

How lactic acid regulates insulin/glucagon secretion; How hypoxia causes elevated blood pressure, obesity, and diabetes mellitus

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Introduction

In islets, the lactate efflux rate is linear with glucose level⁽¹⁾, suggesting close relationship between rate of lactic acid production and glucose level. In 1989, Leonard BEST et al⁽¹⁾ demonstrated that lactate efflux is a possible determinant of islet-cell depolarization by glucose. Later researches showed that islet β cell produces very small amount of lactic acid^(2, 3), making lactate look unlikely to be able to regulate insulin secretion. However, I stress hereby that scarce rate of lactic acid production is exactly what differentiates β cell from α cell and the reason why β cell behaves the way differently from α cell.

Mere facilitated uptake of Arginine, which is electrogenic, can trigger action potential of β cell membrane, invoking influx of Ca^{2+} and secretion of insulin⁽⁴⁾. Likewise, this paper will start by elaborating two ways of lactate efflux, both electrogenic but in opposing direction. Subsequently, mechanism of proton-driven exocytosis, which is independent of Ca^{2+} influx, is proposed. And then genesis of elevated blood pressure, obesity, and diabetes by hypoxia is discussed. Lastly, there is a brief mentioning of adrenaline on glucose regulation.

Two ways of electrogenic lactate efflux on membrane electrical activity

Lactic acid is product of anaerobic glycolysis, with pKa of 3.86, readily dissociated in physiologic pH range into negatively charged lactate and positively charged proton (H^+). Lactate comes out of a cell facilitated diffusion either through volume-sensitive anion channel or by monocarbohydrate transporter (MCT)⁽⁵⁾, which is coupled with proton. Obviously diffusion of lactate anion generates inward current (I_{an}), i.e. net efflux of negative charge, promoting membrane depolarization. On the contrary, diffusion of proton-lactate by MCT, which is also electrogenic while at high pH milieu, generates outward current (I_{mct}), i.e. net efflux of positive charge^(6, 7, 8), promoting hyperpolarization. The aggregated electrogenic effect brought by lactate efflux is expressed as equ. 1:

$$I_{\text{lac}} = I_{\text{an}} - (n-1)I_{\text{mct}} \quad (\text{equ. 1})$$

Abbreviations:

MCT: monocarbohydrate transporter; LPR: Lactate Production Rate; LDH: lactate dehydrogenase; mGPDH: mitochondrial Glycerol Phosphate Dehydrogenase; K^+ -ATPase: ATP sensitive potassium channel

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Where I_{lac} is electric current generated by aggregate lactate efflux; I_{an} , rate of lactate efflux through anion channel; $n>1$, the stoichiometry of H:lactate facilitated by MCT; and I_{mct} , the rate of lactate efflux through by MCT.

If $I_{\text{lac}}>0$, it stands for inward current depolarizing cell membrane, and Vice versa.

Figure 1 is a schematic diagram for equ. 1. If I_{lac} is related to Lactate Production Rate (LPR) as variable it is denoted as $I_{\text{lac}} -L$, and $I_{\text{lac}} -G$, if related to glucose level as variable, to which islet cells are exposed.

I_{lac} curve consists of 5 segments as in figure 1. Two segments, OS and SP, constitute β region; and three segments, PD, DE, and E^+ , constitute α region.

1) β region: inward current grows with increase of LPR, or glucose level.

β region is characterized by smallest LPR. Right on cue, islet β cell is known to produce tiny amount of lactic acid due to low level expression of lactate dehydrogenase(LDH) and high level expression of mitochondrial Glycerol Phosphate Dehydrogenase(mGPDH)^(2, 3). And we can better account for behavior of islet β cell if β region is applied to explain the rule of insulin secretion (compare figure 1 to figure 2).

β -OS segment: lactate efflux predominantly by diffusion through volume-sensitive anion channel; diffusion by MCT expression of which is rare, neglectable. (Compare AB in figure 2.)

For lactate-proton diffusion by MCT, generating outward current, to occur, high concentration of lactate acid, which is untrue in islet β cell, is required to overcome membrane potential, due to that stoichiometry of H^+ :lactate, $n>1$. Therefore lactate diffusion through anion channel decides the membrane potential. Following elevation of LPR, L_{an} goes up to a point, corresponding to D' on OS segment in figure 1, where membrane action potential is fired.

On the sideline, closure of ATP sensitive potassium channel (K^+ -ATPase) is helpful⁽⁹⁾ for tiny amount of lactate efflux through anion channel to trigger membrane action potential. At glucose level of 7~8mM, activity of K^+ -ATPase peaks⁽¹⁰⁾.

Further augmentation of membrane electric activity by further elevated glucose should not be contributed to closure of K^+ -ATPase.

β -SP segment: elevation of LPR begets lactate-proton efflux by MCT, generating outward current eating into inward current generated by the lactate efflux through anion channel. (Compare BC in figure 2.)

In β -SP segment, increment in LPR, or in glucose, is not responded by as much increment in inward current as in β -OS segment due to occurrence of opposing current

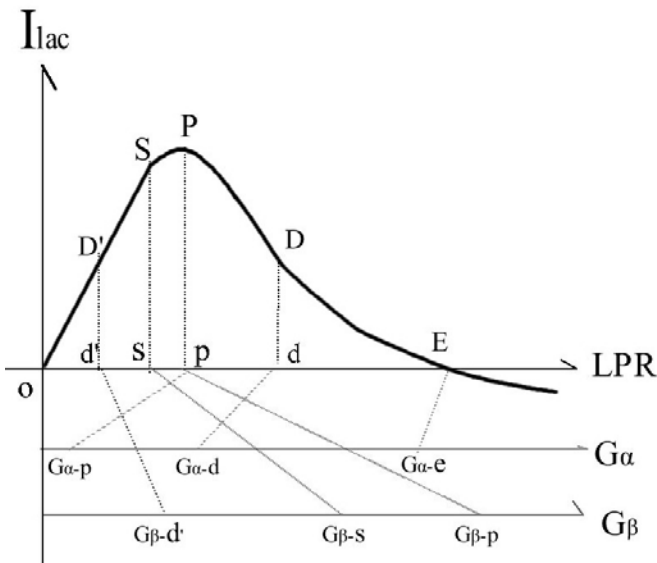


Figure 1: I_{lac} to LPR; LPR to glucose level at α cell and β cell

(G_α and G_β representing glucose level in α cell and β cell respectively)

OS segment: lactate efflux predominantly through volume-sensitive anion channel, generating inward current, with no meaningful lactate-proton efflux by MCT. At point D', action potential is fired.

SP segment: LPR grows up to a point where H^+ -lactate diffusion by MCT is born generating outward current.

Note: Segments, OS and SP, constitute β region.

PD segment: outward current grows faster than inward current with growing LPR. While $LPR > od$, no meaningful action potential is made due to electrogenic cancel-out effect between two way of lactate efflux.

DE segment: lactate-proton efflux still grows at higher rate than the one through anion channel; action potential is no longer triggered.

E^+ segment: membrane is hyperpolarized.

Segments, PD, DE, and E^+ , constitute α segment. α cell functioning at both DE and E^+ segment secretes glucagon through proton-driven exocytosis rather than by firing action potential.

at α cell, glucose at level $G_{\alpha-p}$ produces lactate at rate, op , expressed on LPR axis, $G_{\alpha-d}$ at od , and $G_{\alpha-e}$ at oE .

at β cell, glucose at level $G_{\beta-d'}$ produces lactate at rate, od' expressed on LPR axis, $G_{\beta-s}$ at os , and $G_{\beta-p}$ at op .

I_{lac-L} : I_{lac} to LPR as variable; I_{lac-G} : I_{lac} to glucose as variable.

LPR: lactate production rate; I_{lac} : the electric current generated by efflux of lactate.

$G_{\alpha,s}$ the glucose level to which islet α cell is exposed. $G_{\beta,s}$ the glucose level to which islet β cell is exposed

generated by lactate-proton efflux by MCT.

In rat, membrane electric activity and Ca^{2+} influx at islet β cell peaks at glucose level of 20mM^(11,12), suggesting point P of SP segment in figure 1.

As shown in figure 1, electric current generated by efflux of lactate goes down hill while $G_\beta > G_{\beta-p}$, which means too much production of lactate is to perturb insulin secretion, as demonstrated experimentally by over-expressing LDH in islet β cell^(13, 14, 15).

2) *α region:* with increasing LPR or glucose level, the amount of lactate-proton efflux goes up, resulting in

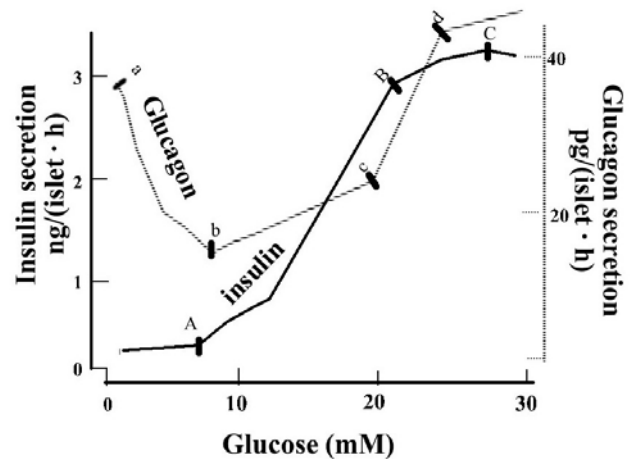


Figure 2: glucagon and insulin secretion from mouse pancreatic islets

Curve insulin (the solid line): AB is compared to D'S in figure 1; BC to SP.

Curve glucagon (the dashed line): ab is compared to PD in figure 1, bd to DE and to E^+ .

In bc and cd, no action potential is triggered by lactate efflux, and granule exocytosis is driven by proton efflux through lactate- H^+ facilitated diffusion which alkalizes nearby docking site to disrupt Ca^{2+} store in granule.

Diagram is reconstructed according to data from DIABETES, VOL. 55, AUGUST 2006⁽¹⁶⁾

tapering-off of net inward current and eventual hyperpolarization by net outward current

α region is applicable when LPR is at high level. Islet α cell is known to prefer anaerobic glycolysis⁽³⁾, and we can account for rule of glucagon secretion better if α region is applied to the electrical behavior of Islet α cell while the blood sugar level is within physiologic range. (Compare figure 1 to figure 2)

α -SD segment: (Compare segment ab in figure 2).

Outward current grows faster than inward current with growing LPR. The more higher the glucose level, the lower the probability of action potential firing and Ca^{2+} influx. This pattern is consistent with glucagon secretion of α cell in physiologic glucose range^(16, 17). As triggering of action

potential is predominant method for glucagon secretion in this segment, closure of K^+ ATPase is still helpful⁽¹⁸⁾.

While $LPR > od$, no meaningful action potential is made.

α -DE segment: no action potential is realized, neither Ca^{2+} influx.

To note, although no action potential is fired glucagon is still secreted, suggesting existence of granule exocytosis mechanism without Ca^{2+} influx.

α - E^+ segment: lactate-proton efflux by MCT generates more electric current than lactate efflux through anion channel, and the membrane is hyperpolarized.

α - E^+ segment coincides with the fact that high level of glucose hyperpolarizes islet α cell membrane^(16, 17, 19, 20), driving down cytosolic Ca^{2+} level⁽²⁰⁾.

Hyperpolarization is caused by surplus in proton efflux than total lactate efflux. What role does this excessive H^+ efflux play?

Proton-driven granule exocytosis

Although there is no Ca^{2+} influx in α cell at high glucose level granule exocytosis is still observed⁽¹⁶⁾ (compare DE and E^+ in figure 1 to *bc* and *cd* in figure 2). Islet β cell can also be experimentally designed to have granule exocytosis without Ca^{2+} influx⁽²¹⁻²³⁾ by manipulating efflux of proton. Proton in that context may play pivotal role, taking two distinct steps.

1) *Lactic acid promotes granule priming by donating proton*
Granules of neuron and secretory cells are rich^(24, 25) in Ca^{2+} . Artificial depletion of Ca^{2+} store in granule effectively inhibits exocytosis⁽²⁴⁾, suggesting importance of Ca^{2+} store in granules for exocytosis. Meanwhile, these granules are heavily acidified, with pH value sustained at about 5.5^(26, 27, 28, and 29), which is result of work done by vacuolar H^+ -ATPase. In islet cell, metabolism of glucose results in increased value of ATP/ADP, which in turn acutely drives down granule pH value by pumping H^+ from cytosol. This process is accompanied by uptake of Cl^- to keep electroneutrality of the granule⁽²⁹⁾ and acutely elevated cytosol pH⁽³⁰⁾, facilitating generation of outward electric current by proton-lactate efflux by MCT. The proton gradient, created by vacuolar H^+ -ATPase, supplies energy for H^+ - Ca^{2+} exchanger to sequester^(31, 32) cytosol Ca^{2+} , building up Ca^{2+} inside the granule. Without proton gradient, H^+ - Ca^{2+} exchanger is also likely to act as Ca^{2+} -ATPase⁽³²⁾. H^+ is also known to promote loading^(33, 34, 35) and docking⁽³⁶⁾ of granule as well.

Lactic acid as metabolite of anaerobic glycolysis can donate

protons ceaselessly to promote priming, loading, and docking of a granule.

2) *Facilitated diffusion of lactate- H^+ triggers granule exocytosis by alkalizing local site.*

On granules docking at plasma membrane, small amount of Ca^{2+} leaked from them is able to build up enough local concentration to invoke Ca^{2+} -induced- Ca^{2+} release from the granule⁽³⁷⁾, which in turn triggers granule exocytosis. Michelle L. et al⁽³⁸⁾ demonstrated that weak amine base can disrupt vesicular Ca^{2+} stores by alkalizing docking site and trigger exocytosis. Similarly, efflux of proton through facilitated diffusion of lactate- H^+ surely alkalizes nearby docking site and induces leakage of Ca^{2+} store, building up local cytosolic concentration sufficient enough to trigger Ca^{2+} -induced- Ca^{2+} release and exocytosis.

α cell should secrete glucagon by way of proton-driven exocytosis when functioning at α -DE and α - E^+ segment. With growing LPR, more and more proton is extruded by MCT, especially at α - E^+ where membrane is hyperpolarized, which potentiates granule secretion, the only explanation is proton-driven granule exocytosis (look at segment *cd* in figure 2; Note that *cd* doesn't necessarily coincide with segment E^+ in figure 1, nether *bc* to *DE*).

Free fatty lipid and amino acid: These nutrients may influence the secretion of insulin/glucagon by 1) electrogenic uptake, and subsequent depolarizing; 2) promoting production of lactic acid by competing for oxygen with glucose.

Inefficient oxygen supply and left-shift of I_{lac} -G curve

So far, our discussion has not covered topic of oxygen. Obviously, hypoxia promotes production of lactic acid, left-shifting I_{lac} -G curve in figure 1. Is hypoxia prevalent in our daily life? Although not widely recognized the answer is "yes".

Gas exchange takes place at capillary bed. As diameter of erythrocyte is 7~8 μm on average while luminal diameter of most capillaries is between 4~6 μm , erythrocyte has to be squeezed into capillary vessel by blood pressure. Acidity, generated by CO_2 by forming carbonic acid, of interstitial fluid helps erythrocyte release oxygen, which then diffuses to tissue cells. The benefit of this mechanism is that mechanical friction between erythrocyte and capillary wall prolongs passage time, which is helpful for erythrocyte to have sufficient time to release oxygen. The flip side is that erythrocyte is required to be good at deformability so as to be squeezed into capillary at normal blood pressure.

Otherwise, erythrocyte has to flow directly to vein through thoroughfare, bypassing the capillary nearby, and much less releasing oxygen. Obviously, membrane rigidity results in decreased efficiency of erythrocyte in terms of oxygen delivery.

There are 4 major factors that are common in our daily life and can influence deformability of erythrocyte.

1) *Glycation decreases erythrocyte deformability*⁽³⁹⁻⁴¹⁾

At hyperglycemia, glucose attaches to the membrane of erythrocyte non-enzymatically, resulting in decreased deformability of erythrocyte. This process is spontaneously reversible when glucose level comes down. Postprandial sleepiness and the recover after 60 minutes are believed to be a good example.

Glycation-induced hypoxia, augmenting LPR is primarily responsible for acutely elevated insulin secretion after meal. One other contributing factor is probably enteric glucose uptake through lymphatic pathway. In rat, higher than 10% of glucose from small intestine is poured into lymphatic system⁽⁴²⁾. Given the geographical proximity between pancreas and jejunum, glucose supply to islet cells through lymphatic way is supposed to be far higher than that of arterial blood immediately after absorption.

2) *Oxidation by free radicals impairs deformability of erythrocyte*⁽⁴³⁻⁴⁵⁾: those factors promoting production of free radicals or dwarfing antioxidant mechanisms will impair deformability of erythrocyte, such as stress in workplace or life, gestation, menopause, and so on.

3) *Hyperlipidemia impairs deformability by changing membrane lipid composition of erythrocyte*⁽⁴⁶⁻⁴⁸⁾.

4) *adrenalin improves deformability of erythrocyte*⁽⁴⁹⁻⁵¹⁾.

Adrenaline improves deformability of erythrocyte through cAMP dependant pathway, consuming ATP^(49,50). Therefore, Adrenaline should even improve deformability of those erythrocytes impaired by hyperlipidemia, glycation, or oxidation. This scenario may be instructive: when you are sleepy after meal, what is going to happen to your sleepiness if a tiger jumps over before you?

Now we can tell that long term exposure to hyperglycemia, hyperlipidemia, or overproduction of free radicals makes erythrocyte less deformable, resulting in hypoxia and left-shifted I_{lac} -G curve in figure 1. Adrenaline counteracts against these impairing effect.

Hypoxic left-shift of I_{lac} -G curve on elevated blood pressure, obesity and diabetes mellitus

Hypoxia and elevated blood pressure: to compensate

impaired deformability of erythrocyte, there are several options, such as 1) elevating blood pressure to help squeeze less deformable erythrocyte into capillary; 2) increase blood flow to deliver more erythrocytes; and 3) produce more erythrocyte to increase the number of erythrocyte per unit of whole blood volume, i.e. increased hematocrit. Each of the 3 options requires elevated blood pressure to work. Our body elevates blood pressure partly by contracting peripheral vessels, which implicates that elevated blood pressure may worsen the oxygen uptake at peripheral tissue while brain may get as much as necessary.

Left-shift of I_{lac} -G curve on islet β cell:

Within the whole β region in figure1, left-shift of I_{lac} -G curve augments insulin secretion. Over-shifting will bring β cell functioning in β -SP segment, in which increment in glucose level is responded by attenuated increment in insulin secretion, although total insulin level is at highest level. As a result of too much left-shifting of I_{lac} -G curve, it is theoretically possible that β cell may function in α -PD segment, especially the upper part, in which β cell secretes less insulin with increased glucose level.

Left-shift of I_{lac} -G curve on islet α cell

1) Mild left-shift of I_{lac} -G curve most likely results in attenuated glucagon secretion under normoglycemia. At the same condition, insulin secretion is increased, resulting in decreased blood sugar, which is to invoke feel of hunger.

2) With increased left-shift of I_{lac} -G curve, α cell is eventually to depend on proton-driven exocytosis, where increased glucose level is responded by increased glucagon secretion, especially at α -E⁺ segment. Meanwhile, insulin is secreted at high level as well. This is exactly the mechanism underlying the Glucose Intolerance.

Hypoxia stimulates secretion of glucocorticoid as well^(52,53) so as to promote gluconeogenesis.

Obesity and insulin: left-shift of I_{lac} -G curve augments insulin secretion, making it sustained at high level. The distribution pattern of insulin receptors dictates that hyperinsulinemia cause obesity. To sum up, hypoxia causes obesity.

The expression of insulin receptors in membrane of adipocyte is second highest in all types of cells, next only to hepatocyte⁽⁵⁴⁾, approximately 200~300 x 10³. About half of them in adipocyte are of high affinity insulin receptor isoform with exon 11, which is uncommon in skeletal muscle⁽⁵⁴⁾. Major target of glucagon is hepatocyte. Therefore, adipocyte is affected mostly by insulin without

meaningful counteraction from glucagon. As a result, under hyperinsulinemia, adipocyte undergoes anabolic metabolism faster than catabolic one, building up fat.

Diabetes mellitus by failure of islet β cell

Left-shift of I_{lac} -G curve sustains secretion of insulin at high level per each β cell. What makes aggregated insulin level down is likely by β cell failure. It is reported that morphometric studies on postmortem pancreases of people with type 2 diabetes show that β -cell mass is reduced to ~50% of that of control subjects⁽⁵⁵⁻⁵⁷⁾. The mechanism for apoptosis of β -cell is not clear. But hypoxia clearly presents oxidative stress to β -cell. β -cell is characterized by energy metabolism preferably through oxidative phosphorylation. With lack of oxygen supply, the advantage of redundancy in mGPDH can no longer be in play, leaving more and more amount of NADH and pyruvate for tiny amount of LDH to handle, putting β cell constantly under strong oxidative stress.

Hypoxia as culprit for diabetes mellitus is implicated by high rate lactate appearance in plasma^(58, 59) of type 2 diabetes patients. WILLIAMSON et al developed "pseudo-hypoxia theory" to account for diabetes and complications^(60, 61). Lastly, anemia is an obvious target of diabetes, which is confirmed by the finding that anemia is common among type 2 diabetes patients⁽⁶²⁾.

Adrenaline and glucose regulation

Adrenaline is able to improve hypoxia condition by improving deformability of erythrocyte⁽⁴⁹⁻⁵¹⁾. However, rampant elevation of adrenaline may result in, theoretically, another type of diabetes.

1) Adrenaline hyperpolarizes membrane of islet β cell through α_2 adrenergic receptor⁽⁶³⁾. As Islet β cell secretes insulin mostly by triggering action potential, the membrane hyperpolarization by adrenaline can acutely inhibit insulin secretion, leaving the blood sugar level less controllable.

Obese people are low in plasma adrenaline⁽⁶⁴⁾, which makes sense. On one hand, adrenaline prevents fat from building

up at adipocyte by inhibiting insulin secretion directly through α_2 adrenergic receptor. On the other hand, adrenaline prevents plasma insulin elevation indirectly by improving hypoxia to prevent left-shift of I_{lac} -G curve.

2) Adrenaline acutely stimulates secretion of glucagon through β -adrenergic receptor in islet α cell⁽⁶⁵⁾, promoting blood glucose.

3) Adrenaline stimulates secretion of glucocorticoid through α_2 adrenergic receptor⁽⁶⁶⁾, augmenting mobilization of protein and fat and gluconeogenesis. Adrenaline itself is known to be able to mobilize fat as well.

4) Adrenaline directly works on hepatocyte to promote gluconeogenesis and glycogenolysis, resulting in the elevated blood glucose level.

Obviously, sustained high level of plasma adrenaline should demonstrate symptom of hyperglycemia. Over-secretion of adrenaline from adrenal should not only be attributed to hyperfunction of adrenal, but also that of sympathetic center, like hypothalamus. Unfortunately, relevant literatures are unavailable. In case over-secretion lays root in brain, adrenaline-induced diabetes mellitus, if any, should be characterized by short temper, energetic, leaner day by day, good shape of hemorheology, and hypoinsulinemia without autoimmune marker.

Treatment

It is recommended that improvement of deformability of erythrocyte, or hemorheology, is paramount in treating diabetes mellitus. Antioxidant, aerobic exercise and appropriate dietary are all helpful.

Dedication:

This paper is dedicated to my endearing daughter, Zhu Dan.