Effects of insulin per se on neuroendocrine and metabolic counter-regulatory responses to hypoglycaemia

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ABSTRACT

We examined and compared findings from studies aimed at detecting and quantifying an effect of insulin per se on counter-regulatory responses to hypoglycaemia. The experimental protocols used in many of these studies were very different with regard to study design and patient population, resulting at times in inconsistencies and discrepancies. Taken together, the results from this extensive body of work clearly indicate that, at similar levels of hypoglycaemia, greater hyperinsulinaemia results in enhanced counter-regulatory responses. This enhancement includes higher circulating levels of counter-regulatory hormones (adrenaline, noradrenaline, cortisol and growth hormone, but not glucagon), more intense activation of hypoglycaemic symptoms (both neural-sympathetic and adrenal-sympathetic), and greater deterioration of neuro-psychological skills. The insulin-induced enhancement of counter-regulatory responses is not influenced by gender, is present in several animal species, and applies to healthy subjects as well as to patients with Type I diabetes. The underlying mechanisms remain speculative, and possibly include a direct neuromodulatory effect and/or suppression of glucose utilization in various areas of the brain, which either independently or in a hierarchical fashion trigger the sequence of downstream counter-regulatory events.

INTRODUCTION

The presence of hypoglycaemia triggers a complex pattern of profound physiological changes aimed at limiting and reversing falling blood glucose levels. These processes are referred to as the counter-regulatory responses to hypoglycaemia. Traditionally, the depth [1,2] and duration [3–11] of hypoglycaemia have been considered as the primary factors regulating the magnitude of counter-regulatory responses. More recently other parameters, such as age [12], gender [13–15], rate of fall of blood glucose at the onset of hypoglycaemia [3,16] and prior exposure to metabolic perturbations (euglycaemic hyperinsulinaemia) [17,18], have also been observed to influence hypoglycaemic counter-regulation. The possibility that insulin per se may play a direct role, independent of the prevailing glucose level, in regulating counter-regulatory responses had been overlooked. The purpose of the present review is to discuss the considerable amount of data documenting the effects of insulin per se on the neuroendocrine and metabolic responses to hypoglycaemia.

Key words: autonomic nervous system, catecholamines, counter-regulatory responses, endogenous glucose production, hypoglycaemia, insulin.

Abbreviations: ACTH, adrenocorticotrophic hormone (corticotropin); EGP, endogenous glucose production; PET, positron-emission tomography; \( R_d \), rate of disappearance.

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THE PHYSIOLOGICAL COUNTER-REGULATORY RESPONSE TO HYPOGLYCAEMIA

When blood glucose drops to a sufficiently low level, subjects become aware of the presence of hypoglycaemia through a series of warning symptoms. ‘Neuroglycopenic’ symptoms, attributed to insufficient glucose utilization in cerebral cortical areas, include loss of concentration, confusion, difficulty thinking, tiredness, tingling of the extremities, hunger and blurred vision [19]. ‘Adrenergic’ symptoms include sweating, tremor, palpitations, agitation, heat and thirst, and are the consequence of hypoglycaemia-induced activation of the sympatho-chromaffin system [19]. Counter-regulatory hormones secreted in response to hypoglycaemia include catecholamines, glucagon, growth hormone and cortisol. Secretion of adrenaline (epinephrine) and noradrenaline (norepinephrine) is the direct result of the hypoglycaemia-induced increase in sympathetic activity. Control of glucagon secretion during acute hypoglycaemia, on the other hand, is complex, and may involve partial regulation by the autonomic nervous system as well as direct $\alpha$-cell sensing. Hypothalamic sensing of hypoglycaemia results in increased release of corticotropin-releasing factor, which stimulates the secretion of growth hormone and adrenocorticotrophic hormone (ACTH; corticotropin). The latter increases circulating levels of cortisol, which not only has a direct counter-regulatory action, but also further stimulates catecholamine secretion via an intra-adrenal effect.

EFFECTS OF INSULIN PER SE ON COUNTER-REGULATORY RESPONSES TO HYPOGLYCAEMIA

Catecholamines and the autonomic nervous system

The advent of the glucose clamp technique, in its original euglycaemic [20] and subsequent hypoglycaemic [21] forms, has allowed the effects of insulin on neuroendocrine and metabolic responses to be determined. Several studies have demonstrated that hyperinsulinaemia in the presence of euglycaemic conditions has effects on neuroendocrine responses. Levels of glucagon and pancreatic polypeptide decrease, those of adrenaline and growth hormone are unaffected, while plasma cortisol and noradrenaline increase under euglycaemic/hyperinsulinaemic conditions [22–28]. Furthermore, at least two studies [25,29] have shown that, in humans, muscle sympathetic nerve activity increases in a dose-dependent manner in response to circulating insulin. Parenthetically, it should be noted that the increased sympathetic vasoconstrictive tone in skeletal muscle was not paralleled by increased blood pressure, suggesting that insulin may not be, as was proposed earlier [30,31], one of the factors responsible for arterial hypertension in patients with insulin resistance.

Early studies provided conflicting results regarding a direct effect of insulin on counter-regulatory responses to hypoglycaemia [18,32,33]. The reasons for these conflicting data will be analysed below. It took several years, and numerous further investigations, to establish the present consensus that, indeed, an effect of insulin per se on hypoglycaemic counter-regulatory responses exists, and primarily involves augmentation of adrenaline, noradrenaline and autonomic nervous system responses. To date, at least 15 studies have investigated the effects of different concentrations of insulin in the presence of equivalent hypoglycaemia. Except for this common feature, the studies have varied considerably with respect to experimental design (see Table 1). One study was performed in dogs, eight in healthy humans, and six in patients with Type I diabetes. The gender composition was also variable, ranging from studies containing exclusively men or women to various combinations of the two sexes. The number of subjects per study has also been extremely variable, ranging from 1 to 30. The depth of hypoglycaemia used during the hypoglycaemic plateau varied from moderate (4.1 mM) to severe (2.0 mM), with a mean ($\pm$ S.E.M.) of 2.9 $\pm$ 0.1 mM. Low-dose insulin levels have varied from 132 $\pm$ 6 pM to 794 $\pm$ 52 pM (mean $\pm$ S.E.M. 369 $\pm$ 47 pM), and the higher hyperinsulinaemic levels ranged from 402 $\pm$ 18 pM to 27956 $\pm$ 1901 pM (mean $\pm$ S.E.M. 1641 $\pm$ 304 pM).

Despite these marked differences, examination of these data reveals several common patterns. Noradrenaline levels were measured in 11 of the above studies (Table 2). In all instances the reported levels were greater at the higher insulin level, with a mean increase of 28 $\pm$ 5% ($P = 0.0002$). Adrenaline levels were reported in 12 studies (Table 2). Again, in the majority of instances adrenaline levels were greater at the higher insulin level, with a mean increase (high- compared with low-dose insulin) of 44 $\pm$ 14% ($P = 0.03$). Taken together, these data overwhelmingly support the concept of a direct stimulatory effect of insulin on catecholamine secretion, independent of the stimulatory effect of hypoglycaemia. This effect is present in healthy human subjects [2,13,18,32,34], in patients with Type I diabetes [35–39], and across different species (humans, dogs [40] and rats).

Based on the consensus of catecholamine responses to hypoglycaemia, earlier inconsistent results can now be put into perspective. Liu et al. [32] and Mellman et al. [18] reported that catecholamine responses to hypoglycaemia were increased during high-dose insulin. Although not statistically significant, the direction and the magnitude of these changes were compatible with significant differences observed later by other investigators. The small number of the subjects studied, milder hypoglycaemia and shorter duration may have generated a reduced experimental signal in the studies by Liu et al.
Table 1  Subjects, depth of hypoglycaemia and hyperinsulinaemic levels
M, male; F, female. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>No. and gender</th>
<th>Hypoglycaemic plateau (nM)</th>
<th>Plasma insulin (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower dose</td>
<td>Higher dose</td>
</tr>
<tr>
<td>Liu et al. [32]</td>
<td>Healthy humans</td>
<td>6 M/3 F</td>
<td>3.4 ± 0.2</td>
<td>208 ± 14</td>
</tr>
<tr>
<td>Diamond et al. [33]</td>
<td>Healthy humans</td>
<td>5 M/7 F</td>
<td>3.1 ± 0.1</td>
<td>265 ± 29</td>
</tr>
<tr>
<td>Mellman et al. [18]</td>
<td>Healthy humans</td>
<td>4 M/3 F</td>
<td>3.5 ± 0.1</td>
<td>352 ± 29</td>
</tr>
<tr>
<td>S. N. Davis et al. [40]</td>
<td>Healthy dogs</td>
<td>19 M + F</td>
<td>2.8 ± 0.1</td>
<td>400 ± 48</td>
</tr>
<tr>
<td>S. N. Davis et al. [34]</td>
<td>Healthy humans</td>
<td>9 M</td>
<td>2.8 ± 0.1</td>
<td>408 ± 33</td>
</tr>
<tr>
<td>S. N. Davis et al. [13]</td>
<td>Healthy humans</td>
<td>9 F</td>
<td>2.8 ± 0.1</td>
<td>794 ± 62</td>
</tr>
<tr>
<td>S. N. Davis et al. [2]</td>
<td>Healthy humans</td>
<td>10 M</td>
<td>3.4 ± 0.1</td>
<td>132 ± 6</td>
</tr>
<tr>
<td>M. R. Davis et al. [34]</td>
<td>Humans with Type I diabetes</td>
<td>3 M/5 F</td>
<td>3.1 ± 0.1</td>
<td>329 ± 62</td>
</tr>
<tr>
<td>S. N. Davis et al. [42]</td>
<td>Humans with Type I diabetes</td>
<td>7 M</td>
<td>2.8 ± 0.1</td>
<td>742 ± 212</td>
</tr>
<tr>
<td>Lingenfeher et al. [37]</td>
<td>Humans with Type I diabetes</td>
<td>18 M/9 F</td>
<td>3.5 ± 0.02</td>
<td>270 ± 20</td>
</tr>
<tr>
<td>Kerr et al. [38]</td>
<td>Humans with Type I diabetes</td>
<td>5 M/4 F</td>
<td>2.8 ± 0.04</td>
<td>360 ± 24</td>
</tr>
<tr>
<td>Liu et al. [39]</td>
<td>Humans with Type I diabetes</td>
<td>8M/1F</td>
<td>3.3 ± 0.2</td>
<td>180 ± 14</td>
</tr>
<tr>
<td>Freuhwald-Shultes et al. [46]</td>
<td>Healthy humans</td>
<td>30 M</td>
<td>2.5 ± 0.01</td>
<td>635 ± 34</td>
</tr>
<tr>
<td>Freuhwald-Shultes et al. [46a]</td>
<td>Healthy humans</td>
<td>30 M</td>
<td>4.1 ± 0.02</td>
<td>522 ± 18</td>
</tr>
</tbody>
</table>

Table 2  Plasma catecholamines
Values are means ± S.E.M. P values are for the comparison between lower and higher insulin doses; NS, not significant.

<table>
<thead>
<tr>
<th>Study</th>
<th>Plasma adrenaline (nM)</th>
<th>Plasma noradrenaline (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With lower insulin dose</td>
<td>With higher insulin dose</td>
</tr>
<tr>
<td>Liu et al. [32]</td>
<td>0.9 ± 0.3</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>Diamond et al. [33]</td>
<td>2.3 ± 0.5</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Mellman et al. [18]</td>
<td>1.6 ± 0.3</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>S. N. Davis et al. [40]</td>
<td>4.4 ± 1.0</td>
<td>14 ± 1.4</td>
</tr>
<tr>
<td>S. N. Davis et al. [34]</td>
<td>5.5 ± 0.7</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>S. N. Davis et al. [13]</td>
<td>3.9 ± 0.6</td>
<td>5.7 ± 0.9</td>
</tr>
<tr>
<td>S. N. Davis et al. [2]</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>M. R. Davis et al. [34]</td>
<td>0.8 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>S. N. Davis et al. [42]</td>
<td>6.1 ± 1.1</td>
<td>6.9 ± 1.3</td>
</tr>
<tr>
<td>Lingenfeher et al. [37]</td>
<td>0.6 ± 0.1</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Kerr et al. [38]</td>
<td>1.0 ± 0.2</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Liu et al. [39]</td>
<td>2.1 ± 0.4</td>
<td>2.8 ± 0.7</td>
</tr>
</tbody>
</table>

[32] and Mellman et al. [18], resulting in lack of a statistical difference in hormonal responses between high- and low-dose insulin protocols. The depth of hypoglycaemia appears to be an important regulator determining the ability of insulin to amplify adrenaline and noradrenaline responses [17] (see Figure 1). Therefore experimental conditions using mild hypoglycaemia may have confounded the ability to discern the stimulatory effects of insulin per se on counter-regulatory responses.

Somewhat more puzzling were the results reported by Diamond et al. [33], who not only did not observe

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increased catecholamine/autonomic nervous system responses to hypoglycaemia with high-dose insulin, but in fact reported a significant suppression of adrenaline in the presence of greater hyperinsulinaemia. The data from this study are surprising because the insulin levels (much higher than in Liu et al. [32] or Mellman et al. [18]) were very similar to those used in the classical study of Garber et al. [41] and in several studies by Davis et al. [2,13,34,40,42], all of which showed substantial increases in circulating levels of both adrenaline and noradrenaline under comparable hypoglycaemic conditions. Although the reasons for this discrepancy remain unclear, it should be noted that, in the study of Diamond et al. [33], glucose levels dropped substantially faster during low-dose than during high-dose insulin, thereby reaching the glycaemic threshold for adrenaline much quicker. On matching the adrenaline data for the time at which equivalent glycaemia occurred, the difference in adrenaline levels between high- and low-dose insulin becomes much smaller.

Although increased catecholamine levels indicate greater activation of the autonomic nervous system at higher insulin levels, concurrent direct measurements of sympathetic activity were never performed, and even the degree of activation of adrenergic symptoms during hypoglycaemia was recorded in only a subset of studies. The effects of differing insulin levels on the cardiovascular system were reported in seven studies [2,13,34,37,38,40,42]; in five studies heart rate was increased at greater hyperinsulinaemia [2,13,34,37,40], and in three instances blood pressure was also increased [2,13,37]. Levels of pancreatic polypeptide, an indirect index of parasympathetic activation, were measured in seven studies, and in all but one instance [34] no differences were reported between the high- and low-dose insulin protocols. Hypoglycaemic symptoms were measured only in the studies of Kerr et al. [38] and Lingenfelser et al. [37], both in patients with Type 1 diabetes, with contrasting results. While Kerr et al. [38] reported that facial flushing, trembling, sweating and hunger were significantly attenuated at higher insulin levels, Lingenfelser et al. [37] observed an increase, during the higher insulin level, of autonomic and neuroglycopaenic symptom scores.

### Glucagon

In contrast with that of catecholamines, the glucagon response to hypoglycaemia appears to be attenuated by greater hyperinsulinaemia. Arterial plasma levels (Table 3) of this hormone were decreased by 15–39% at higher compared with lower insulin levels in seven studies [2,32–34,39,40,42] (although this difference was significant in only three instances), were unchanged in one study [13] and were increased in one study [18] (+18%; not significant). Overall, therefore, the shift from lower to higher insulin resulted in an 18±4% decrease in circulating glucagon ($P = 0.03$). Although the arterial glucagon concentration appears to be inversely correlated with the degree of hyperinsulinaemia ($r = 0.46$), the correlation was much weaker than that observed with catecholamines (see Figure 1), and was unrelated to differences in the depth of hypoglycaemia. A decrease in the glucagon response in the presence of greater hyperinsulinaemia is consistent with studies on the regulation of pancreatic hormone secretion [43–45].

### Anterior pituitary hormones

Consistent with the adrenergic response to hypoglycaemia, growth hormone appears to increase more during high-dose than during low-dose hyperinsulinaemia (Table 4). In the 10 studies in which growth hormone was measured, values were 12% higher during high-dose insulin treatment. ACTH was only measured by Fruehwald-Shultes et al. [46] and by Lingenfelser et al. [37]. In the former study identical concentrations were observed during the two insulin levels, while in the latter non-significant increases occurred during high-dose insulin. Interestingly, Lingenfelser et al. [37] also reported increased levels, at greater hyperinsulinaemia, of β-endorphin and prolactin. Although these two hormones are not known to play any role in glucose counter-regulation, their increased secretion is likely to reflect greater autonomic activity in the hypothalamus due to higher insulin concentrations.

### Cortisol

Despite the variable ACTH response to increased hyperinsulinaemia, cortisol appears to be consistently higher at high-dose compared with low-dose insulin (Table 4). The possible reasons for this apparent discrepancy between ACTH and cortisol may be due to diurnal changes in both adrenal sensitivity to ACTH [47] and ACTH sensitivity to glucocorticoid feedback [48]. Plasma
Table 4  Plasma cortisol and growth hormone

Values are means ± S.E.M.  P values are for the comparison between lower and higher insulin doses; NS, not significant.

<table>
<thead>
<tr>
<th>Study</th>
<th>Plasma cortisol (nM)</th>
<th>Plasma growth hormone (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With lower insulin dose</td>
<td>With higher insulin dose</td>
</tr>
<tr>
<td>Liu et al. [32]</td>
<td>Not measured</td>
<td>18 ± 0</td>
</tr>
<tr>
<td>Diamond et al. [33]</td>
<td>Not measured</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>Mellman et al. [18]</td>
<td>552 ± 91</td>
<td>497 ± 99</td>
</tr>
<tr>
<td>S. N. Davis et al. [40]</td>
<td>160 ± 33</td>
<td>334 ± 41</td>
</tr>
<tr>
<td>S. N. Davis et al. [34]</td>
<td>611 ± 72</td>
<td>811 ± 36</td>
</tr>
<tr>
<td>S. N. Davis et al. [13]</td>
<td>826 ± 129</td>
<td>918 ± 55</td>
</tr>
<tr>
<td>S. N. Davis et al. [3]</td>
<td>330 ± 30</td>
<td>410 ± 50</td>
</tr>
<tr>
<td>Mellman et al. [18]</td>
<td>552 ± 91</td>
<td>497 ± 99</td>
</tr>
<tr>
<td>S. N. Davis et al. [40]</td>
<td>160 ± 33</td>
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<td>330 ± 30</td>
<td>410 ± 50</td>
</tr>
<tr>
<td>Lingenfelser et al. [37]</td>
<td>408 ± 33</td>
<td>442 ± 35</td>
</tr>
<tr>
<td>Fruehwald-Shultes et al. [46]</td>
<td>445 ± 20</td>
<td>538 ± 25</td>
</tr>
<tr>
<td>Kerr et al. [38]</td>
<td>423 ± 67</td>
<td>365 ± 63</td>
</tr>
<tr>
<td>Fruehwald-Shultes et al. [46a]</td>
<td>490 ± 80</td>
<td>588 ± 80</td>
</tr>
</tbody>
</table>

Figure 1  Plots of plasma adrenaline (A, B) and noradrenaline (C, D) levels against insulin levels in the studies listed in Table 1

Epinephrine = adrenaline; norepinephrine = noradrenaline. Data points in (A) and (C) are group averages from protocols utilizing mild hypoglycaemia (plasma glucose 3.1–3.5 mM). Data points in (B) and (D) are group averages from protocols utilizing deeper hypoglycaemia (plasma glucose 2.0–2.8 mM).
cortisol increased by an average of 18% \((P = 0.002)\), in the presence of high-dose compared with low-dose insulin, in the 11 studies in which it was measured.

Interestingly, cortisol responses occurred earlier with high-dose insulin, resulting in peak differences in cortisol levels between the first and the second hour of the hypoglycaemic clamps. At later times, differences in cortisol levels were reduced, or even abolished [46]. It should also be noted that, in one study reporting no differences in cortisol levels [38], hypoglycaemia was preceded by 60 min of hyperinsulinaemic euglycaemia, a condition known to diminish the subsequent cortisol response to hypoglycaemia [17]. This may have resulted in the discrepancy between the data reported by Kerr et al. [38] and other studies.

**Summary**

In summary, differences in the experimental protocols of the numerous studies investigating a putative effect of insulin *per se* on counter-regulatory responses to hypoglycaemia have prevented the gathering of conclusive evidence from any individual piece of work. When observed as a whole, the above studies appear to generate a common pattern of responses. In particular, at comparable levels of hypoglycaemia, adrenaline, noradrenaline, cortisol, growth hormone and cardiovascular responses appear to be increased with greater hyperinsulinaemia, while the glucagon response is proportionally decreased. The magnitude of these hormonal responses displays a significant direct correlation with the level of hyperinsulinaemia. This is best illustrated by the catecholamine responses \((r = 0.72\) and \(0.79\) for adrenaline and noradrenaline respectively) (Figure 1). Interestingly, with the exception of adrenaline, the correlation between neuroendocrine responses and insulin levels was not affected by the depth of hypoglycaemia. For adrenaline, a strong positive correlation with insulin levels \((r = 0.75)\) was detected only in the presence of deeper hypoglycaemia \((2.0–2.8 \text{ mM})\), whereas virtually no correlation with insulin levels was measured \((r = 0.02)\) with milder hypoglycaemia \((3.1–3.5 \text{ mM})\).

**GLUCOSE TURNOVER, ENDOGENOUS GLUCOSE PRODUCTION (EGP) AND LIPOLYSIS**

Under euglycaemic conditions, hyperinsulinaemia is known to suppress EGP and lipolysis, and to stimulate the rate of disappearance \((R_d)\) of glucose. Nevertheless, in the majority of the studies summarized above, EGP was greater with high-dose than with low-dose insulin. This apparent paradox may be explained by the fact that the suppressive effect of insulin on EGP is overcome during hypoglycaemia by the stimulatory effect of the autonomic nervous system, which, as shown above, shows increased activity at higher insulin levels. Similarly, lipolysis, which under euglycaemic conditions is totally suppressed by only mild hyperinsulinaemia, was also significantly increased during high-dose compared with low-dose insulin. Again, the profound insulin-induced stimulation of the autonomic nervous system was able to offset the usual inhibitory effects of the hormone on lipolysis.

The stimulatory effect of insulin on glucose \(R_d\) on the other hand, was only partially overcome. This phenomenon can be explained by the fact that the maximal suppressive effect on glucose \(R_d\) is reached at comparatively low levels of counter-regulatory hormones, \((i.e.\) adrenaline at \(~1.5 \text{ nM},\) or \(20\%\) of the peak levels obtained during some high-insulin infusions). When the level of hyperinsulinaemia was increased, higher levels of counter-regulatory hormones could not further suppress glucose \(R_d\), and the stimulatory effect of insulin became predominant, resulting in a greater \(R_d\) during high-dose compared with low-dose insulin infusions.

This pattern of metabolic responses to greater hyperinsulinaemic hypoglycaemia \((\text{increases in EGP, lipolysis and glucose } R_d)\) was consistently observed in healthy subjects when supra-physiological insulin levels were used. Interestingly, insulin-induced suppression of EGP and lipolysis were not overcome by counter-regulatory responses if physiological insulin levels were used [2], or if the study was performed in patients with Type I diabetes [42]. Counter-regulatory failure is a well known characteristic of Type I diabetes; it is not surprising, therefore, that in these patients counter-regulatory responses may not have been strong enough to overcome the suppressive effect of insulin on EGP and lipolysis.

**COGNITIVE FUNCTION**

Deterioration of cognitive function is a well known physiological event that occurs progressively with increasing depth of hypoglycaemia. Kerr et al. [38] reported a progressive deterioration in neuropsychological skills \((\text{as determined by three standard cognition tests})\) in a group of patients with Type I diabetes when blood glucose was lowered to \(2.8 \text{ mM}\) and then to \(2.0 \text{ mM}\). Nevertheless, these authors failed to detect any differences in the deterioration of cognitive function when, for equivalent hypoglycaemia, the level of hyperinsulinaemia was increased by more than 3-fold.

A later study conducted on a much larger \((n = 27)\) and more homogeneous group of patients with Type I diabetes by Lingenfelser et al. [37] yielded conflicting results. In that study a greater deterioration in the performance of psychometric tests was consistently
observed with high-dose than with low-dose insulin protocols. The subjective psychometric testing results were paralleled by a deterioration in brain electrophysiological activity, as detected by recording of middle-latency auditory-evoked potentials, a technique that can objectively detect even subclinical deteriorations in cerebral function. Because only these two studies directly assessed the deterioration of cognitive function at variable hyperinsulinaemia, and since the results were somewhat conflicting, clear detection of an effect of insulin on this aspect of counter-regulation will require additional work.

THE ISSUE OF GENDER DIFFERENCES

Gender differences have been reported in many aspects of glucose metabolism [49]. For example, plasma glucose levels drop more sharply during fasting in females than in males, and remain significantly lower if fasting is prolonged for up to 84 h [50]; basal and insulin-stimulated glucose transport across cell membranes is significantly greater in males than in females (due to a greater number of transporters, higher glucose transport rates and greater glucose transporter intrinsic activity [51]); and female adolescents with Type 1 diabetes are less insulin-sensitive at puberty than males [52]. Furthermore, sympathetic-adrenal responses have been reported to be greater during mental stress and exercise in men than in women [53–55].

Not surprisingly, therefore, gender-related differences have also been reported in counter-regulatory responses to hypoglycaemia [14,15,56]. In a recent study by Drake et al. [15], for instance, hypoglycaemia (2 mM) was induced via a bolus injection of insulin in rats. Over the ensuing 60 min, male rats displayed a 5-fold greater adrenaline response than females (P < 0.001). A similar pattern of responses was observed in humans by Amiel et al. [56]. In that study, after 60 min of fixed hypoglycaemia (2.5 mM), adrenaline and noradrenaline responses were 44% and 17% greater respectively in men than in women. Diamond et al. [14] also studied gender difference in humans at various levels of hyperinsulinaemia (~ 200 to ~ 2400 pM) and hypoglycaemia (~ 2.8 to ~ 3.3 mM). The catecholamine, glucagon, cortisol and growth hormone responses were consistently lower in females than in males. Similar conclusions were drawn by Davis et al. [13] from a study in which counter-regulatory responses to hypoglycaemia in nine healthy females were compared with those of males at two hyperinsulinaemic levels during equivalent hypoglycaemia. At both insulin levels, the adrenaline, noradrenaline, glucagon, growth hormone and EGP responses to hypoglycaemia were significantly greater in males than in females. On the other hand, the cortisol and lipolytic responses were greater in females than in males at both insulin levels.

The latter two studies [13,14] also allow quantification of the effect of gender on the ability of insulin to modulate hypoglycaemic counter-regulatory responses. In the study by Davis et al. [13], when insulin levels were increased from ~ 800 to ~ 3600 pM in females during equivalent hypoglycaemia of 2.8 mM, most counter-regulatory responses were amplified. The adrenaline response increased by 46%, noradrenaline by 44%, cortisol by 11%, growth hormone by 28%, hepatic glucose production by 90% and lipolysis (glycerol) by 91%. These differences are similar to those observed in male subjects for comparable changes in hyperinsulinaemia. This suggests that, despite the documented quantitative gender-related differences in hormonal and metabolic responses to hypoglycaemia, insulin per se exerts similar incremental effects on counter-regulatory responses to hypoglycaemia in males and females.

In summary, a marked sexual dimorphism has been observed in hormonal and metabolic adaptation to hypoglycaemia, both in laboratory animals and in humans. When compared with males, females appear to have quantitatively less pronounced counter-regulatory responses to hypoglycaemia. Despite these differences, females, similar to males, retain the ability to amplify the counter-regulatory response to equivalent hypoglycaemia when challenged with greater levels of hyperinsulinaemia. This amplification is manifested as increases in catecholamines, cortisol, growth hormone secretion, hepatic glucose production and lipolysis.

POTENTIAL CLINICAL IMPLICATIONS

Extrapolating the concepts expressed above to the practical field of clinical practice may require some caution. Experimental conditions in clinical studies are often artificial and different from the metabolic or physiological environments in which patients live. Stressing physiological systems beyond the range of normal physiology, on the other hand, remains a useful tool for gaining understanding of the mechanisms of disease. Along this line of thought, it is clear that the effects of insulin per se on counter-regulatory responses to hypoglycaemia reported above are particularly marked at supraphysiological hyperinsulinaemia. Nevertheless, the enhancing effect of insulin on counter-regulatory responses to hypoglycaemia was still present in the studies in which more physiological insulin concentrations were used, both in dogs and in humans. These effects of insulin per se, therefore, may be also relevant in the day-to-day management of patients with diabetes (both Type I and Type II), who often experience hyperinsulinaemic hypoglycaemia, particularly if undergoing intensive treatment for glycaemic control. Hypoglycaemic symptoms, for instance, are likely to be
activated to a greater degree if, during equivalent hypoglycaemia, higher levels of insulin are present. Any mechanism that may increase hypoglycaemia awareness in diabetic patients has potential clinical usefulness.

The implications of the enhancing effects of insulin per se on counter-regulatory responses to hypoglycaemia may also extend to pathologies other than diabetes. Insulin tolerance tests, for instance, are used for the diagnosis and quantification of impaired pituitary gland function. During these tests plasma insulin levels are likely to vary considerably due to the range of insulin dosages routinely used (0.15–0.25 units/kg), and to individual differences in the rate of insulin clearance and insulin release from the injection site. Unless the level of plasma hyperinsulinaemia is known, therefore, care should be taken in quantitative comparisons of insulin tolerance tests, as the progression or improvement of impaired pituitary function may be clouded by the confounding effect of differing hyperinsulinaemia.

PUTATIVE MECHANISMS OF THE INTRINSIC EFFECTS OF INSULIN ON COUNTER-REGULATORY RESPONSES TO HYPOGLYCAEMIA

The individual pieces of evidence provided by the studies summarized in this review may at times lack homogeneity, mostly due, as discussed extensively above, to major differences in study design, statistical power and the range of subjects studied. When this fairly comprehensive body of work is analysed as a whole, however, it clearly reveals that insulin per se plays a substantial role in modulating the neuroendocrine counter-regulatory responses to hypoglycaemia. To some extent, the intensity of hypoglycaemic symptoms and the degree of deterioration of cognitive function also appear to be affected by the level of hyperinsulinaemia. These effects occur similarly in male and female healthy subjects and in patients with Type I diabetes. The enhancement of counter-regulatory responses at higher insulin levels appears to extend across the spectrum of neuroendocrine hormones (adrenal and pituitary) and hypoglycaemic symptoms (neuroglycopaenic and adrenergic) [19]. This ‘co-ordinated homogeneity’ in the modulatory effect of insulin on hypoglycaemic responses suggests that, rather than multiple direct peripheral effects (alteration of adrenal catecholamine metabolism or β-adrenergic sensitivity) [57,58], a centrally co-ordinated modulation of autonomic activity is probably the underlying mechanism. Indeed, several authors [59–63] have reported that alterations in glucose homeostasis, lipid metabolism and cardiovascular function could be induced in anaesthetized dogs and rats by insulin injection into the carotid arteries or into the hypothalamus and pre-optic areas, even in the absence of hypoglycaemia. Taken together, therefore, the body of observations reported above points towards the presence of an intrinsic effect of insulin on the central nervous system. As discussed extensively above, the very presence of this effect has been the object of much debate, and as a consequence the possible underlying mechanisms remain unclear and largely speculative.

EVIDENCE OF DIRECT INSULIN ACTIVITY INSIDE THE BRAIN

The first step in determining a direct effect of insulin on cerebral tissue implies documenting an interaction between insulin and its receptor downstream of the blood–brain barrier. Failure to document such an interaction had led to the early assumption that the brain is largely insulin-insensitive [64]. However, several lines of recent evidence now clearly demonstrate that insulin can indeed penetrate the brain. Insulin of pancreatic origin has been detected by radioimmunological techniques in various brain regions, particularly the hypothalamus [65,66]. The autoradiographic or immunocytochemical identification of insulin receptors in the same hypothalamic areas characterized these sites as specific targets for direct insulin action [67]. The glucoregulatory function of the hypothalamus has been known for decades. For example, the sympathetic activity of the ventromedial hypothalamus results in the suppression of pancreatic insulin secretion [68], stimulation of the secretion of glucagon [69], growth hormone [70] and adrenaline [71], and stimulation of hepatic glucose production [72]. Direct injection of small amounts of insulin into the hypothalamus, on the other hand, results in hypoglycaemia [73]. The localization of insulin molecules and insulin receptors in this area of the brain establishes a direct link between insulin action and ventromedial sympathetic efferent impulses.

The pathway by which insulin reaches its target sites in the hypothalamus and elsewhere in the brain has been the subject of much debate. van Houten et al. [74] detected strong autoradiographic signals after administration of 125I-insulin in various circumventricular brain structures (hypothalamic arcuate and ventromedial nuclei, area postrema, paravagal region). Because no autoradiographic evidence of insulin binding to the neuronal parenchyma was found in any other region of the brain (neocortex, thalamus, cerebellum), the authors concluded that insulin fails to penetrate the blood–brain barrier, but manages to reach the circumventricular organs of the brain where the blood–brain barrier is virtually absent. It was later demonstrated by Pardridge et al. [75] that glycoprotein insulin receptors are present on the endothelium of cerebral microvessels forming the blood–brain barrier. Binding of insulin to this endothelial surface triggers a receptor-mediated active transport mechanism,
allowing blood-borne insulin to cross the blood–brain barrier and penetrate the brain.

**INTERACTION OF INSULIN WITH TARGET CEREBRAL REGIONS**

**Intracellular effects of the insulin–insulin-receptor interaction**
The molecular mechanism by which insulin exerts its effects at its target sites is still partly unclear. Several lines of evidence indicate that insulin decreases glucose utilization at specific sites, inducing local neuroglycopenia, which triggers autonomic activation. The predominant cerebral glucose transporter, GLUT-1, is largely insensitive to insulin. The insulin-induced decrease in cerebral glucose utilization, therefore, must derive from either decreased glucose transport across the blood–brain barrier and/or a direct intracellular effect of insulin on glucose metabolism; direct evidence in favour of both mechanisms has been provided. Namba et al. [76] reported decreased transport of 3-O-[14C]methylglucose across the blood–brain barrier during hyperglycaemia. In two separate studies, Grundstein et al. [22] and Marfaing et al. [77] observed similar decreases in glucose utilization (as assessed by the 2-[^3H]deoxy-β-glucose method) in various cerebral regions, including the hypothalamus and the cortex, under euglycaemic/hyperinsulinaemic conditions. A direct effect of insulin at the cortical level, in particular, could explain the reported suppression of electrocortical activity at higher insulin but equivalent blood glucose concentrations [37], and could provide the physiological basis (severe cortical neuroglycopenia) for the more pronounced deterioration of neuropsychological skills in the presence of greater hyperinsulinaemia [37]. However, it should be noted that the effect of insulin on decreasing glucose uptake has not been demonstrated in humans. In fact, two recent studies [78,79] using positron-emission tomography (PET) detected no apparent effect of physiological hyperinsulinaemia during euglycaemia on net blood–brain barrier transport [79] or regional brain glucose uptake [78,79]. Future studies using PET to investigate the effects of different insulin levels during equivalent hyperglycaemia on brain glucose metabolism would be interesting.

A suppression of intracellular glucose metabolism, based on the interaction of insulin with the neuronal cell membrane, may not be the only mechanism by which insulin exerts its effects at its cerebral interaction sites. It has in fact been postulated that insulin interferes with neuronal activity in multiple brain regions, including the hypothalamus, possibly via direct binding to interneuronal glial connections [80]. Insulin has also been hypothesized to exert intracellular effects in select brain regions (arcuate, paraventricular nuclei) via mediation of neuropeptide Y, the most potent endogenous orexigenic signal. Although many aspects of this interaction still need clarification, circulating neuropeptide Y levels are decreased by insulin [81], and hypothalamic neuropeptide Y is increased in insulin-dependent diabetic models [82,83], possibly providing the physiological basis for diabetes-induced hyperphagia [84].

**Efferent response to the insulin–brain interaction**
Regardless of the mechanism by which it communicates with its individual cerebral interaction sites, insulin ultimately activates both components of the sympathetic–chromaffin system (adrenal medulla and sympathetic nervous system) [85], as well as the pituitary–adrenocortical system (resulting in secretion of growth hormone, ACTH and cortisol). The hypothesis that this activation is not hyperglycaemia-driven is supported by the fact that hyperinsulinaemia enhances sympathetic nervous system activity even under euglycaemic conditions [24].

Although several of the individual cerebral sites with which insulin may have a direct interaction have been identified, it still remains largely speculative as to how local interactions evolve in the final co-ordinated response to hyperinsulinaemia. One possibility is that the whole-body response is just the summation of multiple localized responses to insulin stimulation of individual target sites, including the following. (a) The hypothalamus. Neuroglycopenia in hypothalamic glucoregulatory centres would result in autonomic activation, downstream release of catecholamines and the onset of adrenergic symptoms. Also, release of hypothalamic pituitary releasing factors may stimulate the secretion of growth hormone, ACTH and other hormones. (b) The hippocampus. This region, very rich in insulin receptors [86], exerts an inhibitory effect on ACTH release from the hypophysis [87]. Insulin may decrease this inhibition, thereby stimulating secretion of ACTH and cortisol. It should be noted that cortisol secretion may also be stimulated directly by insulin via extracerebral pathways, such as direct insulin stimulation of the adrenal gland (where insulin receptors have been identified) [88], and insulin-induced conversion of cortisone into cortisol in adipose tissue [89]. (c) The hypophysis. Insulin receptors have been identified widely dispersed in the pituitary gland [90], and may determine ACTH and growth hormone release via direct insulin interaction with this gland. (d) The cortex. The ability of insulin to interact with the cerebral cortex and suppress local glucose metabolism is now well documented (see above).

Alternatively, the generalized response to hyperinsulinaemia could derive from a co-ordinated series of events stemming from the activation of one initial trigger
site, possibly the cerebral cortex, which would then relay impulses to ‘second level’ centres (hypothalamic nuclei, pituitary gland, etc.). These centres would, in turn, amplify or modulate the response and determine the downstream counter-regulatory events.

In summary, the hypothetical mechanism by which greater insulin levels, at comparable hypoglycaemia, enhance hypoglycaemic counter-regulatory responses implies several steps. Higher insulin levels reach the brain (either crossing the blood–brain barrier or accessing directly the circumventricular region) and impair intracellular glucose utilization, inducing local neuroglycopaenia, and/or have a direct neuromodulatory effect at multiple cerebral sites. In the cortex, this results in greater deterioration of neuropsychological skills. Insulin then stimulates other cerebral sites, either by direct interaction or through impulses relayed by the cortex or other cerebral areas. Through the hypothalamus, efferent sympathetic activity is stimulated. Through the pituitary gland, secretion of growth hormone and ACTH is increased, the latter increasing cortisol levels. Sympathetic stimulation of the adrenal medulla increases adrenaline and possibly noradrenaline levels. Both adrenal-sympathetic and neural-sympathetic hypoglycaemic symptoms are accentuated. The combined action of increased circulating levels of adrenaline, noradrenaline, growth hormone and cortisol stimulate lipolysis and EGP, and decrease peripheral glucose utilization.

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