insulin dose. This suggests that the body might be able to perceive the hypoglycemic effect of the insulin dose, and adjust the counterregulatory responses accordingly – independent of the actual blood glucose level.
Figure 5. Final levels of glucose, insulin and the counterregulatory responses in normally fed rats at the end of the infusion period (t=90), plotted against the administered insulin dose (x-axis). Glucose levels (○) are plotted against the left y-axis, insulin and the counter-regulatory responses (●) to the right y-axis.

Such a precise regulation of blood glucose independent of the insulin dose requires a fast recognition of an upcoming insulin-induced hypoglycemia. In the current study it was demonstrated that the glucagon response was very rapid and in the appropriate magnitude to slow down the decline in glucose. It means that the body had somehow activated the counterregulatory responses appropriately already before glucose levels were significantly affected. Such a “feed-forward” system for the tight regulation of glucose levels during insulin-induced hypoglycemia needs another factor than blood glucose levels to determine
the magnitude of the required counterregulatory responses. The obvious candidate is plasma insulin, which did reach different levels in the different groups. It has been reported earlier that, at similar glucose levels, higher insulin levels lead to higher responses (18). Also supportive of this is the finding that treatment with an insulin sensitizer increases the counterregulatory responses to hypoglycemia (17). It remains to be resolved if these graded counterregulatory responses are caused by a direct effect of insulin on the counterregulatory response-producing organs (such as the pancreas (34) or the central nervous system (1, 3, 28)), or by an indirect effect via one of the consequences of high insulin levels (such as the changes in glucose homeostasis, the suppression of endogenous insulin secretion, or a possible sensitization of glucosensing mechanisms (16, 29, 40)). Recent findings in mice lacking brain insulin receptors seem to support the former possibility (14), presumed that peripheral insulin enters the involved brain areas.

Fasting enhances all counterregulatory responses

The second aim of this study was to investigate the effect of the nutritional state on the counterregulatory responses to hypoglycemia. In a fasted situation, insulin-induced hypoglycemia might pose a bigger risk to the organism because of the already decreased glucose levels, the limited glycogen reserves, and the enhanced insulin sensitivity. The role of insulinemia in the regulation of the counterregulatory responses might also be different in the fasted state than in the fed state. These questions were studied by fasting rats for 48 hours before subjecting them to insulin-induced hypoglycemia.

Blood glucose levels were indeed significantly lower in fasted animals compared to normally fed animals. Baseline glucose levels were in the fasted rats in the range of 4-5 mM (fed rats 6 mM), and the glucose nadir during hypoglycemia levels was around 2.0-2.5 mM (while in the fed state around 3.5 mM). Baseline insulin levels were lower too after fasting (fasted rats 0.1-0.4 ng/ml versus fed rats 1.9-3.5 ng/ml). At similar insulin doses, plasma insulin levels in the fasted rats were comparable to those in the fed rats, with a tendency to being higher (at the 5.5 mU.kg⁻¹.min⁻¹ dose, fed rats reached a plasma insulin level of 4.9 ± 1.1 ng/ml while fasted rats reached 6.6 ± 1.4 ng/ml (p=0.34), at the 11 mU.kg⁻¹.min⁻¹ dose this was 10.3 ± 0.6 ng/ml versus 15.8 ± 2.8 ng/ml (p=0.07)).

The major finding is that the magnitude of the counterregulatory responses was remarkably greater in fasted rats than in fed rats, despite comparable insulin levels. An example is the glucagon response in the 5.5 mU.kg⁻¹.min⁻¹ group, where glucagon rose to a peak level of 201 ± 49 pg/ml at t=30, while in the fed rats the same dose of insulin only caused a moderate increase to maximally 76 ± 5 pg/ml at t=45.

Such enhanced counterregulatory responses in the fasted state were to be expected. Blood glucose was already decreased before the insulin infusion started and therefore there was a greater risk for glucose becoming so low that it impairs brain functioning. Furthermore insulin sensitivity is increased after fasting so that similar plasma insulin levels could result in stronger glucose disposal in the fasted rats. Glycogen supplies are also limited, therefore more glucagon and adrenaline might be needed to access the remaining glycogen, further stimulate gluconeogenesis, and inhibit glucose disposal. It has been
indicated both for hypoglycemia (38) and for other metabolic challenges (35, 36) that fasting enhances the sympathoadrenal responses as well.

The counterregulatory responses started earlier, too. In the fasted rats, adrenaline and corticosterone were already markedly increased within 30 minutes after the onset of insulin-induced hypoglycemia. This is in sharp contrast to the fed rats where there were no adrenaline or corticosterone responses in the first hour. As a steroid hormone, corticosterone's secretion is not rapid but depends on synthesis of new hormone rather than release of intracellular stores. Furthermore, its production needs activation of the hypothalamo-pituitary-adrenal axis first. Therefore the corticosterone response most likely already started when glucose levels were still around 3-4 mM, a level which in the fed state does not result in a high corticosterone response. Since this corticosterone response in addition also occurred with low insulin doses (which resulted in lower plasma insulin levels than in the fed rats), this implies that the counterregulatory responses to hypoglycemia are not only dependent on the blood glucose levels, or the insulin levels, but that also other factors such as the nutritional state in itself determine the regulation of counterregulatory responses.

A third important observation is that just like in the fed rats, different doses of insulin resulted in similar glucose levels, but with clear differences in secretion of e.g. glucagon and adrenaline (Figure 3). Therefore, the phenomenon of similar glucose nadir levels despite different insulin levels, but with different counterregulatory response levels, seems to apply in the fasted state as well. This is also illustrated in Figure 6, where the t=90 levels of glucose, insulin, and counterregulatory responses are plotted against the insulin dose. All counterregulatory responses depended on the dose of insulin (with corticosterone already reaching its maximal response at the dose of 5.5 mU.kg⁻¹.min⁻¹). These correlations are very similar to those in the fed state, except that the glucagon and noradrenaline t=90 response levels now kept increasing with increasing insulin dose, again suggesting that hypoglycemia in the fasted state indeed poses a bigger risk to the organism.

Summary

In summary, it appears that both in the fed and in the fasted state blood glucose levels are defended at or above a certain level, despite large amounts of infused insulin. The counterregulatory responses are however greater with larger amounts of insulin, and it is suggested that the magnitude of the counterregulatory responses is adjusted to keep blood glucose above this certain level. From the data it appears that the activation and magnitude of the counterregulatory responses controlling glucose levels during hypoglycemia are determined by multiple factors, including at least glucose levels, insulin levels, and the nutritional state. Also the hierarchy between the counterregulatory responses, with glucagon coming first and adrenaline later and/or at higher insulin doses, might be influenced by these factors. Such a complicated regulatory system may also mean that the later responses may serve as backup for the former, and that impairing one counterregulatory response will lead to stronger activation of the others.

Combined, these findings suggest that hypoglycemia is not an absolute but a relative state, and its effects and severity depend on several separate factors, including the glucose level, the insulin level, and the availability of other energy sources. It remains to be resolved
how these factors are integrated by the neuronal systems controlling the counterregulatory responses to insulin-induced hypoglycemia.

Figure 6. Final levels of glucose, insulin and the counterregulatory responses in 48h fasted rats at the end of the infusion period (t=90), plotted against the administered insulin dose (x-axis). Glucose levels (○) are plotted against the left y-axis, insulin and the counterregulatory responses (●) to the right y-axis.
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References


5. V.J. Briscoe and S.N. Davis; Hypoglycemia in Type 1 and Type 2 diabetes: physiology, pathophysiology, and management. Clinical Diabetes (2006) 24: 115-121


22. W.S. Hoffmann; A rapid method for the determination of glucose in blood and urine. Journal of Biological Chemistry (1937) 120: 51-55
35. G. van Dijk; Central and peripheral mechanisms involved in fuel homeostasis. Fbedruk, Enschede, 1995