
1 Normal Glucose Metabolism and Responses to Hypoglycaemia

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NORMAL GLUCOSE HOMEOSTASIS

Humans evolved as hunter-gatherers, and, unlike people today, did not consume regular meals. Mechanisms therefore evolved for the body to store food when it was in abundance, and to use these stores to provide an adequate supply of energy, in particular in the form of glucose when food was scarce. Cahill (1971) originally described the 'rules of the metabolic game' which man had to follow to ensure his survival. These were modified by Tattersall (personal communication) and are:

1. Maintain glucose within very narrow limits.
2. Maintain an emergency energy source (glycogen) that can be tapped quickly in time of need; 'Fight or Flight' response.
3. Waste not, want not, i.e. store (fat and protein) in times of plenty.
4. Use every trick in the book to maintain protein reserves.

Insulin and glucagon are the two key hormones controlling glucose homeostasis, and are therefore critical to the mechanisms enabling these 'rules' to be followed. The most important processes governed by these hormones are:

- *Glycogen synthesis and breakdown (glycogenolysis)*: Glycogen, a carbohydrate, is the most readily accessible energy store and is mostly found in liver and skeletal muscle. Liver glycogen is broken down to provide glucose for all tissues, whereas the breakdown of muscle glycogen results in lactate formation.
- *Gluconeogenesis*: This is the production of glucose in the liver from precursors: glycerol, lactate and amino acids (in particular alanine). The process can also occur in the kidneys, but this site is not important under most physiological conditions.
- *Glucose uptake and metabolism (glycolysis)* by skeletal muscle and adipose tissue.

The actions of insulin and glucagon are summarised in Boxes 1.1 and 1.2, respectively. Insulin is an anabolic hormone, reducing glucose output by the liver (hepatic glucose

Box 1.1 Actions of insulin

Liver

- ↑ glycogen synthesis (↑ glycogen synthetase activity)
- ↑ glycolysis
- ↑ lipid formation
- ↑ protein formation
- ↓ glycogenolysis (↓ phosphorylase activity)
- ↓ gluconeogenesis
- ↓ ketone formation

Muscle

- ↑ uptake of glucose
- ↑ uptake of amino acids
- ↑ uptake of ketone
- ↑ uptake of potassium
- ↑ glycolysis
- ↑ synthesis of glycogen
- ↑ synthesis of protein
- ↓ protein catabolism
- ↓ release of amino acids

Adipose tissue

- ↑ uptake of glucose
- ↑ uptake of potassium
- ↑ storage of triglyceride

Box 1.2 Actions of glucagon

Liver

- ↑ glycogenolysis
- ↑ gluconeogenesis
- ↑ extraction of alanine
- ↑ ketogenesis
- No significant peripheral action

output), increasing uptake of glucose by muscle and adipose tissue (increasing peripheral uptake) and increasing protein and fat formation. Glucagon opposes the actions of insulin in the liver. Thus insulin tends to reduce, and glucagon increase blood glucose concentrations.

The metabolic effects of insulin and glucagon and their relationship to glucose homeostasis are best considered in relationship to fasting and the postprandial state (Siegal and Kreisberg 1975). In both these situations it is the relative and not absolute concentrations of these hormones that are important.

Fasting (Figure 1.1a)

During fasting, insulin concentrations are reduced and glucagon increased, which maintains blood glucose concentrations in accordance with rule 1 above. The net effect is to reduce peripheral glucose utilisation, increase hepatic glucose production and to provide non-

glucose fuels for tissues not entirely dependent on glucose. After a short (e.g. overnight) fast, glucose production needs to be 5–6 g/h to maintain blood glucose concentrations, with the brain using 80% of this. Glycogenolysis provides 60–80% and gluconeogenesis 20–40% of the required glucose. In prolonged fasts, glycogen becomes depleted and glucose production is primarily from gluconeogenesis, with an increasing proportion from the kidney as opposed to the liver. In extreme situations renal gluconeogenesis can contribute as much as 45% of glucose production. Thus glycogen is the short-term or ‘emergency’ fuel source (rule 2), with gluconeogenesis predominating in more prolonged fasts. The following metabolic alterations enable this increase in glucose production to occur.

- *Muscle*: Glucose uptake and oxidative metabolism are reduced and fatty acid oxidation increased. Amino acids are released.
- *Adipose tissue*: There are reductions in glucose uptake and triglyceride storage. The increase in the activity of the enzyme hormone-sensitive lipase, results in hydrolysis of triglyceride to glycerol (a gluconeogenic precursor) and fatty acids, which can be metabolised.
- *Liver*: Increased cAMP concentrations result in increased glycogenolysis and gluconeogenesis thus increasing hepatic glucose output. The uptake of gluconeogenic precursors (i.e. amino acids, glycerol, lactate and pyruvate) is also increased. Ketone bodies are produced in the liver from fatty acids. This process is normally inhibited by insulin and stimulated by glucagon, thus the hormonal changes during fasting lead to an increase in ketone production. Fatty acids are also a metabolic fuel used by the liver as a source of energy needed for the reactions involved in gluconeogenesis.

The reduced insulin:glucagon ratio favours a catabolic state, but the effect on fat metabolism is greater than protein, and thus muscle is relatively preserved (rule 4). These adaptations meant that not only did the hunter-gatherer have sufficient muscle power to pursue his next meal, but that brain function was optimally maintained to help him do this.

Fed state (Figure 1.1b)

In the fed state, in accordance with the rules of the metabolic game, excess food is stored as glycogen, protein and fat (rule 3). Rising glucose concentrations after a meal result in an increase in insulin and reduction in glucagon secretion. This balance favours glucose utilisation, reduction of glucose production and increases glycogen, triglyceride and protein formation. The following changes enable these processes to occur:

- *Muscle*: Insulin increases glucose transport, oxidative metabolism and glycogen synthesis. Amino acid release is inhibited and protein synthesis increased.
- *Adipose tissue*: In the fat cells, glucose transport is increased, while lipolysis is inhibited. At the same time the enzyme lipoprotein lipase, located in the capillaries, is activated and causes triglyceride to be broken down to fatty acids and glycerol. The fatty acids are taken up into the fat cells and re-esterified to triglyceride (using glycerol phosphate derived from glucose) before being stored.
- *Liver*: Glucose uptake is increased in proportion to plasma glucose, a process that does not need insulin. However, insulin does decrease cAMP concentrations, which result in an increase in glycogen synthesis and the inhibition of glycogenolysis and gluconeogenesis. These effects ‘retain’ excess glucose as glycogen in the liver.

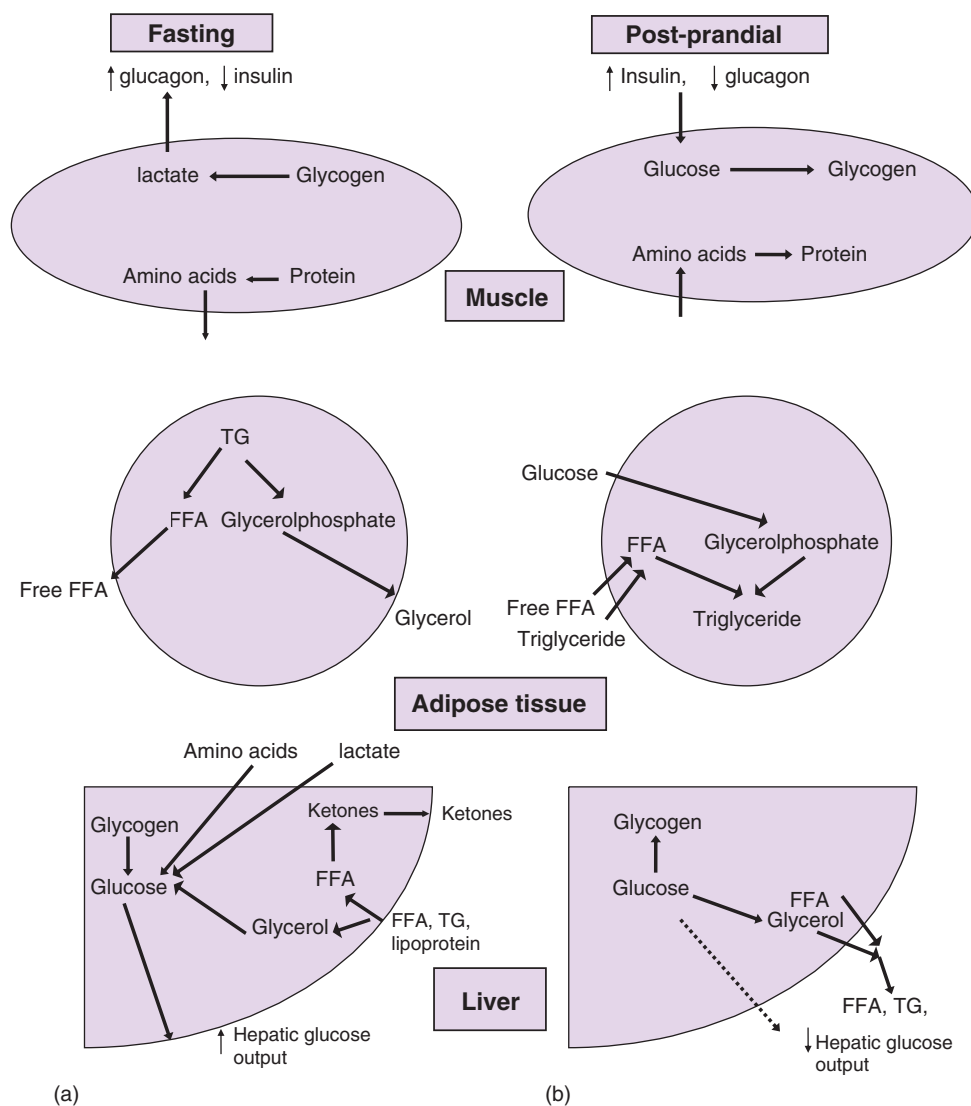


Figure 1.1 Metabolic pathways in glucose homeostasis in muscle, adipose tissue and liver. (a) Fasting; (b) post-prandial. FFA, free fatty acids; TG, triglyceride.

While insulin and glucagon are the key hormones involved in glucose homeostasis in the fed state, there are a number of other glucoregulatory hormones released in response to an oral glucose load including Gastric Inhibitory Peptide, Glucagon-like peptide 1 (GLP-1), cholecystikinin Peptide Y and Ghrelin.

GLP-1, for example is made in the L-cells of the distal gut as well as in the brain. Peripheral GLP-1 is produced in response to a glucose load. Through vagally-mediated central and peripheral mechanisms, GLP-1 augments glucose-stimulated insulin production, reduces glucagon secretion, slows gastric emptying and promotes satiety.

A detailed discussion of the role of these peptides in glucose homeostasis is beyond the scope of this chapter, but has been reviewed by Heijboer *et al.* (2006), Drucker (2007) and

Maggs *et al.* (2008). Some recently introduced antidiabetes drugs act through the GLP-1 receptor, either through a direct action (GLP-1 agonism) or through prevention of endogenous GLP-1 break-down (dipeptidyl peptidase 4 inhibitors), and via this action stimulate insulin release. Concerns that this might lead to problems with gluco-regulation during exercise do not seem to be well founded, as Khoo *et al.* (2010) showed no detrimental effects of the GLP-1 agonist, exenatide, on glycaemia during exercise.

This complex interplay between insulin and glucagon as well as gut peptides maintains euglycaemia and enables the rules of the metabolic game to be followed, ensuring not only the survival of the hunter-gatherer, but also of modern humans.

EFFECTS OF GLUCOSE DEPRIVATION ON CENTRAL NERVOUS SYSTEM METABOLISM

The brain constitutes only 2% of body weight, but consumes 20% of the body's oxygen and receives 15% of its cardiac output (Sokaloff 1989). It is almost totally dependent on carbohydrate as a fuel and since it cannot store or synthesise glucose, depends on a continuous supply from the blood. The brain contains the enzymes needed to metabolise fuels other than glucose such as lactate, ketones and amino acids, but under physiological conditions their use is limited by insufficient quantities in the blood or slow rates of transport across the blood-brain barrier. When arterial blood glucose falls below 3 mmol/l, cerebral metabolism and function decline.

Metabolism of glucose by the brain releases energy, and also generates neurotransmitters such as gamma amino butyric acid (GABA) and acetylcholine, together with phospholipids needed for cell membrane synthesis. When blood glucose concentration falls, changes in the synthesis of these products may occur within minutes because of reduced glucose metabolism, which can alter cerebral function. This is likely to be a factor in producing the subtle changes in cerebral function detectable at blood glucose concentrations of 3 mmol/l, a degree of hypoglycaemia that is actually not sufficiently low to cause a major depletion in ATP or creatine phosphate, the brain's two main sources of energy (McCall 1993).

Isotope techniques and Positron Emission Tomography (PET) allow the study of metabolism in different parts of the brain and show regional variations in metabolism during hypoglycaemia. The neocortex, hippocampus, hypothalamus and cerebellum are most sensitive to hypoglycaemia, whereas metabolism is relatively preserved in the thalamus and brainstem. Changes in cerebral function are initially reversible, but during prolonged severe hypoglycaemia, general energy failure (due to the depletion of ATP and creatine phosphate) can cause permanent cerebral damage. Pathologically this is caused by selective neuronal necrosis most likely due to 'excitotoxin' damage. Local energy failure induces the intrasynaptic release of glutamate or aspartate, and failure of reuptake of the neurotransmitters increases their concentrations. This leads to the activation of N-methyl-D-aspartate (NMDA) receptors causing cerebral damage. One study in rats has shown that an experimental compound called AP7, which blocks the NMDA receptor, can prevent 90% of the cerebral damage associated with severe hypoglycaemia (Wieloch 1985). In humans with fatal hypoglycaemia, protracted neuroglycopenia causes laminar necrosis in the cerebral cortex and diffuse demyelination. Regional differences in neuronal necrosis are seen, with the basal ganglia and hippocampus being affected, but the hypothalamus and cerebellum relatively spared (Auer and Siesjö 1988; Sieber and Traysman 1992).

Transport of glucose across blood-brain barrier

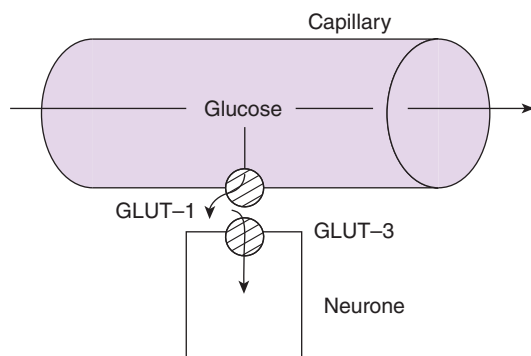


Figure 1.2 Transport of glucose into the brain across the blood–brain barrier.

The brain is very sensitive to acute hypoglycaemia, but can adapt to chronic fuel deprivation. For example, during starvation, it can metabolise ketones for up to 60% of its energy requirements (Owen *et al.* 1967). Glucose transport can also be increased in the face of hypoglycaemia. Normally, glucose is transported into tissues using proteins called glucose transporters (GLUT) (Bell *et al.* 1990). This transport occurs down a concentration gradient faster than it would by simple diffusion and does not require energy (facilitated diffusion). There are several of these transporters, with GLUT 1 being responsible for transporting glucose across the blood–brain barrier and GLUT 3 into neurones (Figure 1.2). Chronic hypoglycaemia in animals (McCall *et al.* 1986) and in humans (Boyle *et al.* 1995) increases global cerebral glucose uptake, which is thought to be due to an increase in the production and action of GLUT 1 protein. It has not been established whether this adaptation is of major benefit in protecting brain function during hypoglycaemia or whether this adaptation also occurs in response to repeated, rather than just chronic (up to 3 days), hypoglycaemia.

COUNTERREGULATION DURING HYPOGLYCAEMIA

The potentially serious effects of hypoglycaemia on cerebral function mean that not only are stable blood glucose concentrations maintained under physiological conditions, but if hypoglycaemia occurs, mechanisms have developed to combat it. In clinical practice, the principal causes of hypoglycaemia are iatrogenic (as side-effects of insulin and sulfonylureas used to treat diabetes) and excessive alcohol consumption. Insulin-secreting tumours (such as insulinoma) are rare. The mechanisms that correct hypoglycaemia are called counterregulation, because the hormones involved oppose the action of insulin and are thus the counterregulatory hormones. The processes of counterregulation were identified in the mid-1970s and early 1980s, using either a bolus injection or continuous infusion of insulin to induce hypoglycaemia (Cryer 1981; Gerich 1988). The response to the bolus injection of 0.1 U/kg insulin in a normal subject is shown in Figure 1.3. Blood glucose concentrations

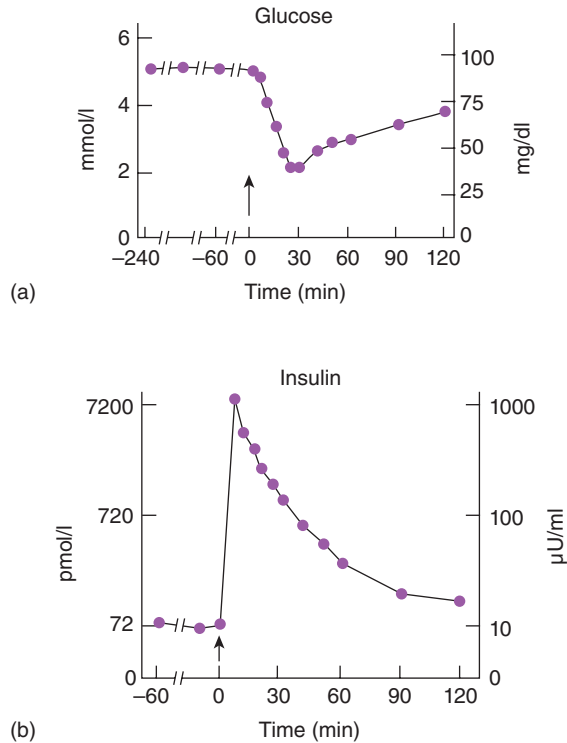


Figure 1.3 Glucose (a) and insulin (b) concentrations after intravenous injection of insulin 0.1 U/kg at time 0. (Source: Garber AJ 1976. Reproduced with permission of the American Society for Clinical Investigation).

decline within minutes of the administration of insulin and reach a nadir after 20–30 minutes, then gradually rise to near normal by 2 hours after the insulin was administered. The fact that blood glucose starts to rise when plasma insulin concentrations are still 10 times the baseline values means that it is not simply the reduction in insulin that reverses hypoglycaemia, but active counterregulation must also occur. Many hormones are released when blood glucose is lowered (see below), but glucagon, the catecholamines, growth hormone and cortisol are regarded as being the most important.

Several studies have determined the relative importance of these hormones by producing isolated deficiencies of each hormone (by blocking its release or action) and assessing the subsequent response to administration of insulin. These studies assessed the relative importance of glucagon, adrenaline (epinephrine) and growth hormone in the counterregulation of short-term hypoglycaemia. Somatostatin infusion was used to block glucagon and growth hormone secretion and significantly impaired glucose recovery. When growth hormone was replaced in the same model to produce isolated glucagon deficiency or glucagon replaced to produce isolated growth hormone deficiency, it is clear that it was glucagon and not growth hormone that was responsible for acute counterregulation. Combined alpha- and beta-adrenoceptor blockade using phentolamine and propranolol infusions or studies of patients who had undergone an adrenalectomy were used to evaluate the role of the catecholamines. Overall these studies demonstrate that glucagon is the most important counterregulatory hormone while catecholamines provide a backup if glucagon

is deficient (e.g. in type 1 diabetes, Chapters 3 and 6). Cortisol and growth hormone are probably more important to prolonged hypoglycaemia. Therefore, if glucagon and catecholamines are both deficient, as in longstanding type 1 diabetes, counterregulation is seriously compromised, and the individual is nearly defenceless against acute hypoglycaemia (Cryer 1981).

Glucagon and catecholamines increase glycogenolysis and stimulate gluconeogenesis. Catecholamines also reduce glucose utilisation peripherally and inhibit insulin secretion. Cortisol and growth hormone increase gluconeogenesis and reduce glucose utilisation. The role of the other hormones (see below) in counterregulation is unclear, but they are unlikely to make a significant contribution. Finally, there is evidence that in profound hypoglycaemia (blood glucose below 1.7 mmol/l), hepatic glucose output is stimulated directly, although the mechanism is unknown. This is termed *hepatic autoregulation*.

The depth, as well as the duration of hypoglycaemia is important in determining the magnitude of the counterregulatory hormone response. Studies using 'hyperinsulinaemic clamps' show a hierarchical response of hormone production. In this technique, insulin is infused at a constant rate and a glucose infusion rate varied to maintain blood glucose concentrations within ± 0.2 mmol/l of target concentrations. This permits the controlled evaluation of the counterregulatory hormone response at varying degrees of hypoglycaemia. It also demonstrates that in human subjects without diabetes, glucagon, catecholamines and growth hormone start to be produced at a blood glucose concentration of 3.5–3.7 mmol/l, with cortisol produced at a lower glucose of 3.0 mmol/l (Mitrakou *et al.* 1991). The counterregulatory response is initiated before impairment in cerebral function is first evident, usually at a glucose concentration of approximately 3.0 mmol/l (Heller and Macdonald 1996).

The magnitude of the hormonal response also depends on the length of the hypoglycaemic episode. The counterregulatory hormonal response commences up to 20 minutes after hypoglycaemia is achieved and continues to rise for 60 minutes (Kerr *et al.* 1989). In contrast, this response is attenuated if it occurs within 2–3 days of a previous episode of hypoglycaemia (Heller and Macdonald 1996) or if it follows prolonged exercise the day before hypoglycaemia is induced. Galassetti *et al.* (2001) showed in non-diabetic subjects that 3 hours of moderate intensity exercise the previous day markedly decreased the counterregulatory response to hypoglycaemia induced by the infusion of insulin, and that the reduced counterregulatory response was more marked in men than in women. It is also now apparent that antecedent bouts of exercise can affect the counterregulatory response to subsequent hypoglycaemia (Sandoval *et al.* 2006).

While the primary role of the counterregulatory hormones is on glucose metabolism, any effects on fatty acid utilisation can have an indirect effect on blood glucose. Thus, the increase in plasma adrenaline (epinephrine) (and activation of the sympathetic nervous system) that occurs in hypoglycaemia can stimulate lipolysis of triglyceride in adipose tissue and muscle and release fatty acids which can be used as an alternative fuel to glucose, making more glucose available for the central nervous system (CNS). Enoksson *et al.* (2003) demonstrated that adults with type 1 diabetes who had lower plasma adrenaline responses to hypoglycaemia than non-diabetic controls also had reduced rates of lipolysis in adipose tissue and skeletal muscle, making them more dependent on glucose as a fuel and therefore at risk of developing more severe hypoglycaemia.

The complex counterregulatory and homeostatic mechanisms described above are thought to be mostly under CNS control. Evidence for this comes from studies in dogs, where glucose was infused into the carotid and vertebral arteries to maintain euglycaemia

in the brain. Despite peripheral hypoglycaemia, glucagon did not increase, and responses of the other counterregulatory hormones were blunted. This, and other studies in rats, led to the hypothesis that the ventromedial nucleus of the hypothalamus (VMH) acts as the primary glucose sensor and coordinates counterregulation during hypoglycaemia (Borg *et al.* 1997). However, other parts of the brain are also involved in mediating counterregulation, and it is likely that the VMH is part of an integrated network of specialised glucose-sensing brain regions.

It is now clear that glucose-sensing neurones use glucokinase and ATP-sensitive K^+ channels to transduce the glucose signal into changes in neuronal activity (Levin *et al.* 2004). In rats, the VMH has ATP-sensitive K^+ channels that seem to be involved in the counterregulatory responses to hypoglycaemia, as injection of the sulfonylurea, glibenclamide, directly into the VMH, suppressed hormonal responses to systemic hypoglycaemia (Evans *et al.* 2004), whereas diazoxide amplified hormonal responses (McCrimmon *et al.* 2005).

The existence of hepatic autoregulation suggests that some peripheral control also exists. Studies producing central euglycaemia and hepatic-portalvenous hypoglycaemia in dogs have provided evidence for hepatic glucose sensors and suggest that these sensors, as well as those in the brain, are important in the regulation of glucose (Hamilton-Wessler *et al.* 1994). However, this topic is somewhat controversial and more recent studies on dogs have failed to demonstrate an effect of hepatic sensory nerves on the responses to hypoglycaemia (Jackson *et al.* 2000). Moreover, studies in human subjects by Heptulla *et al.* (2001) showed that providing glucose orally rather than intravenously during a hypoglycaemic hyperinsulinaemic clamp actually enhanced the counterregulatory hormone responses rather than reduced them.

HORMONAL CHANGES DURING HYPOGLYCAEMIA

Hypoglycaemia induces a change in various hormones some of which also contribute to symptom generation (Chapter 2), counterregulation, and many of the physiological changes that occur as a consequence of lowering blood glucose. The stimulation of the autonomic nervous system is central to many of these changes.

Activation of the autonomic nervous system

The autonomic nervous system comprises sympathetic and parasympathetic components (Figure 1.4). Fibres from the sympathetic division leave the spinal cord with the ventral roots from the first thoracic to the third or fourth lumbar nerves to synapse in the sympathetic chain or visceral ganglia, and the long postganglionic fibres are incorporated in somatic nerves. The parasympathetic pathways originate in the nuclei of cranial nerves III, VII, IX and X and travel with the vagus nerve. A second component, the sacral outflow, supplies the pelvic viscera via the pelvic branches of the second to fourth spinal nerves. The ganglia in both cases are located near the organs supplied, and the postganglionic neurones are therefore short.

Activation of both components of the autonomic system occurs during hypoglycaemia. The sympathetic nervous system in particular is responsible for many of the physiological changes during hypoglycaemia, and the evidence for its activation can be obtained indirectly by observing functional changes such as cardiovascular responses (considered

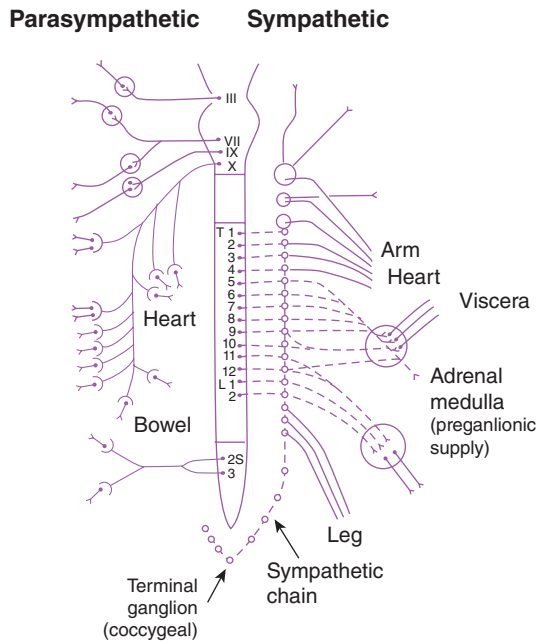
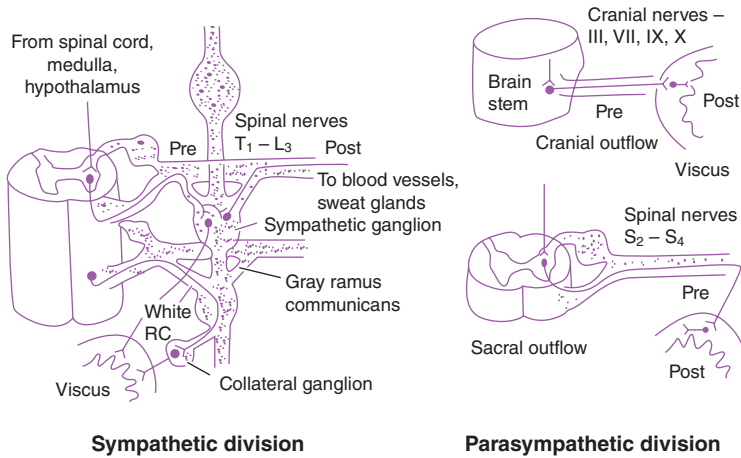


Figure 1.4 Anatomy of the autonomic nervous system. Pre, preganglionic neurones; post, postganglionic neurones; RC, rami communicans.

below), measuring plasma catecholamines, which gives a general index of sympathetic activation, or by directly recording sympathetic activity.

Direct recordings are possible from sympathetic nerves supplying skeletal muscle and skin. Sympathetic neural activity in skeletal muscle involves vasoconstrictor fibres that innervate blood vessels and are involved in controlling blood pressure. During hypoglycaemia (induced by insulin), the frequency and amplitude of muscle sympathetic activity are increased as blood glucose falls, with an increase in activity 8 minutes after insulin is

injected intravenously, peaking at 25–30 minutes coincident with the glucose nadir, and persisting for 90 minutes after euglycaemia is restored (Fagius *et al.* 1986). During hypoglycaemia, a sudden increase in skin sympathetic activity is seen, which coincides with the onset of sweating. This sweating leads to vasodilatation of skin blood vessels, which is also contributed to by a reduction in sympathetic stimulation of the vasoconstrictor components of skin arterio-venous anastomoses (Berne and Fagius 1986). These effects (at least initially) increase total skin blood flow and promote heat loss from the body.

Activation of both muscle and skin sympathetic nerve activity is thought to be centrally mediated. Tissue neuroglycopenia can be produced by 2-deoxy-D-glucose, a glucose analogue, without increasing insulin. Infusion of this analogue causes stimulation of muscle and skin sympathetic activity demonstrating that it is hypoglycaemia and not the insulin used to induce it that is responsible for the sympathetic activation (Fagius and Berne 1989).

The activation of the parasympathetic nervous system (vagus nerve) during hypoglycaemia cannot be measured directly. The most useful index of parasympathetic function is the measurement of plasma pancreatic polypeptide (PP), the peptide hormone secreted by the PP cells of the pancreas, which is released in response to vagal stimulation.

Neuroendocrine activation (Box 1.3)

Insulin-induced hypoglycaemia was used to study pituitary function as early as the 1940s. The glucose response to insulin was used to measure insulin resistance, and thereby evaluate conditions such as Cushing's syndrome and hypopituitarism. The development of assays for adrenocorticotrophic hormone (ACTH) and growth hormone (GH) allowed the direct measurement of pituitary function during hypoglycaemia in the 1960s, and many of the processes governing these changes were unravelled before elucidation of the counterregulatory system. The studies are comparable to those evaluating counterregulation, in that potential regulatory factors are blocked to measure the hormonal response to hypoglycaemia with and without the regulating factor.

Hypothalamus and anterior pituitary gland

ACTH, GH and prolactin concentrations increase during hypoglycaemia, but there is no change in thyrotrophin or gonadotrophin secretion. The secretion of these pituitary hormones is controlled by releasing factors that are produced in the median eminence of the hypothalamus and secreted into the hypophyseal portal vessels and then pass to the pituitary gland (Figure 1.5). The mechanisms regulating the releasing factors are incompletely understood but may involve the VMH and other hypothalamic regions where brain glucose sensors are situated (Fish *et al.* 1986).

- *ACTH*: Secretion is governed by release of corticotrophin releasing hormone (CRH) from the hypothalamus; alpha adrenoceptors stimulate CRH release, and beta adrenoceptors have an inhibitory action. A variety of neurotransmitters control the release of CRH into the portal vessels, including serotonin and acetylcholine, which are stimulatory, and GABA, which is inhibitory. The increase in ACTH causes cortisol to be secreted from the cortices of the adrenal glands.
- *Beta endorphins* are derived from the same precursors as ACTH and are co-secreted with it. The role of endorphins in counterregulation is uncertain, but they may influence the secretion of the other pituitary hormones during hypoglycaemia.

Box 1.3 Neuroendocrine activation

Hypothalamus
 ↑ CRH
 ↑ GHRH
 Ant Pituitary
 ↑ ACTH
 ↑ β endorphin
 ↑ GH
 ↑ Prolactin
 \leftrightarrow TSH
 \leftrightarrow Gonadotrophins
 Post Pituitary
 ↑ Vasopressin
 ↑ Oxytocin
 Pancreas
 ↑ Glucagon
 ↑ Somatostatin-28
 ↑ Pancreatic polypeptide
 ↓ Insulin
 Adrenal
 ↑ Cortisol
 ↑ Adrenaline (epinephrine)
 ↑ Aldosterone
 Others
 ↑ PTH
 ↑ Gastrin

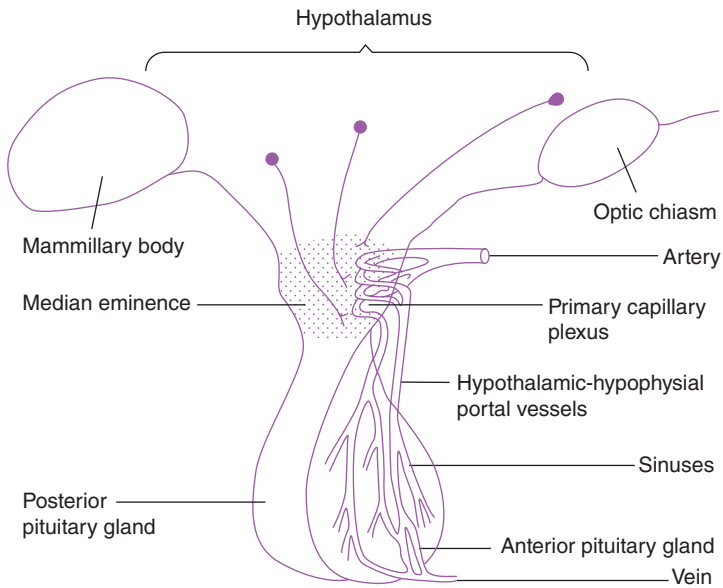


Figure 1.5 Anatomy of the hypothalamus and pituitary.

- *GH*: Growth hormone secretion is governed by two hypothalamic hormones: growth hormone releasing hormone (GHRH), which stimulates GH secretion, and somatostatin, which is inhibitory. GHRH secretion is stimulated by dopamine, GABA, opiates and through alpha adrenoceptors, while it is inhibited by serotonin and beta adrenoceptors. A study in rats showed that bio-assayable GH and GHRH are depleted in the pituitary and hypothalamus respectively after insulin-induced hypoglycaemia (Katz *et al.* 1967).
- *Prolactin*: The mechanisms are not established. Prolactin secretion is normally under inhibitory control of dopamine, but there is also evidence for releasing factors during hypoglycaemia. Prolactin does not contribute to counterregulation.

Posterior pituitary gland

Vasopressin and oxytocin both increase during hypoglycaemia (Fisher *et al.* 1987). Their secretion is under hormonal and neurotransmitter control in a similar way to the hypothalamic hormones. Vasopressin has glycolytic actions and oxytocin increases hepatic glucose output in dogs, but their contribution to glucose counterregulation is uncertain.

Pancreas

- *Glucagon*: The mechanisms of glucagon secretion during hypoglycaemia are still not fully understood. Although activation of the autonomic nervous system stimulates its release, this pathway has been shown to be less important in humans. A reduction in glucose concentrations may have a direct effect on the glucagon-secreting pancreatic alpha cells, or the reduced beta cell activity (reduced insulin and/or cofactors such as zinc or GABA secretion), which also occurs with low blood glucose, may release the tonic inhibition of glucagon secretion. In type 1 diabetes, where hypoglycaemia is normally associated with high plasma insulin levels, this mechanism is disturbed, and the failure of insulin to suppress ('switch-off') may contribute to the selective defect in glucagon secretion that is characteristic of C-peptide-negative type 1 diabetes. However, this remains to be proven (Cryer 2012).
- *Somatostatin*: This is thought of as a pancreatic hormone produced from D cells of the islets of Langerhans but it is also secreted in other parts of the gastrointestinal tract. There are a number of structurally different polypeptides derived from prosomatostatin: the somatostatin-14 peptide is secreted from D cells, and somatostatin-28 from the gastrointestinal tract. The plasma concentration of somatostatin-28 increases during hypoglycaemia (Francis and Ensink 1987). The normal action of somatostatin is to inhibit the secretion both of insulin and glucagon, but somatostatin-28 inhibits insulin 10 times more effectively than glucagon, and thus may have a role in counterregulation by suppressing insulin release.
- *Pancreatic polypeptide*: This peptide has no role in counterregulation, but its release during hypoglycaemia is stimulated by cholinergic fibres through muscarinic receptors and is a useful marker of parasympathetic activity.

Adrenal gland and renin – angiotensin system

The processes governing the increase in cortisol during hypoglycaemia are discussed above. A rise in catecholamines, in particular adrenaline from the adrenal medulla that

occurs when blood glucose is lowered, is controlled by sympathetic fibres in the splanchnic nerve. The increase in renin, angiotensin and aldosterone during hypoglycaemia, results primarily from the intra-renal actions of catecholamines, mediated through beta adrenoceptors, although the hypoglycaemia-induced increase in ACTH and hypokalaemia may contribute (Trovati *et al.* 1988; Jungman *et al.* 1989). These changes do not have a significant role in counterregulation, although angiotensin II has glycolytic actions *in vitro*.

PHYSIOLOGICAL RESPONSES

Haemodynamic changes (Box 1.4)

The haemodynamic changes during hypoglycaemia (Hilsted 1993) are mostly caused by the activation of the sympathetic nervous system and an increase in circulating adrenaline. An increase in heart rate (tachycardia) and cardiac output occurs, which is mediated through beta₁ adrenoceptors, but increasing vagal tone counteracts this so the increase is transient. Peripheral resistance, estimated from mean arterial pressure divided by cardiac output, is reduced. A combination of increased cardiac output and reduced peripheral resistance results in an increase in systolic and a small decrease in diastolic pressure, in other words, widening of pulse pressure. However, central blood pressure falls during hypoglycaemia accompanied by an increase in arterial elasticity, as demonstrated by changes in the Augmentation Index (Sommerfield *et al.* 2007).

Changes in regional blood flow (Box 1.5 and Figure 1.6)

- *Cerebral blood flow*: Early work produced conflicting results, but these studies were in subjects receiving insulin shock therapy, and the varying effects of convulsions and altered level of consciousness may have influenced the outcome. Subsequent studies have consistently shown an increase in cerebral blood flow during hypoglycaemia

Box 1.4 Haemodynamic changes

↑ Heart rate
 ↑ Blood pressure (peripheral systolic); ↓ central blood pressure
 ↑ Cardiac output
 ↓ Peripheral resistance

Box 1.5 Changes in regional blood flow

↑ cerebral flow
 ↑ total splanchnic flow
 ↑ muscle flow
 skin flow variable
 ↓ renal flow
 ↓ splenic flow

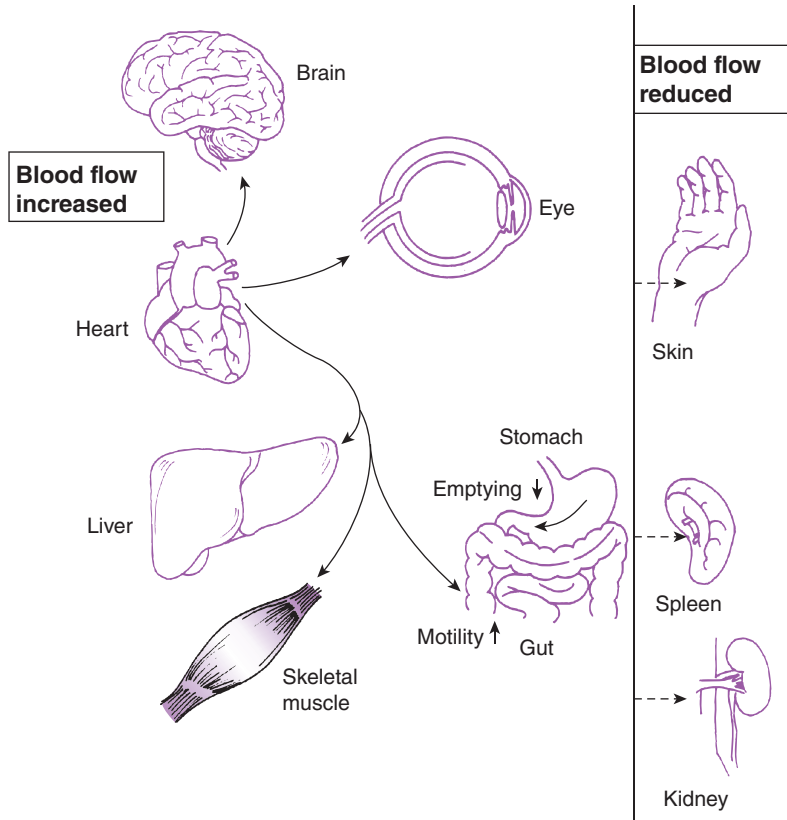


Figure 1.6 Changes in regional blood flow during hypoglycaemia.

despite the use of different methods of measurement (isotopic, single photon emission computed tomography (SPECT), and Doppler ultrasound). In most of the studies blood glucose concentration was less than 2 mmol/l before a change was observed. In the last decade the use of PET and Magnetic Resonance Imaging (MRI) has allowed a more detailed study of cerebral blood flow, showing regional changes even with minimal changes in glucose concentration. Using PET, Teves *et al.* (2004) demonstrated a 6.8% reduction in cerebral blood flow in the brainstem, cerebellum and hemispheres, and an increase in the thalamus, globus pallidus and medial frontal cortex at glucose concentrations of 3 mmol/l. The subsequent differences in substrate delivery may explain why certain areas of the brain such as the cerebellum are more vulnerable to hypoglycaemia-induced cerebral damage. More recently, Page *et al.* (2009) used MRI to demonstrate an increase in hypothalamic blood flow when glucose was reduced from 5.3 to 4.3 mmol/l. In animals, hypoglycaemia is associated with loss of cerebral autoregulation (the ability of the brain to maintain cerebral blood flow despite variability in cardiac output) through beta adrenoceptor stimulation, but the exact mechanisms are unknown (Bryan 1990; Sieber and Traysman 1992).

- *Gastrointestinal system:* Total splanchnic blood flow (that supplying the intestines, liver, spleen and stomach) is increased and splanchnic vascular resistance reduced as assessed by the bromsulphthalein extraction technique (Bearn *et al.* 1952). Superior

mesenteric artery blood flow measured using Doppler ultrasound increases during hypoglycaemia due to beta adrenoceptor stimulation (Braatvedt *et al.* 1993). Radioisotope scanning has demonstrated a reduction in splenic activity during hypoglycaemia (Fisher *et al.* 1990), which is thought to be due to alpha adrenoceptor-mediated reduction in blood flow. All these changes would all be expected to increase hepatic blood flow, although this has not been confirmed experimentally.

- *Skin*: The control of blood flow to the skin is complex and different mechanisms predominate in different areas. Studies of the effect of hypoglycaemia on skin blood flow are inconsistent partly because different methods have been used for blood flow measurement and induction of hypoglycaemia, as well as differences in the part of the body studied. Definitive conclusions are therefore not possible. Studies using the dorsum of the foot, cheek and forehead have consistently shown an initial vasodilatation and increase in blood flow followed by later vasoconstriction at a blood glucose of 2.5 mmol/l (Maggs *et al.* 1994a). These findings are consistent with the clinical picture of initial flushing and later pallor, with an early rise in skin blood flow and a later fall.
- *Muscle blood flow*: A variety of techniques have been used to study muscle blood flow (including venous occlusion plethysmography, isotopic clearance techniques and the use of thermal conductivity meters). All studies have consistently shown an increase in muscle blood flow during hypoglycaemia irrespective of skin blood flow. This change is mediated by beta₂ adrenoceptors (Allwood *et al.* 1959; Abramson *et al.* 1966).
- *Kidney*: Inulin and sodium hippurate clearance can be used to estimate glomerular filtration rate and renal blood flow, respectively. Both decrease during hypoglycaemia (Patrick *et al.* 1989) and catecholamines and renin are implicated in initiating the changes.

The changes in blood flow in various organs, like the haemodynamic changes, are mostly mediated by the activation of the sympathetic nervous system or circulating adrenaline. The majority either protect against hypoglycaemia or increase substrate delivery to vital organs. The increase in cerebral blood flow increases substrate delivery to the brain. Increasing muscle flow enhances the release and washout of gluconeogenic precursors. The increase in splanchnic blood flow and reduction in splenic blood flow serve to increase hepatic blood flow to maximise hepatic glucose production. Meanwhile blood is diverted away from organs such as the kidney that are not required in the acute response to the metabolic stress.

Functional changes (Box 1.6)

- *Sweating*: Sweating is mediated by sympathetic cholinergic nerves, although other neurotransmitters such as vasoactive intestinal peptide and bradykinin may also be involved. The activation of the sympathetic innervation of the skin as described above results in the sudden onset of sweating. Sweating is one of the first physiological responses to occur during hypoglycaemia and can be demonstrated within 10 minutes of achieving a blood glucose of 2.5 mmol/l (Maggs *et al.* 1994b). It coincides with the onset of other measures of autonomic activation, such as an increase in heart rate and tremor (Figure 1.7).
- *Tremor*: Trembling and shaking are characteristic features of hypoglycaemia and result from an increase in physiological tremor. The rise in cardiac output and vasodilatation occurring during hypoglycaemia increase the level of physiological tremor and this is

Box 1.6 Functional changes

- ↑ sweating (sudden onset)
- ↑ tremor
- ↓ core temperature
- ↓ intraocular pressure
- ↑ jejunal activity
- ↑ gastric emptying

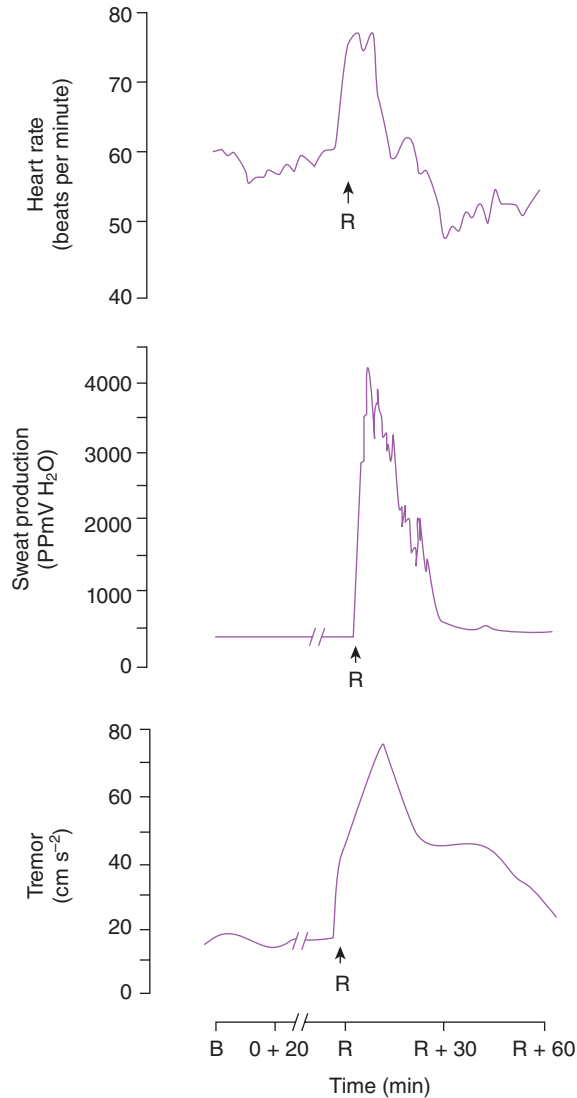


Figure 1.7 Sudden onset of sweating, tremor and increase in heart rate during the induction of hypoglycaemia.

exacerbated by beta adrenoceptor stimulation associated with increased adrenaline concentrations (Kerr *et al.* 1990). Since adrenalectomy does not entirely abolish tremor, other components such as the activation of muscle sympathetic activity must be involved.

- *Temperature*: Despite a beta adrenoceptor-mediated increase in metabolic rate, core temperature falls during hypoglycaemia. The mechanisms by which this occurs depend on whether the environment is warm or cold. In a warm environment, heat is lost because of sweating and increased heat conduction from vasodilatation. Hypoglycaemia reduces core temperature by 0.3°C and skin temperature by up to 2°C (depending on the part of the body measured) after 60 minutes (Maggs *et al.* 1994b). In the cold, shivering is reduced, and this together with vasodilatation and sweating causes a substantial reduction in core temperature (Gale *et al.* 1983). In rats, mortality was increased in animals whose core temperature was prevented from falling during hypoglycaemia (Buchanan *et al.* 1991). In humans there is anecdotal evidence from subjects undergoing insulin shock therapy that those who had a rise in body temperature showed delayed neurological recovery (Ramos *et al.* 1968). These findings support the hypothesis that the fall in core temperature reduces metabolic rate, allowing hypoglycaemia to be better tolerated, and thus the changes in body temperature are of survival value. The beneficial effects are likely to be limited, particularly in the cold, where the impairment of cerebral function means subjects may not realise they are cold, causing them to be at risk of severe hypothermia.
- Other functional changes include a reduction in intra-ocular pressure, greater jejunal but not gastric motility and inconsistent abnormalities of liver function tests. An increase in gastric emptying occurs during hypoglycaemia (Schvarcz *et al.* 1995), which may be protective in that carbohydrate delivery to the intestine is increased, enabling faster glucose absorption and reversal of hypoglycaemia.

CONCLUSIONS

- Homeostatic mechanisms exist to maintain glucose concentration within narrow limits despite a wide variety of circumstances.
- The dependence of the central nervous system on glucose has led to a complex series of biochemical, functional and haemodynamic changes aimed at restoring glucose concentrations, generating symptoms and protecting the body in general and central nervous system in particular against the effects of a low blood glucose (Figure 1.8).
- Many symptoms of hypoglycaemia result from the activation of the autonomic nervous system and help to warn the individual that their blood glucose is low. This encourages the ingestion of carbohydrate, so helping to restore glucose concentrations in addition to counterregulation.
- Faster gastric emptying and the changes in regional blood flow, which also occur as a result of the activation of the autonomic nervous system, increase substrate delivery.
- The greater cerebral blood flow increases glucose delivery to the brain (although loss of autoregulation is undesirable), and the increased splanchnic flow results in a greater delivery of gluconeogenic precursors to the liver.
- Activation of the autonomic nervous system also increases sweating, and this, together with the inhibition of shivering predisposes to hypothermia, which may be neuroprotective.

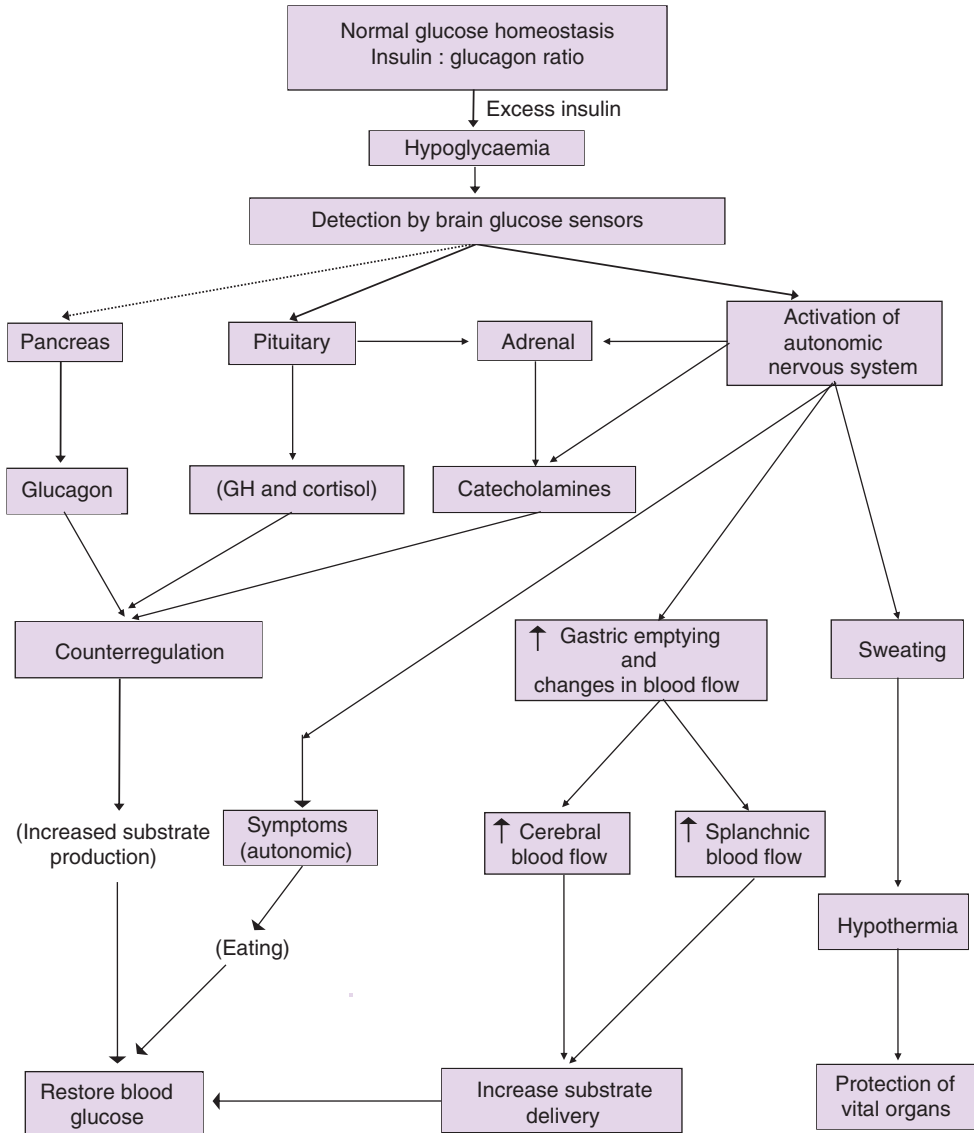


Figure 1.8 Glucose homeostasis and the correction of hypoglycaemia.

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