REVIEW

Endocrine cells and blood vessels work in tandem to generate hormone pulses

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Abstract

Hormones are dynamically collected by fenestrated capillaries to generate pulses, which are then decoded by target tissues to mount a biological response. To generate hormone pulses, endocrine systems have evolved mechanisms to tightly regulate blood perfusion and oxygenation, coordinate endocrine cell responses to secretory stimuli, and regulate hormone uptake from the perivascular space into the bloodstream. Based on recent findings, we review here the mechanisms that exist in endocrine systems to regulate blood flow, and facilitate coordinated cell activity and output under both normal physiological and pathological conditions in the pituitary gland and pancreas.

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Introduction

Finely tuned secretion of hormones is essential for regulating a multitude of basic body functions such as growth, metabolism, reproduction, and lactation. The pulsatile pattern of secretion of most hormones, resulting in burst like or episodic increases in circulating concentrations, is necessary for the generation of an appropriate downstream physiological response as well as the regulation of target cell function in both health and disease (Wildt *et al.* 1981, Matthews *et al.* 1983, Giustina & Veldhuis 1998, Robinson & Hindmarsh 1999). The generation of hormone pulses requires not only the energy demands of thousands of highly active endocrine cells to be met but also the temporally precise entry of hormone into the bloodstream. To achieve this, endocrine cell activity and output has to be temporally correlated with changes in blood flow, resulting in the replenishment of cell energy stores and the coordinated passage of hormone into the vasculature. Therefore, organisms have evolved mechanisms to tightly regulate blood perfusion and oxygenation, coordinate endocrine cell responses to secretory stimuli, and promote hormone uptake by blood vessels. This has prompted recent studies to explore whether endocrine systems possess different regulatory mechanisms to adjust blood flow velocity in

response to metabolic demand, structurally organized populations of endocrine cells that favor coordinated responses, and specific mechanisms that permit hormone storage in the perivascular space before diffusion into the bloodstream (Bonnefont *et al.* 2005, Nyman *et al.* 2008, 2010, Hodson *et al.* 2010, Lafont *et al.* 2010, Sanchez-Cardenas *et al.* 2010). Since endocrine systems must display marked plasticity in response to varying physiological demand, these mechanisms must rapidly and reversibly adapt to the prevailing conditions (Robinson & Hindmarsh 1999, Islam 2010). Moreover, defaults in hormone levels and their rhythms are signatures of many socioeconomically costly hormonal disorders (e.g. diabetes, hypopituitarism and infertility) (Knobil *et al.* 1994, Carroll *et al.* 1998, Islam 2010). Consequently, during the past 50 years, an impressive number of studies have been conducted to understand how hormone secretion is regulated and how hormone output can be modified/restored to reverse common hormonal disorders. Despite this, practically nothing is known about how hormones, locally secreted by endocrine cells, are captured by fenestrated capillaries and how blood vessel dynamics contribute to the formation of hormone pulses in the bloodstream. In light of recent findings (Nyman *et al.* 2008, 2010, Lafont *et al.* 2010), the aims of the current review are to describe the mechanisms which exist in the pituitary

gland and the pancreas to: 1) regulate blood flow to adapt to varying metabolic demand during periods of plasticity in hormone release; 2) facilitate coordinated cell activity and output; and 3) regulate hormone entry into the bloodstream. In addition, we will detail the potential role that these mechanisms play in the development of pathologies related to endocrine dysfunction.

Vascular system

Structure and common features

Endocrine organs are typically irrigated by a network of fenestrated capillaries that allow transendothelial transit of medium-sized molecules (i.e. incoming secretagogues and released hormones) (Henderson & Moss 1985). Pancreatic islets, for example, are embedded in a very dense microvascular network, that is, almost two- to three-fold denser than in surrounding exocrine tissue (Fig. 1). In addition, endocrine cells in islets are closely apposed to and polarized toward islet vascular endothelial cells (Bonner-Weir 1988), and produce factors that promote the formation of fenestrae (Lammert *et al.* 2003). In the pituitary gland, blood supply is provided by the hypothalamo-hypophyseal portal vasculature (Harris 1955, Daniel 1966, 1976), which ramifies into a specialized perfusion system to deliver hypothalamic signals to target pituitary cells (Harris 1947). Some pituitary cell types also form topologically organized networks in close apposition to the pituitary vasculature (Bonnefont *et al.* 2005, Lafont *et al.* 2010). What would be the benefit of such close relationships between vasculature and organized cell networks? As an example, proximity to highly fenestrated vessels may be of considerable advantage for

cells needing to respond synchronously to changes in metabolic demand. In fact, small gaseous molecules, such as oxygen, can freely diffuse from the blood to the tissue, following their gradient (Birol *et al.* 2007). However, to accommodate the increased metabolic demand observed in endocrine cells during secretory episodes, oxygen diffusion may not be rapid enough, and blood flow may require local adjustment to increase delivery. Although proper oxygen supply is essential for endocrine function, very little is known about how oxygen intake and consumption are temporally regulated during hormone release in the pituitary. Similarly, the mechanisms by which signaling molecules are distributed to endocrine cells remain elusive. The circulation of stimulatory or inhibitory molecules may be restricted by vessel pore size and require additional transport mechanisms. Indeed, fenestrations contain a diaphragm composed of radial fibrils converging on a central knob (Bearer & Orci 1985, Stan *et al.* 1999), and may act to regulate molecule diffusion from the blood to the perivascular space, influencing the rate of transcapillary transport of hormones toward the bloodstream. The microvasculature in endocrine organs may therefore, in addition to dispersing incoming signaling molecules and clearing secreted products, function as a gatekeeper that shapes the distribution and the clearance of hormones from the gland.

Regulation of blood flow

It is generally believed that an increase in endocrine cell activity during hormone secretion leads to increased blood flow to meet metabolic demand. Although the exact mechanisms remain unclear, release of vasoactive substances most likely plays an important role. During an acute deficit in oxygen supply, the coordinated release of highly diffusible vasoactive factors, such as nitric oxide (NO), is triggered (Prabhakar 2006, Kemp *et al.* 2009), inducing changes in mural cell tone. Mural cells, usually vascular smooth muscle cells (VSMC), line vessel walls and regulate blood flow. However, VSMC are absent from the pituitary, and are only found on large efferent arterioles in murine pancreatic islets (Lai *et al.* 2007). Nevertheless, the microvasculature in these tissues possesses specific regulatory mechanisms to allow the control of blood flow (Satoh *et al.* 1989). Pericytes, another type of mural cell related to VSMC, are putative candidates since they can induce vessel constriction and modify vascular diameter and blood flow (Rucker *et al.* 2000, Peppiatt *et al.* 2006). These cells have been detected in both the pituitary and pancreatic islets (Farquhar 1961, Nakamura *et al.* 1995, Richards *et al.* 2010) and may play an important role in blood flow regulation (Gaengel *et al.* 2009). NO, the main regulator of vascular tone, can be produced by a variety

Figure 1 Vessels density in endocrine and exocrine pancreas. (A) Representative image of a mouse pancreatic islet. Green, blood vessels labeled with gelatin-FITC; red, insulin-producing cells. The image corresponds to a 30 μm thick z-projection obtained using a Zeiss LSM 510 confocal microscope. (B) Percentage volume occupied by vessels in endocrine and exocrine pancreas measured using Velocity software (Improvision, Coventry, UK). Statistical analysis was performed by a Student's *t*-test (***) $P < 0.001$.

of cell types. In the pituitary, different endocrine cells express neuronal NO synthase (nNOS; Hokfelt *et al.* 1994, Andric *et al.* 2001, Tsumori *et al.* 2002), and vascular endothelial cells express endothelial NO synthase (eNOS; Garcia-Cardena *et al.* 1996). Both nNOS and eNOS produce NO using L-arginine as substrate. Various peptides, including hypothalamic neuropeptides and pituitary hormones, can modulate NOS activity and vascular tone (Boger 1999, Tsumori *et al.* 2002). NO, in turn, can modulate secretory activity and hormone output (Gonzalez-Hernandez & Gonzalez 2000). In pancreatic islets, both endothelial cells and β -cells express NOS (Corbett *et al.* 1992a,b, Alm *et al.* 1999) and NO influences both regulation of islet perfusion and hormone secretion (Salehi *et al.* 1996). In rodents, glucose considerably increases blood flow specifically at the level of islets (Jansson & Hellerstrom 1983, Nyman *et al.* 2010) through the induction of vasodilation by the central nervous system as well as local NO synthesis (Jansson & Hellerstrom 1986, Carlsson *et al.* 1999). On the contrary, blood flow rate remains unchanged in the exocrine pancreas. This differential regulation of blood flow may implicate sphincter-like activity of endothelial cells and extra-islet gates (McCuskey & Chapman 1969, Moldovan & Brunicardi 2001). Shunting may be partly achieved by pericytes, which are present in the pancreas (Richards *et al.* 2010), although this remains to be investigated. In addition, insulin decreases islet blood flow (Jansson *et al.* 2007) through induction of normoglycemia as well as direct affects on vascular tone (Sparrow & Beckingham 1989, Montagnani *et al.* 2001). Figure 2 summarizes potential mechanisms for blood flow regulation in the islets.

Blood flow measurement and oxygen consumption

Direct *in vivo* measurement of blood flow in endocrine organs has been particularly challenging due to lack of appropriate models which allow identification of cells *in situ* and technical constraints on imaging deep structures. Only recently have high temporal and spatial resolutions been combined to measure islet blood flow velocities under differing states of glycemia (Nyman *et al.* 2010). In the pituitary, direct measurements of differences in blood flow rates in individual vessels or the relationship between local blood flow and secretion of hormone has remained unresolved. By taking advantage of transgenic mice expressing eGFP-tagged GH cells (Magoulas *et al.* 2000) and a novel imaging approach using long working distance objectives, we have been able to measure blood supply dynamics and oxygen consumption in relation to GH pulse generation in a physiologically relevant context (Lafont *et al.* 2010; Fig. 3). We were particularly interested in how blood flow and tissue oxygen partial pressures (P_{tiss}O₂) varied

Figure 2 Potential mechanisms for blood flow regulation in the pancreatic islets. Stimulation of glucose-sensitive neurons increases islet blood flow through indirect actions on parasympathetic outflow (vagus) and direct actions on islet vasoactive substance release (acetylcholine and NO). Glucose, acetylcholine, low pO₂ and insulin can stimulate NOS expression in endothelial cells, eliciting relaxation of pericytes and vascular smooth muscle cells (presumably located on the internal and/or external islet gates) and leading to increased blood flow. ACh, acetylcholine; Enos, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; VSM, vascular smooth muscle. Red arrows indicate blood flow direction.

during hormone release following stimulation with GHRH, the major GH secretagogue. Using plasma fluorescently labeled with large molecular weight dextrans, we were able to measure red blood cell (RBC) velocity (appearing as shadows) and found that blood flow was independent of vessel diameter and variable within the pituitary. Oxygen partial pressures (P_{tiss}O₂) were measured using Clark-type O₂ microsensors (Bergsten *et al.* 2002), and revealed little variation in resting conditions. On the contrary, following GHRH challenge, RBC velocity, GH cell electrical activity, and P_{tiss}O₂ deflections increased, coincident with the appearance of a GH pulse in the bloodstream (Fig. 3). To confirm that GH cell coordinated activity was positively correlated with increased oxygen consumption, acute pituitary slices, in which oxygen supply is stable, were used (Lafont *et al.* 2010). Therefore, we demonstrated for the first time *in vivo* that GHRH-induced GH secretion was associated with coordinated GH cell activity, increases in local blood flow and changes in partial oxygen pressure within GH cell clusters (Lafont *et al.* 2010; Fig. 3). It should be noted, however, that the ability of GHRH to activate nNOS and increase NO production, as opposed to a compensatory increase in blood flow in direct response to tissue hypoxia, may account for the observed increases in blood flow (Boger 1999, Tsumori *et al.* 2002). Potential regulatory mechanisms that may exist in the pituitary to modify blood flow following GHRH stimulation have been proposed (Schaeffer *et al.* 2010).

These include upregulation of inducible NO synthase (iNOS), eNOS and cyclooxygenase activity in pituitary cells, resulting in release of NO and prostaglandins. These potent vasodilators may then induce relaxation of pericytes, leading to the increased blood flow which is observed in response to the GHRH-induced decrease in $P_{tiss}O_2$. In islets, comparable episodes of oxygen consumption were observed simultaneously with increases in β -cell calcium-spiking activity (Jung *et al.* 2000). However, NOS inhibition in islets does not affect oxygen tension following glucose challenge suggesting that increases in islet blood flow cannot be simply explained by metabolic demand for oxygen (Carlsson *et al.* 2002a). As insulin has known vasodilator properties (Sparrow & Beckingham 1989, Montagnani *et al.* 2001), local blood flow increase may be a consequence of hormone secretion as opposed to an increase in oxygen consumption. Other mechanisms, such as adenosine production associated with islet metabolism, may favor local increases in blood flow velocity (Carlsson *et al.* 2002b).

Figure 3 GHRH stimulation induces a local increase in red blood cell velocity, and a modification of oxygen consumption by GH cells. (A) GH cell clusters in the pituitary of a living GH-eGFP transgenic mouse. Green, GH-eGFP cells; red, blood vessels labeled with Rhodamine B-conjugated dextran injected intravenously. (B) Red blood cell (RBC) velocity and GH serum levels increase after i.v. injection of GHRH (1 μ g). Mean velocity (red line) and S.E.M. (gray lines) from six individual vessels is represented. (C) Variation in RBC velocity 5 min after i.v. injections of either NaCl or GHRH (1 μ g). (D) O_2 deflection amplitude 5 min after i.v. injections of either NaCl or GHRH (1 μ g). Statistical analysis was performed using Student's *t*-test (***) $P < 0.001$).

Endocrine cell coordination

Release of hormones from endocrine cells is evoked by humoral, neural, or hormonal stimuli. Most secretory stimuli are carried by the blood and must diffuse rapidly into the endocrine parenchyma to reach their targets. We modeled the entry of such stimuli by monitoring the distribution of 4 kDa dextrans (approximate size of most hypothalamic secretagogues) from the bloodstream into the parenchyma (Lafont *et al.* 2010). Following i.v. injection, the 4 kDa marker spread rapidly and radially from the vessels and the extent of diffusion of the 4 kDa fluorescent marker was limited in distance to $< 100 \mu$ m from the injection site due to rapid capture by vessels. These results suggest that groups of pituitary cells are exposed to differential secretagog concentration gradients and that other mechanisms must exist to allow coordinated cell responses in the face of such heterogeneous stimulation.

For many years, it was assumed that endocrine cell types in the pituitary gland were randomly distributed throughout the parenchyma and that changes in the secretion of hormone-releasing factors from hypothalamic nerve terminals were primarily responsible for the observed plasticity in pituitary hormone secretion. However, this does not fit with studies in dispersed pituitary cells, where the ability of hypothalamic releasing hormones to evoke endocrine secretion is 100- to 1000-fold lower than that observed in the intact tissue (Tannenbaum *et al.* 1976, Sartor *et al.* 1985). We have previously shown that GH-producing cells in the pituitary gland are arranged into a topologically organized network in close association with the pituitary vasculature. By virtue of this three-dimensional organization, GH cells are able to mount large-scale coordinated responses following arrival of secretagog, increasing the amplitude and efficiency of cell signaling events and hormone secretion (Bonnefont *et al.* 2005, Sanchez-Cardenas *et al.* 2010). These responses are severely impaired when the GH cell network is 'broken' using transgenic approaches (Waite *et al.* 2010), implicating structural integrity in the coordinated transmission of cell-cell information. Therefore, endocrine cell networks may represent a physical mechanism that facilitates coordinated activity following encounter with heterogeneous stimuli. Like their counterparts in the pituitary, endocrine cells in the islets of Langerhans are topologically organized in a non-random manner. Murine islets consist of a β -cell core, principally responsible for the maintenance of blood glucose concentrations through the secretion of insulin, surrounded by a mantle of α -, δ -, and PP-cells (Bonner-Weir *et al.* 1993). Of particular note, β -cells are homotypically linked by gap junctions, allowing emergence of coordinated calcium oscillations which

drive calcium-dependent secretory bursts of insulin in response to glucose stimulus (Santos *et al.* 1991, Ravier *et al.* 1999, 2005, Kjems *et al.* 2002, Benninger *et al.* 2008). Furthermore, intimate molecular crosstalk between β -cells and endothelial cells is essential for endocrine pancreas function (Kragl & Lammert 2010).

Regulation of hormone uptake

Endocrine cells respond to stimuli by generating coordinated changes in signaling molecules (i.e. cytosolic calcium) that are responsible for driving secretion of hormones into the extracellular space (Schlegel *et al.* 1987, Mollard & Schlegel 1996, Stojilkovic *et al.* 2005). Hormones are dynamically collected by fenestrated capillaries to make pulses, which are then decoded by target tissues to mount a biological response. The exocytosis of hormones packed into docked granules is a very fast event (ms–s range) and quickly stops after cessation of stimulus (Tse *et al.* 1993, Mollard *et al.* 1995). However, while the pituitary GH cell network discharges signaling and exocytotic events rapidly in response to GHRH, the appearance of GH pulses in the bloodstream requires several to dozens of minutes (Clark *et al.* 1987). Such enigma is widely observed in most, if not all endocrine glands. As another example, human islets display a wide variety of electrical and calcium-spiking activities *ex vivo*, but their roles in mediating pulsatile secretion of insulin (pulses at ~ 5 min intervals) remain unanswered since the fate of secreted insulin between the β -cells and the lumen of fenestrated capillaries is not known (Islam 2010). Although a perivascular space was identified in the 1950s (Rinehart & Farquhar 1955), its role remains widely unexplored, and it has been proposed to act as a modulator of hormone uptake by fenestrated capillaries. For instance, GH released upon GHRH stimulation may be stored in the perivascular space long enough to play a role in the control of blood vessel density and maintenance (Sonntag *et al.* 2000).

To model hormone uptake by capillaries and investigate the role of the perivascular space in hormone storage in the pituitary gland, we locally injected fluorescent markers of different sizes (4 kDa for ACTH; 20 kDa for GH, PRL) into the endocrine parenchyma of anesthetized animals (Lafont *et al.* 2010). We found that, although passage toward blood vessels was rapid, significant amounts of larger markers remained sequestered in what appeared to be a perivascular compartment (Fig. 4). These results suggest that GH pulse generation is not simply a GH cell network response, but is shaped by the tissue microenvironment, involving a role of the perivascular space as a gatekeeper or rate-limiting step for hormone uptake by fenestrated pituitary capillaries. Although fluorescent sugars present the advantage of possessing

Figure 4 Clearance of products injected into the extracellular space at the vascular level. (A–C) Single pulse injection by iontophoresis of a 20 kDa fluorescent dextran into the pituitary parenchyma of a living mouse. Time post-injection is indicated. Accumulation of the molecule around the blood vessels is visible at $t=30$ s post-injection (C). (D) Schematic representation of the vessels (red) around the injection zone. (E) Clearance of the 20 kDa molecule measured by variation in fluorescence intensity across a vessel (red line in C and black line in D). Fluorescence increases up to 21 s post-injection and declines thereafter, although it still remains detectable 45 s post-injection, suggesting the molecule is stored in the perivascular space.

a large number of fluorescent markers per molecule, development in the future of cost-effective fluorescently labeled bioactive hormones, with emission properties detectable using *in vivo* imaging, will provide invaluable tools to study diffusion and transport of hormones in their native environment. Furthermore, such methods will help resolve whether the mechanisms of hormone transport across the endothelial barrier involve passive diffusion through fenestrae and/or saturable receptor-mediated processes, a matter still debated for most transcapillary hormone transports (King & Johnson 1985, Steil *et al.* 1996, Hamilton-Wessler *et al.* 2002).

Importance in pathology

There is increasing evidence that vasculopathies may precede a range of endocrine diseases such as diabetes mellitus and pituitary tumors. Extended literature in the past decade has provided evidence for dynamic interactions between blood vessels and pancreatic β -cells, which are pivotal for enhancing insulin expression and β -cell proliferation in response to increased insulin demand during body growth, pregnancy, and virtually all conditions associated with insulin resistance. A failure in this adaptive response might contribute to the onset of diabetes (Eberhard *et al.* 2010). In addition, abnormalities in islet blood flow regulation have been described in animal models of functional stress on β -cells (Atef *et al.* 1992, Jansson 1994, Svensson *et al.* 1994, 2000, Carlsson *et al.* 1998), as well as following rejection of experimentally transplanted islet grafts (Olsson *et al.* 2000). Lastly, impairment of pericyte recruitment, which may interfere with blood flow regulation, has also been implicated in

diabetes (Clee *et al.* 2006). In the pituitary gland, deregulation of pituitary blood flow has been observed in adenomas (Turner *et al.* 2003) and acquired hypopituitarism (Dusick *et al.* 2008). The former are a significant cause of morbidity in both rodents and humans, and increases in angiogenic factor expression, NOS immunoreactivity and blood flow have been detected in adenomatous tissue (Kemeny *et al.* 1987, Heaney *et al.* 2002, Kruse *et al.* 2002). Altogether, the exact pathophysiological mechanisms involved in these modifications remain unclear. Hence, the study of the microcirculation may help unveil the contribution of flow abnormalities to the development of endocrine pathology.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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References

- Alm P, Ekstrom P, Henningsson R & Lundquist I 1999 Morphological evidence for the existence of nitric oxide and carbon monoxide pathways in the rat islets of Langerhans: an immunocytochemical and confocal microscopical study. *Diabetologia* **42** 978–986. (doi:10.1007/s001250051256)
- Andric SA, Kostic TS, Tomic M, Koshimizu T & Stojilkovic SS 2001 Dependence of soluble guanylyl cyclase activity on calcium signaling in pituitary cells. *Journal of Biological Chemistry* **276** 844–849. (doi:10.1074/jbc.M004406200)
- Atef N, Ktorza A, Picon L & Penicaud L 1992 Increased islet blood flow in obese rats: role of the autonomic nervous system. *American Journal of Physiology* **262** E736–E740.
- Bearer EL & Orci L 1985 Endothelial fenestral diaphragms: a quick-freeze, deep-etch study. *Journal of Cell Biology* **100** 418–428. (doi:10.1083/jcb.100.2.418)
- Benninger RK, Zhang M, Head WS, Satin LS & Piston DW 2008 Gap junction coupling and calcium waves in the pancreatic islet. *Biophysical Journal* **95** 5048–5061. (doi:10.1529/biophysj.108.140863)
- Bergsten P, Westerlund J, Liss P & Carlsson PO 2002 Primary *in vivo* oscillations of metabolism in the pancreas. *Diabetes* **51** 699–703. (doi:10.2337/diabetes.51.3.699)
- Biról G, Wang S, Budzynski E, Wangsa-Wirawan ND & Linsenmeier RA 2007 Oxygen distribution and consumption in the macaque retina. *American Journal of Physiology. Heart and Circulatory Physiology* **293** H1696–H1704. (doi:10.1152/ajpheart.00221.2007)
- Boger RH 1999 Nitric oxide and the mediation of the hemodynamic effects of growth hormone in humans. *Journal of Endocrinological Investigation* **22** 75–81.
- Bonnefont X, Lacampagne A, Sanchez-Hormigo A, Fino E, Creff A, Mathieu MN, Smallwood S, Carmignac D, Fontanaud P, Travo P *et al.* 2005 Revealing the large-scale network organization of growth hormone-secreting cells. *PNAS* **102** 16880–16885. (doi:10.1073/pnas.0508202102)
- Bonner-Weir S 1988 Morphological evidence for pancreatic polarity of beta-cell within islets of Langerhans. *Diabetes* **37** 616–621. (doi:10.2337/diabetes.37.5.616)
- Bonner-Weir S, Baxter LA, Schuppert GT & Smith FE 1993 A second pathway for regeneration of adult exocrine and endocrine pancreas. A possible recapitulation of embryonic development. *Diabetes* **42** 1715–1720. (doi:10.2337/diabetes.42.12.1715)
- Carlsson PO, Sandler S & Jansson L 1998 Pancreatic islet blood perfusion in the nonobese diabetic mouse: diabetes-prone female mice exhibit a higher blood flow compared with male mice in the prediabetic phase. *Endocrinology* **139** 3534–3541. (doi:10.1210/en.139.8.3534)
- Carlsson PO, Iwase M & Jansson L 1999 Stimulation of intestinal glucoreceptors in rats increases pancreatic islet blood flow through vagal mechanisms. *American Journal of Physiology* **276** R233–R236.
- Carlsson PO, Jansson L & Palm F 2002a Unaltered oxygen tension in rat pancreatic islets despite dissociation of insulin release and islet blood flow. *Acta Physiologica Scandinavica* **176** 275–281. (doi:10.1046/j.1365-201X.2002.01049.x)
- Carlsson PO, Olsson R, Kallskog O, Bodin B, Andersson A & Jansson L 2002b Glucose-induced islet blood flow increase in rats: interaction between nervous and metabolic mediators. *American Journal of Physiology. Endocrinology and Metabolism* **283** E457–E464. (doi:10.1152/ajpendo.00044.2002)
- Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, Clemmons D, Hintz R, Ho K, Laron Z, Sizonenko P *et al.* 1998 Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. *Journal of Clinical Endocrinology and Metabolism* **83** 382–395. (doi:10.1210/jc.83.2.382)
- Clark RG, Carlsson LM & Robinson IC 1987 Growth hormone secretory profiles in conscious female rats. *Journal of Endocrinology* **114** 399–407. (doi:10.1677/joe.0.1140399)
- Clee SM, Yandell BS, Schueler KM, Rabaglia ME, Richards OC, Raines SM, Kabara EA, Klass DM, Mui ET, Stapleton DS *et al.* 2006 Positional cloning of Sorcs1, a type 2 diabetes quantitative trait locus. *Nature Genetics* **38** 688–693. (doi:10.1038/ng1796)
- Corbett JA, Wang JL, Hughes JH, Wolf BA, Sweetland MA, Lancaster JR Jr & McDaniel ML 1992a Nitric oxide and cyclic GMP formation induced by interleukin 1 beta in islets of Langerhans. Evidence for an effector role of nitric oxide in islet dysfunction. *Biochemical Journal* **287** 229–235.
- Corbett JA, Wang JL, Sweetland MA, Lancaster JR Jr & McDaniel ML 1992b Interleukin 1 beta induces the formation of nitric oxide by beta-cells purified from rodent islets of Langerhans. Evidence for the beta-cell as a source and site of action of nitric oxide. *Journal of Clinical Investigation* **90** 2384–2391. (doi:10.1172/JCI116129)
- Daniel PM 1966 Blood supply of hypothalamus and pituitary gland. *British Medical Bulletin* **22** 202–208.
- Daniel PM 1976 Anatomy of the hypothalamus and pituitary gland. *Journal of Clinical Pathology* **7** 1–7. (doi:10.1136/jcp.s1-7.1.1)
- Dusick JR, Wang C, Cohan P, Swerdloff R & Kelly DF 2008 Chapter 1: pathophysiology of hypopituitarism in the setting of brain injury. *Pituitary* **11** 123–126. (doi:10.1007/s11102-008-0130-6)
- Eberhard D, Kragl M & Lammert E 2010 ‘Giving and taking’: endothelial and beta-cells in the islets of Langerhans. *Trends in Endocrinology and Metabolism* **21** 457–463. (doi:10.1016/j.tem.2010.03.003)
- Farquhar MG 1961 Fine structure and function in capillaries of the anterior pituitary gland. *Angiology* **12** 270–292. (doi:10.1177/00031976101200704)

- Gaengel K, Genove G, Armulik A & Betsholtz C 2009 Endothelial-mural cell signaling in vascular development and angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **29** 630–638. (doi:10.1161/ATVBAHA.107.161521)
- Garcia-Cardena G, Oh P, Liu J, Schnitzer JE & Sessa WC 1996 Targeting of nitric oxide synthase to endothelial cell caveolae via palmitoylation: implications for nitric oxide signaling. *PNAS* **93** 6448–6453. (doi:10.1073/pnas.93.13.6448)
- Giustina A & Veldhuis JD 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocrine Reviews* **19** 717–797. (doi:10.1210/er.19.6.717)
- Gonzalez-Hernandez T & Gonzalez MC 2000 Gender differences and the effect of different endocrine situations on the NOS expression pattern in the anterior pituitary gland. *Journal of Histochemistry and Cytochemistry* **48** 1639–1648. (doi:10.1177/002215540004801207)
- Hamilton-Wessler M, Ader M, Dea MK, Moore D, Loftager M, Markussen J & Bergman RN 2002 Mode of transcapillary transport of insulin and insulin analog NN304 in dog hindlimb: evidence for passive diffusion. *Diabetes* **51** 574–582. (doi:10.2337/diabetes.51.3.574)
- Harris GW 1947 The blood vessels of the rabbit's pituitary gland, and the significance of the pars and zona tuberalis. *Journal of Anatomy* **81** 343–351. (doi:10.1002/aja.1000810303)
- Harris GW 1955 The function of the pituitary stalk. *Bulletin of the Johns Hopkins Hospital* **97** 358–375.
- Heaney AP, Fernando M & Melmed S 2002 Functional role of estrogen in pituitary tumor pathogenesis. *Journal of Clinical Investigation* **109** 277–283. (doi:10.1172/JCI0214264)
- Henderson JR & Moss MC 1985 A morphometric study of the endocrine and exocrine capillaries of the pancreas. *Quarterly Journal of Experimental Physiology* **70** 347–356.
- Hodson DJ, Molino F, Fontanaud P, Bonnefont X & Mollard P 2010 Investigating and modelling pituitary endocrine network function. *Journal of Neuroendocrinology* **22** 1217–1225. (doi:10.1111/j.1365-2826.2010.02052.x)
- Hokfelt T, Ceccatelli S, Gustafsson L, Hulting AL, Verge V, Villar M, Xu XJ, Xu ZQ, Wiesenfeld-Hallin Z & Zhang X 1994 Plasticity of NO synthase expression in the nervous and endocrine systems. *Neuropharmacology* **33** 1221–1227. (doi:10.1016/0028-3908(94)90020-5)
- Islam MS (Ed) 2010 *The Islets of Langerhans. Volume 654 of Advances in Experimental Medicine and Biology*. Dordrecht: Springer. (doi:10.1007/978-90-481-3271-3)
- Jansson L 1994 The regulation of pancreatic islet blood flow. *Diabetes/Metabolism Reviews* **10** 407–416. (doi:10.1002/dmr.5610100405)
- Jansson L & Hellerstrom C 1983 Stimulation by glucose of the blood flow to the pancreatic islets of the rat. *Diabetologia* **25** 45–50. (doi:10.1007/BF00251896)
- Jansson L & Hellerstrom C 1986 Glucose-induced changes in pancreatic islet blood flow mediated by central nervous system. *American Journal of Physiology* **251** E644–E647.
- Jansson L, Andersson A, Bodin B & Kallskog O 2007 Pancreatic islet blood flow during euglycaemic, hyperinsulinaemic clamp in anaesthetized rats. *Acta Physiologica* **189** 319–324. (doi:10.1111/j.1748-1716.2006.01666.x)
- Jung SK, Kauri LM, Qian WJ & Kennedy RT 2000 Correlated oscillations in glucose consumption, oxygen consumption, and intracellular free Ca(2+) in single islets of Langerhans. *Journal of Biological Chemistry* **275** 6642–6650. (doi:10.1074/jbc.275.9.6642)
- Kemeny AA, Jakubowski J, Stawowy A, Smith C & Timperley WR 1987 Changes of blood flow in oestrogen-induced hyperplastic anterior pituitary lobe following bromocriptine administration. *British Journal of Neurosurgery* **1** 243–250. (doi:10.3109/02688698709035308)
- Kemp PJ, Telezhkin V, Wilkinson WJ, Mears R, Hanmer SB, Gadeberg HC, Muller CT, Riccardi D & Brazier SP 2009 Enzyme-linked oxygen sensing by potassium channels. *Annals of the New York Academy of Sciences* **1177** 112–118. (doi:10.1111/j.1749-6632.2009.05025.x)
- King GL & Johnson SM 1985 Receptor-mediated transport of insulin across endothelial cells. *Science* **227** 1583–1586. (doi:10.1126/science.3883490)
- Kjems LL, Ravier MA, Jonas JC & Henquin JC 2002 Do oscillations of insulin secretion occur in the absence of cytoplasmic Ca²⁺ oscillations in beta-cells? *Diabetes* **51** (Suppl 1) S177–S182. (doi:10.2337/diabetes.51.2007.S177)
- Knobil E, Neill JD, Greenwald GS, Markert C & Donald W 1994. *The Physiology of Reproduction*, New York: Raven Press.
- Kragl M & Lammert E 2010 Basement membrane in pancreatic islet function. *Advances in Experimental Medicine and Biology* **654** 217–234. (doi:10.1007/978-90-481-3271-3_10)
- Kruse A, Broholm H, Rubin I, Schmidt K & Lauritzen M 2002 Nitric oxide synthase activity in human pituitary adenomas. *Acta Neurologica Scandinavica* **106** 361–366. (doi:10.1034/j.1600-0404.2002.01138.x)
- Lafont C, Desarmenien MG, Cassou M, Molino F, Lecoq J, Hodson D, Lacampagne A, Mennessier G, El Yandouzi T, Carmignac D *et al.* 2010 Cellular *in vivo* imaging reveals coordinated regulation of pituitary microcirculation and GH cell network function. *PNAS* **107** 4465–4470. (doi:10.1073/pnas.0902599107)
- Lai EY, Persson AE, Bodin B, Kallskog O, Andersson A, Pettersson U, Hansell P & Jansson L 2007 Endothelin-1 and pancreatic islet vasculature: studies *in vivo* and on isolated, vascularly perfused pancreatic islets. *American Journal of Physiology. Endocrinology and Metabolism* **292** E1616–E1623. (doi:10.1152/ajpendo.00640.2006)
- Lammert E, Gu G, McLaughlin M, Brown D, Brekken R, Murtaugh LC, Gerber HP, Ferrara N & Melton DA 2003 Role of VEGF-A in vascularization of pancreatic islets. *Current Biology* **13** 1070–1074. (doi:10.1016/S0960-9822(03)00378-6)
- Magoulas C, McGuinness L, Balthasar N, Carmignac DF, Sesay AK, Mathers KE, Christian H, Candell L, Bonnefont X, Mollard P *et al.* 2000 A secreted fluorescent reporter targeted to pituitary growth hormone cells in transgenic mice. *Endocrinology* **141** 4681–4689. (doi:10.1210/en.141.12.4681)
- Matthews DR, Naylor BA, Jones RG, Ward GM & Turner RC 1983 Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes* **32** 617–621. (doi:10.2337/diabetes.32.7.617)
- McCuskey RS & Chapman TM 1969 Microscopy of the living pancreas *in situ*. *American Journal of Anatomy* **126** 395–407. (doi:10.1002/aja.1001260402)
- Moldovan S & Brunicardi FC 2001 Endocrine pancreas: summary of observations generated by surgical fellows. *World Journal of Surgery* **25** 468–473. (doi:10.1007/s002680020339)
- Mollard P & Schlegel W 1996 Why are endocrine pituitary cells excitable? *Trends in Endocrinology and Metabolism* **7** 361–365. (doi:10.1016/S1043-2760(96)00186-5)
- Mollard P, Seward EP & Nowicky MC 1995 Activation of nicotinic receptors triggers exocytosis from bovine chromaffin cells in the absence of membrane depolarization. *PNAS* **92** 3065–3069. (doi:10.1073/pnas.92.7.3065)
- Montagnani M, Chen H, Barr VA & Quon MJ 2001 Insulin-stimulated activation of eNOS is independent of Ca²⁺ but requires phosphorylation by Akt at Ser(1179). *Journal of Biological Chemistry* **276** 30392–30398. (doi:10.1074/jbc.M103702200)
- Nakamura M, Kitamura H, Konishi S, Nishimura M, Ono J, Ina K, Shimada T & Takaki R 1995 The endocrine pancreas of spontaneously diabetic db/db mice: microangiopathy as revealed by transmission electron microscopy. *Diabetes Research and Clinical Practice* **30** 89–100. (doi:10.1016/0168-8227(95)01155-2)
- Nyman LR, Wells KS, Head WS, McCaughey M, Ford E, Brissova M, Piston DW & Powers AC 2008 Real-time, multidimensional *in vivo* imaging used to investigate blood flow in mouse pancreatic islets. *Journal of Clinical Investigation* **118** 3790–3797. (doi:10.1172/JCI36209)
- Nyman LR, Ford E, Powers AC & Piston DW 2010 Glucose-dependent blood flow dynamics in murine pancreatic islets *in vivo*. *American Journal of Physiology. Endocrinology and Metabolism* **298** E807–E814. (doi:10.1152/ajpendo.00715.2009)

- Olsson R, Jansson L, Andersson A & Carlsson PO 2000 Local blood flow regulation in transplanted rat pancreatic islets: influence of adenosine, angiotensin II, and nitric oxide inhibition. *Transplantation* **70** 280–287. (doi:10.1097/00007890-200007270-00007)
- Pepiatt CM, Howarth C, Mobbs P & Attwell D 2006 Bidirectional control of CNS capillary diameter by pericytes. *Nature* **443** 700–704. (doi:10.1038/nature05193)
- Prabhakar NR 2006 O₂ sensing at the mammalian carotid body: why multiple O₂ sensors and multiple transmitters? *Experimental Physiology* **91** 17–23. (doi:10.1113/expphysiol.2005.031922)
- Ravier MA, Gilon P & Henquin JC 1999 Oscillations of insulin secretion can be triggered by imposed oscillations of cytoplasmic Ca²⁺ or metabolism in normal mouse islets. *Diabetes* **48** 2374–2382. (doi:10.2337/diabetes.48.12.2374)
- Ravier MA, Guldenagel M, Charollais A, Gjinovci A, Caille D, Sohl G, Wollheim CB, Willecke K, Henquin JC & Meda P 2005 Loss of connexin36 channels alters beta-cell coupling, islet synchronization of glucose-induced Ca²⁺ and insulin oscillations, and basal insulin release. *Diabetes* **54** 1798–1807. (doi:10.2337/diabetes.54.6.1798)
- Richards OC, Raines SM & Attie AD 2010 The role of blood vessels, endothelial cells, and vascular pericytes in insulin secretion and peripheral insulin action. *Endocrine Reviews* **31** 343–363. (doi:10.1210/er.2009-0035)
- Rinehart JF & Farquhar MG 1955 The fine vascular organization of the anterior pituitary gland; an electron microscopic study with histochemical correlations. *Anatomical Record* **121** 207–239. (doi:10.1002/ar.1091210206)
- Robinson ICAF & Hindmarsh PC 1999 The importance of the secretory pattern of growth hormone for statural growth. In *Handbook of Physiology, Section 7. The Endocrine System*, vol 5, pp 329–395. Ed. JL Kostyo. New York: Oxford University Press.
- Rucker HK, Wynder HJ & Thomas WE 2000 Cellular mechanisms of CNS pericytes. *Brain Research Bulletin* **51** 363–369. (doi:10.1016/S0361-9230(99)00260-9)
- Salehi A, Carlberg M, Henningson R & Lundquist I 1996 Islet constitutive nitric oxide synthase: biochemical determination and regulatory function. *American Journal of Physiology* **270** C1634–C1641.
- Sanchez-Cardenas C, Fontanaud P, He Z, Lafont C, Meunier AC, Schaeffer M, Carmignac D, Molino F, Couty N, Bonnefont X *et al.* 2010 Pituitary growth hormone network responses are sexually dimorphic and regulated by gonadal steroids in adulthood. *PNAS* **107** 21878–21883. (doi:10.1073/pnas.1010849107)
- Santos RM, Rosario LM, Nadal A, Garcia-Sancho J, Soria B & Valdeolmillos M 1991 Widespread synchronous [Ca²⁺]_i oscillations due to bursting electrical activity in single pancreatic islets. *Pflügers Archiv* **418** 417–422. (doi:10.1007/BF00550880)
- Sartor O, Bowers CY & Chang D 1985 Parallel studies of His-DTrp-Ala-Trp-DPhe-Lys-NH₂ and human pancreatic growth hormone-releasing factor-44-NH₂ in rat primary pituitary cell monolayer culture. *Endocrinology* **116** 952–957. (doi:10.1210/endo-116-3-952)
- Satoh H, Inokuchi T, Shimizu M, Obayashi H & Nakashima Y 1989 Ultrastructure of the hypophyseal portal vessel in mature rats – SEM and TEM observations. *Kurume Medical Journal* **36** 91–94.
- Schaeffer M, Hodson DJ, Lafont C & Mollard P 2010 Functional importance of blood flow dynamics and partial oxygen pressure in the anterior pituitary. *European Journal of Neuroscience* **32** 2087–2095. (doi:10.1111/j.1460-9568.2010.07525.x)
- Schlegel W, Winiger BP, Mollard P, Vacher P, Wuarin F, Zahnd GR, Wollheim CB & Dufy B 1987 Oscillations of cytosolic Ca²⁺ in pituitary cells due to action potentials. *Nature* **329** 719–721. (doi:10.1038/329719a0)
- Sonntag WE, Lynch C, Thornton P, Khan A, Bennett S & Ingram R 2000 The effects of growth hormone and IGF-I deficiency on cerebrovascular and brain ageing. *Journal of Anatomy* **197** 575–585. (doi:10.1017/S002187829900713X)
- Sparrow RA & Beckingham IJ 1989 Islet blood flow following insulin administration. *Journal of Anatomy* **163** 75–81.
- Stan RV, Kubitz M & Palade GE 1999 PV-1 is a component of the fenestral and stomatal diaphragms in fenestrated endothelia. *PNAS* **96** 13203–13207. (doi:10.1073/pnas.96.23.13203)
- Steil GM, Ader M, Moore DM, Rebrin K & Bergman RN 1996 Transendothelial insulin transport is not saturable *in vivo*. No evidence for a receptor-mediated process. *Journal of Clinical Investigation* **97** 1497–1503. (doi:10.1172/JCI118572)
- Stojilkovic SS, Zemkova H & Van Goor F 2005 Biophysical basis of pituitary cell type-specific Ca²⁺ signaling-secretion coupling. *Trends in Endocrinology and Metabolism* **16** 152–159. (doi:10.1016/j.tem.2005.03.003)
- Svensson AM, Abdel-Halim SM, Efendic S, Jansson L & Ostenson CG 1994 Pancreatic and islet blood flow in F1-hybrids of the non-insulin-dependent diabetic GK-Wistar rat. *European Journal of Endocrinology* **130** 612–616. (doi:10.1530/eje.0.1300612)
- Svensson AM, Ostenson CG & Jansson L 2000 Age-induced changes in pancreatic islet blood flow: evidence for an impaired regulation in diabetic GK rats. *American Journal of Physiology. Endocrinology and Metabolism* **279** E1139–E1144.
- Tannenbaum GS, Martin JB & Colle E 1976 Ultradian growth hormone rhythm in the rat: effects of feeding, hyperglycemia, and insulin-induced hypoglycemia. *Endocrinology* **99** 720–727. (doi:10.1210/endo-99-3-720)
- Tse A, Tse FW, Almers W & Hille B 1993 Rhythmic exocytosis stimulated by GnRH-induced calcium oscillations in rat gonadotropes. *Science* **260** 82–84. (doi:10.1126/science.8385366)
- Tsumori M, Murakami Y, Koshimura K & Kato Y 2002 Growth hormone-releasing hormone and gonadotropin-releasing hormone stimulate nitric oxide production in 17beta-estradiol-primed rat anterior pituitary cells. *Endocrine* **17** 215–218. (doi:10.1385/ENDO:17:3:215)
- Turner HE, Harris AL, Melmed S & Wass JA 2003 Angiogenesis in endocrine tumors. *Endocrine Reviews* **24** 600–632. (doi:10.1210/er.2002-0008)
- Waite E, Lafont C, Carmignac D, Chauvet N, Couty N, Christian H, Robinson I, Mollard P & Le Tissier P 2010 Different degrees of somatotroph ablation compromise pituitary growth hormone cell network structure and other pituitary endocrine cell types. *Endocrinology* **151** 234–243. (doi:10.1210/en.2009-0539)
- Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE & Knobil E 1981 Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin-secretion in the rhesus-monkey. *Endocrinology* **109** 376–385. (doi:10.1210/endo-109-2-376)

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