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UNRAVELING THE LINK BETWEEN CAROTID BODY, INSULIN AND DOPAMINE

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TABLE OF CONTENTS

RESUMO

Os corpos carotídeos são quimiorreceptores periféricos que respondem à hipóxia com um aumento da sua actividade quimiosensorial no nervo do seio carotídeo (CSN), desencadeando mecanismos de hiperventilação e activando o sistema simpático-adrenal. A actividade do CSN está integrada no núcleo do tracto solitário, induzindo uma variedade de reflexos respiratórios através do aumento da actividade do ramo simpático do sistema nervoso autónomo. As células quimiorreceptoras contêm vários neurotransmissores como as catecolaminas dopamina e norepinefrina, serotonina, acetilcolina, neuropéptidos, adenosina e adenosina trifosfato. Todas estas substâncias conseguem modificar, inibir ou estimular a actividade do CSN. Além disso, actualmente surgiram evidências que as células quimiorreceptoras do corpo carotídeo conseguem identificar baixos niveis de glicose, participando também numa resposta neuroendócrina importante na hiperinsulinemia. A hiperinsulinemia consegue despoletar uma sobre-actividade simpático-adrenal no corpo carotídeo, podendo assim contribuir para o desenvolvimento de insulino-resistência que está presente em doenças como a síndrome metabólica. A insulina actua nos seus receptores nas células quimiorreceptoras do corpo carotídeo, levando a um aumento do cálcio intracelular com consequente libertação de catecolaminas, como a dopamina. Consistente com estes resultados, uma abolição funcional do corpo carotídeo consegue restaurar a sensibilidade à insulina e manter a homeostasia da glicose, afectando de forma positiva as vias de sinalização da insulina no tecido adiposo visceral e no fígado. Assim, o corpo carotídeo pode ser um alvo terapêutico no tratamento de inúmeras doenças mediadas por activação simpática.

Palavras-chave

Corpo carotídeo; Insulina; Dopamina.

ABSTRACT

The carotid bodies (CB) are peripheral chemoreceptors that respond to hypoxia by increasing chemosensory activity in the carotid sinus nerve (CSN), activating mechanisms of hyperventilation and of the sympathoadrenal system. The CSN activity is integrated in the solitary tract nucleus to induce a myriad of respiratory reflexes via an increase in the activity of the sympathetic branch of the autonomic nervous system (SNS). The chemoreceptor cells, also known as glomus or type I cells, are the main cellular constituent of the CB and contain several classical neurotransmitters including the catecholamines dopamine and norepinephrine, serotonin, acetylcholine, neuropeptides, adenosine and adenosine triphosphate. All these substances are capable of modifying, inhibiting or stimulating CSN activity. Besides this role there is emerging evidence that the carotid body chemoreceptors can sense low blood glucose and play an important role in neuroendocrine responses to hyperinsulinemia. Hyperinsulinemia triggers sympathoadrenal overactivity via the carotid chemoreceptor and perhaps contributes to the development of insulin resistance that is typically associated with the metabolic syndrome. Insulin binds to its receptors present in the carotid body chemoreceptor cells eliciting an increase in intracellular calcium and the release of neurotransmitters, such as dopamine. Consistent with these findings, functional abolition of CB activity was shown to restore insulin sensitivity and glucose homeostasis by positively affecting insulin signaling pathways in visceral adipose tissue and liver. Thus, the carotid body could be a therapeutic target for the treatment of a myriad of sympathetically mediated diseases.

Keywords

Carotid Body; Insulin; Dopamine.

ACROYNMS

Ach – Acetylcholine

Ado – Adenosine

AMP – Adenosine Monophosphate

ATP – Adenosine Triphosphate

ABC – ATP-Binding Cassette

CB – Carotid Body

CSN – Carotid Sinus Nerve

cAMP – Cyclic Adenosine Monophosphate

DA – Dopamine

ET-1 – Endothelin

GABA – Gamma Aminobutyric Acid

GLUT – Glucose Transporter

HF – Heart Failure

HT – Hypertension

NBTI – Nitrobenzylthioinosine

NE – Norepinephrine

NEFA – Non-esterified Fatty Acid

PNMT – Phenylethanolamine N-methyltransferase

SAH – S-Adenosyl-L-Homocysteine

SNS – Sympathetic Nervous System

TH – Tirosine Hydroxylase

TRP – Transient Receptor Potential

VEGF – Vascular Endothelial Growth Factor

OBJECTIVES

The CB has an important role on hypoxia but also on glucose homeostasis. When low glucose levels are detected, a compensatory mechanism is activated through sympathoadrenal activity. However, hypoxia and hypoglycemia induce separate signal transduction pathways and such differences contribute to a different approach on target therapeutics. Also, CB are an overstimulated when hyperinsulinemia is present further contributing to the development of insulin resistance. Insulin binds to its receptors present in the carotid body chemoreceptor cells eliciting an increase in intracellular calcium and the release of neurotransmitters, such as dopamine. Consistent with these findings functional abolition of CB activity restores insulin sensitivity and glucose homeostasis by positively affecting insulin signaling pathways.

Due to the essential role of the CB in sympathetic activation, its potential therapeutic usefulness for diseases with sustained hyperinsulinemia and sympathoexcitation, such as obesity, hypertension, sleep apnea, metabolic syndrome, cardiovascular disease, and diabetes should be pursued and this systematic review aims to explain all known mechanisms leading to such therapeutic goal.

METHODS

A systematic review was developed following international guidelines for systematic reviews. Studies were located by searching PubMed, ScieneDirect and Scholar Google databases, using “carotid body”, “insulin” and “dopamine” as keywords. The selection of studies, published from 2000 to 2017 was performed in two phases: first screening of titles and abstracts were conducted and then an assessment of the full papers identified as relevant was performed.

The first stage was a screening of titles and abstracts to identify potentially pertinent articles. When a final decision based on title or abstract wasn’t possible, the full papers were assessed. If full papers weren’t available, the papers were excluded. The second stage was the reckoning of the full papers identified as relevant at the initial screening.

Data extraction was performed by one reviewer only.

INTRODUCTION

Through the centuries, the CB has been studied by the scientific community due to its innumerable metabolic functions where they are involved. In 1743, Taube *et al*. described the CB as "ganglion minutuum" and assigned a ganglion function with motor innervation.[1] However, only on the beginning of the XX century, in 1900, Kohn *et al.* identified the CB as a ganglion with sensorial activity and not motor activity, finding the afferent innervation by the sinusal branch of the IX cranial nerve, the glossopharyngeal.[2] Then Fernando de Castro *et al.* defended that the CB anatomy and location could detect metabolic changes in the blood, with a reflex response on the functional activity of different organs.[3] Soon after in Ghent, Jean-François Heymans and his son, Corneille Heymans, performed perfusion studies in CB of dogs and discover the role of the CB in the ventilatory homeostasis and such achievement won a Nobel Prize in 1938.[4] During the XX century, a lot of descriptions and possible functions of the CB were presented but only on the end of the same century the individualization of CB cells led to the first descriptions on cell response and electrophysiology of type I and II cells.[5] The modern history of the CB begun and today its role on the maintenance of glucose homeostasis is seen as a powerful ally on the battle against metabolic diseases.

The CB are peripheral chemoreceptors located bilateral on the superior termination of the bifurcation of the common carotid artery, along the posteromedial wall, on the interior or immediately adjacent to the adventitious layer. It connects to the artery through Mayer ligaments and it has a relation with the sympathetic and parasympathetic system.[6] Its mixed embryological origin (mesoderm, 3rd braquial arch, ectoderm and neural crest) justifies the relation between the chemoreceptors and the neurons of the sympathetic plexus, specifically IX and X cranial nerves.[7]

It is composed by two types of cells: the predominant type I cells (glomic, chief cells

or epithelioids) and type II cells (support cells with chemoreceptor activity). The type I cells contain catecholamines such as dopamine (DA) and norepinephrine (NE), as well as serotonin, acetylcholine (Ach), neuropeptides (substance P and enkephalins), adenosine (Ado) and ATP and all of them can modify, inhibit or stimulate CB activity (figure 1).[8]

The type I cells are generally oval with a spherical nucleus and only one nucleolus, surrounded by cytoplasm with a variety of structures as mitochondrias, lysosomes, lipidic and glycogen particles and endoplasmic reticulum with several free ribosomes. However, the vesicles associated to the Golgi complex and simultaneously adjacent to the terminal ends of the sensorial nerves are the ones responsible for two types of secretory vesicles.[9] The less dense vesicles still don't have a clear function but it's advocated that they are storage locations of certain no-catecholamines neurotransmitters and modulators.[10] The dense vesicles contain catecholamines and chromogranin neuropeptides, and also nucleotides of adenine and Ca^{2+}, helping the different sensorial activities of the CB.[10] The type II cells have the ability of neurogenesis *in vivo* in response to a stimulus like chronic hypoxia. Unlike the type I cells, they don't have excitatory activity, without any record of ionic activity of sodium or calcium, having only an outward flow of potassium that is inhibitory. Therefore, these cells don't respond, for example, to hypoxia or hypoglycemia based on ionic changes or depolarization.

At cellular organization there is a grouping of 3 to 5 cells of type I, separated by 15 to 20 nm, involved by type II cells. Fibroblasts, ganglion cells and mastocytes are also present in connective tissue but their chemoreceptor activity is unknown.

The CB is highly vascularized and sensible to changes of partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), pH and blood flow.[8] The vascular anatomy has a functional role both in the acute chemotransduction process and/or in facilitating the proliferation and differentiation of progenitor type II cells during hypoxia. Moreover, the high

vascularity helps to sense tissue levels of PaO_2, algo regulating mitogenic and angiogenic factors including vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1).[8]

Its afferent stimulation starts in the carotid sinus nerve (Hering Nerve), passing to the glossopharyngeal nerve and to the nucleus of the solitary tract. The signal transduction is mediated by calcium (Ca^{2+}) and regulated by the release of catecholamines. An acute or chronic change of PaO_2 or $PaCO_2$ (like hypoxia or hypercapnia) cand lead to a secondary signaling with stimulation of the respiratory centers of the rachidian bulb by the glossopharyngeal (IX) and vagus nerve (X), leading to hyperventilation.[11] Simultaneously, an inhibition of the vasomotor bulb center can lead to a parasympathetic response of venous and arterial vasodilation (mediated by nitric oxide), bradycardia and inotropism decrease, with consequent drop in blood pressure.[11]

Type 2 diabetes is a chronic disease characterized by hyperglycemic state where insulin resistance, deficient insulin secretion and excessive glucagon secretion justify the clinical traits.[12] It's a disease of high social and economic impact, with significant rates of mortality and morbidity in the population. The patients, unlike the patients with type 1 diabetes, aren't necessarily insulin-dependents, however there has been an increase in the patients with the need of insulin treatment.[12]

In type 2 diabetes there is a combination of insulin resistance and impaired insulin secretion by pancreatic β cells.[13] The first is attributed to factors such as increased free fatty acid and pro-inflammatory cytokines in the plasma, which conducts to diminished insulin supply signalling in muscle cells, leading to a decrease of glucose uptake, as well as an increase of hepatic glucose production and lipolysis. The role of glucagon in this pathology is the lost of regulation between secretory α cells of glucagon and β secretory cells of insulin, leading to hiperglucagonemia that aggravates the hyperglycemia.

A dysfunctional CB awakes a sympathetic overstimulation that leads to

catecholamines discharge on the blood flow that besides leading to hypertension (HT), also contributes to the pathogenesis of insulin resistance.[13] It is possible to confirm, using computed tomography (CT), that diabetic patients have dilated CBs and besides this pathology they also often have hypertensive states and coronary diseases, all clinical diseases with an involvement of sympathetic overstimulation.[14] The cause of this growth is still under study but in rats it is explained by the extravascular increase. It is also known that patients with insulin resistance, HT and heart failure (HF) have a 20-25% increased size compared to the CBs of the control groups.[14]

The connection between hypertension and insulin resistance is still unclear, but it was suggested that secondary hyperinsulinemia is connected to vasoconstriction by sympathetic stimulation of skeletal muscle.[15] Besides that, atherosclerosis, common in patients with metabolic syndrome, also may lead to decreased perfusion of the CB and a further overstimulation of itself, with a consequent increase of sympathetic stimulation.

Hypercaloric diets, rich in fat or sucrose, lead to increased insulin resistance and hypertension. In animal models where denervation of CSN was performed before initiating the diet, the development of these pathologies was prevented.[16] This denervation also eliminated the hyperventilation, which suggests a excitatory role of the CB in these conditions. However, there was no increase in dopamine basal release, being the increase only registered in hypoxia, suggesting an increase of the sensibility of CB chemoreceptors cells and not an increase on the excitatory activity of the CB.[16]

GLUCOSE REGULATION OF THE CB

The ventromedial and lateral nuclei of the hypothalamus are known neuronal regulatory centers sensitive to glucose, but the primordial activation of a counterregulatory response is in β-pancreatic cells, as well as in the liver. In different organs different low-affinity glucose transports are known, for example at a neuronal and pancreatic levels GLUT2 prevails and GLUT4 is more prevalent in skeletal muscle and adipose tissue.[17] They all influence a different but effective response, although this type of transporters are not found in regulatory structures like the CB.

Nowadays, CB plays an important role in glucose metabolism, specifically in insulin-created hypoglycemia, functioning as a peripheral glucose sensor by initiating a regulatory response when glycemic values fall below the normal limits (70-100 mg dL^{-1}). With a rich irrigation and metabolic implications of the passage of a constant blood flow, the CB becomes a candidate receptor sensitive to metabolic substrates such as glucose.

Type I cells can identify hypoglycemia and activate a reversible mechanism, similar to hypoxia, that will be proportional to the current glycopenia.[18] Therefore, there is a membrane depolarization, influx of extracellular Ca^{2+} and secretion of neurotransmitters with consequent stimulation of the afferent fibers and activation of the sympathetic-adrenal system through the carotid sinus nerve (figure 2). All these alterations have already been observed in animal studies. However, contradictory results have been reported, which may be justified by the variety of CB preparations, as well as limitations of the different techniques. It should be noted that despite all these variations it is possible to infer that CB sensitivity to glucose levels is present in all mammals.

A study conducted in isolated CB of cats with an *in vivo* glucose infusion indicated no increase in its activity but a decrease of 20% in their activity in response to the increase of hypoxia threshold was observed.[19] The same team further showed that the increase in plasma

glucose after chemostimulation is mediated by activation of hepatic arginine vasopressin V1a receptors, an nNOS-activated mechanism in the hypothalamo-pituitary axis and a GABA-mediated mechanism in the nucleus of the tractus solitarius.[20,21,22]

The hypothesis that hypoglycaemia generates a stimulus in CB has been tested in dog CBs where the insulin-mediated counter-regulatory response to a low plasma glucose concentration has been shown to be less effective in animals with bilateral carotid sinus denervation than in control animals.[23] In this study hypoglycemia was the assumed stimulus. However, the role of other counter-regulatory hormones was disregarded. There was also an expected decrease in PaO_2 of 20 mmHg in dogs without CB. Such value is expected because this change is already described in rats with type 1 diabetes, where a continuous infusion of insulin decreases blood glucose levels to a normal level, increasing proportionally to their ventilation and metabolic rate.[24]

In humans, ventilatory responses were studied in 30-minute hypoglycemic states and it was shown that the expected increase in ventilation occurred without altering arterial $PaCO_2$, which may be explained by several hormonal mechanisms against regulatory agents such as glucagon and cortisol.[25] As well, Ortega-Saenz *et al.* (2013) have shown a secretory response of *post mortem* human type I CB cells in hypoglycemia.[26]

If CB can detect a decrease in glucose such sensitivity may also be dependent on a product of glucose metabolism, as glucose analogs didn't prevent the catecholamine release from thin slices of CB in response to glucose absence.[27] In this study, the mechanism found is triggered by a membrane process different from the described in the mechanism of response to hypoxia, allowing to separate two responses that have in common a process of transduction with increased concentration of intracellular Ca^{2+} and release of neurotransmitters.

The decrease of glucose activates an inward Na^+ permeable cationic conductance, with decreased membrane resistance in cococultered cells and such decrease, in individual

cells, induces a membrane depolarization of 10mV by opening transient receptor potential cation channels (TRPC) of type I cells.[27] In CB, several members of subclass C, TRPC are expressed and are non-selective Na^+ permeable channels that are involved in various cellular functions with sensitivity to temperature or osmolarity. The most common TRPCs in CB are TRPC3 and TRPC6, which belong to the subfamily where all members are activated by diacylglycerol, the product of phospholipase C activation. These subtypes can mediate the Na^+ current that is created in type I cells in the presence of glycopenia, which was already demonstrated by inhibiting phospholipase C.[29] By inhibing phopholipase C with U73122 the type I cells did not activate the ionic currents and catecholamines weren't released in the absence of glucose, suggesting that this enzyme may participate in a mechanism of glucose sensitivity.[27]

In addition, a selective inhibition of voltage-dependent K^+ channels at membrane potentials greater than 40mV has already been demonstrated and was similar to a severe hypoxia response, which counteracts or attenuates the decrease in input resistance created by TRPC channel openings.[28] Simultaneously to the inhibition of the K^+ channels, there is an increase of the influx of extracellular Ca^{2+} to the type I cells, which was demonstrated after inhibiting these channels with Cd^{2+}.[29] Thus, in these depolarization mechanisms, where the reversal potential is above 0mV, an increase in cationic current is common and the prevailing carriers are Na^+, in type I cells.

Zhang *et al.* (2007) suggested that Bin-Jaliah's *et al.* (2004) experiments at high PaO_2 used to exclude hypoxia-induced excitation may have stopped a full glucose response, translating an affirmative interaction between hypoxia and glucopenia.[30,31] Moreover, this has raised the question whether low glucose is either an adequate stimulus alone or merely facilitating a sensitivity to hypoxia; it is necessary to understand that tissue PaO_2 values at post-synaptic sites of the afferent action potential will be lower by diffusion/metabolism than

the values detected in superfusion, and may even be compared to stimulation of PaO_2 in single cococultured layers. In the same study, interaction with oxygen was directly tested by measuring the response to low glucose concentration at different PaO_2 values, ranging from hypo to hyperoxia. In both studies, hypoxia did not induce glucose sensitivity (as measured by ATP or catecholamines release). In fact, hypoxia sensitivity appears to decrease in the presence of glucopenia. There is a need to understand the interaction between hypoxia and glucopenia.

It is then possible to demonstrate similarities and differences in CB control in situations of hypoxia and/or glucopenia. The similarities are: inhibition of the channels voltage of K^+; depolarization of the plasma membrane; influx of extracellular Ca^{2+}; release of neurotransmitters; activation of the afferent nerve with sympathetic-adrenergic excitation. However at the beginning of these processes, the first differences are detected. In glycopenia the depolarization potential is dependent on the Na^+ permeable channels, which doesn't happen in hypoxia. Also, the inhibition of K^+ channels in glucopenia has low impact on the release of catecholamines. The type I cells of the CB can respond to both physiological changes, but it is possible to see that there are specific cells for hypoxia and others for glucopenia. For example, rotenone, a mitochondrial complex I inhibitor, can only block the secretion of catecholamines when induced by hypoxia, not having any activity in the presence of low levels of glycemia.[32] As rotenone is involved in oxidative phosphorylation processes, it may be inferred that control of CB in glucopenia does not serve to create a regulatory response.

Thus, the CB can respond to hypoxia and hypoglycemia together as these two factors can potentiate each other and may lead to a different catecholamine release and to afferent activation. In animal studies, it has been shown that in low glucose situations, low PaO_2 stimulates a greater release of catecholamines and currently the same point has proven

in human type I cells where hypoxia (6% O_2) increased catecholamine secretion induced by glucopenia.[33] However, the relationship between the mechanisms of hyperoxia and glucopenia is unknown, with a human sample (Ward *et al*., 2007) suggesting a decrease in the effects of hypoglycemia if hyperoxia is present.[34]

The reason for the fluctuating differences between CBs of different species, between CB preparations and between different tissues of CB and other glucose-sensing systems and the reason why they exist may determine the role of CB in the neuroendocrine axis, showing a physiological role that goes beyond sensitivity to hypoxia. This role will require CB to quickly detect small variations in glucose levels and initiate fast and effective reflex responses.

CB AND INSULIN

An increased sympathetic activity is already a known insulin-resistance mechanism. Therefore, given that the CB is an activation center of sympathetic activity it may play an important role in its development. Moreover, hyperinsulinemia has also been proven as an important factor.

Hyperinsulinemia may be associated with increased in systemic inflammatory activity and adipose mass, type 2 diabetes an HT with endothelial dysfunction.[35] Insulin may have an excitatory activity in the SNS; this was shown in an animal study where an exogenous administration of insulin into the arcuate and paraventricular nuclei led to an increase in the sympathetic response.[36] It was then further tested with the administration of an anti-insulin injection in the same nuclei and the sympathetic response was inhibited.[37] All these studies point to a role of these two hypothalamic nuclei for sympathetic overexcitation in hyperinsulinemia. However, in more recent literature, this hypothesis has been corroborated through animal studies since the use of models with induced obesity or patients with insulin resistance detected that the insulin carriage through the blood-brain barrier was unchanged.

CB induces a sympathetic response and its excessive stimulation in hyperinsulinemia can lead to insulin resistance. In the presence of insulin, phosphorylation of CB insulin receptors was observed. In 2014, Gallego-Martin *et al.* demonstrated that complete CBs incubated with insulin can accumulate more 2-deoxyglucose than the diaphragm muscle.[38] In 2013, Ribeiro *et al.* demonstrated *in vitro* that, in the presence of normal insulin concentrations, CB can have a neurosecretory response with an increase in intracellular Ca^{2+} and a consequent release of dopamine and ATP.[16] Subsequently, these *in vivo* results were confirmed with ventilation. The effect of insulin on ventilation was already studied in 2004, where an infusion of insulin under hypoglycemic conditions increased ventilation and O_2

consumption rate, an effect mediated in its whole by CB.[31] Moreover, Ribeiro *et al.* (2013) have shown very similar results in euglycemic rats, showing that the effects of insulin on ventilation are always present in glucose reduction states.[16] It is established that insulin can activate the CB and mediate its sympathetic activation. In hypercaloric diets, there is a chronic stimulation of the CB that leads to an increase of circulating catecholamines with stimulation of adrenergic receptors and release of NE in the nerve endings. This sets off a set of systemic responses dependent on the expressed adrenoreceptor. If the stimulation is acute, there is activation of the hepatic sympathetic nerves with increased glycogenolysis in states of satiety and gluconeogenesis in fasting states. In the pancreas, this stimulation contributes to an increased release of glucagon into the portal vein with moderate inhibition of insulin. In adipose tissue, there is lipolysis and release of NEFAs in the circulation. The kidney releases renin with sodium retention and vasoconstriction and adrenal medulla releases adrenaline. Chronic conditions may lead to insulin resistance and metabolic disorders (figure 3).

Prolonged dysregulation of hepatic glucose production and increased secretion of glucagon lead to increased serum glucose levels. However, the release of NEFAs affects the insulin transduction pathway, decreasing its effect. In 2013, a study where all these alterations were shown, a bilateral CSN resection prevented these consequences, showing once again the importance of CB in glucose homeostasis.[16] Shin *et al.* (2014) also observed, in rats subjected to intermittent chronic hypoxia for 4-6 weeks, increased fasting glucose levels, higher hepatic glucose output and insulin resistance.[39] Denervation prevented hyperglycemia in them, with increased baseline hepatic glucose output. There is a concordance between studies with animal models in hypercaloric diets and in environments of intermittent hypoxia. Limberg *et al.* (2014) showed that in hyperoxic environments an inhibition of CB chemoreceptors leds to lower muscle sympathetic activity under conditions of hyperinsulinemia.[40] In addition, Vera-Cruz *et al.* showed that CB suppression with hyperbaric oxygen therapy (100% O_2, 2.5 atm,

70min, 20 sessions) improved fasting glycemia and postprandial glucose tolerance in patients with type 2 diabetes.[41] Similarly, healthy humans CBs subjected to hyperoxia were involved in a hormonal counter-regulatory response. More recent studies have shown that hyperoxia may affect the response of CBs to hypoglycemia. However the lack of alterations, in patients with CB bilateral tumor resection, could indicate physiological adaptation over time, which may help to understand the study (Koyama Y *et al.*,2001) where dogs with CB resection, after 16 days, had the same answer.[42]

Taken together all these studies suggest an insulin-dependent sympathetic excitation of the CB that is involved in multiple metabolic diseases, making CB a therapeutic target.

NEUROTRANSMITTERS ACTIVITY

The activity of different neurotransmitters has a complex but very important role on sensorial transmission of the CB between type I cells (pre-synapse) and terminal nerve endings (pos-synapse). This stimulus is generated by type I cells and can be excitatory or inhibitory, resulting from different substances released and different availability of the receptors.

The multiplicity of released neurotransmitters is subject to factors such as age or genetics, which makes it difficult to organize them in excitatory or inhibitory terms and in a consequent therapeutic attitude. Its modulation with a pharmacological purpose has therefore a poor basis since the heterogeneity of reactions depending on the different factors may influence the affinities of the endogenous receptors, as well as their response. Upon isolation of type I cells, by cryodestruction, the chemosensitivity is lost but the nerve response is maintained.[43] This result may indicate that the nerve endings and the potentials generated by them may be dependent on the released neurotransmitters and not on the changes in the medium, which is present in an intact chemosensitivity.

The enzymatic synthesis of catecholamines involves tyrosine hydroxylase (TH) being one as the most important in biosynthesis. TH is present in all cells capable of synthesizing catecholamines and uses molecular oxygen and tyrosine as substrates and tetrahydrobiopterin as a cofactor in the production of L-DOPA. L-DOPA is transformed into dopamine by decarboxylase, which is the last step in the formation of dopaminergic neurotransmitters. In adrenergic and noreadrenergic neurons, the next step passes through DBH in a reaction where ascorbate is reduced to dehydroascorbate, an oxidase that is on the inner membrane of vesicles containing catecholamines. Both are released by neurons and cells of the adrenal medulla. However, in cells with epinephrine production the phenylethanolamine N-methlytransferase (PNMT) catalyzes the final step, being an enzyme

present in spinal cells where epinephrine is the neurotransmitter present, as well as in cells of the adrenal medulla where it is the main neurohormone. In addition, the heterogeneity of catecholamine receptors and their locations translates into more discrepancies in the literature regarding its activation. In a study using rabbit CB, DA is the main neurotransmitter in response to hypoxia, triggering action potentials on afferent axons.[44] However, in other species this excitatory role gives rise to a modulating role and the different intensity of hypoxia used in the different studies may justify these conclusions.

Dopamine is the most abundant neurotransmitter in CB, being located in the nucleus of the dense vesicles of type I cells. Its synthesis increases in the presence of hypoxia. However, several studies have shown that only in chronic or prolonged hypoxia these results are verified. Exogenous administration of dopamine in the rabbit generates an excitatory response; in other species, as in rats, it is perceived that the exogenous administration alone is not sufficient and the intensity of hypoxia used is crucial to regulate the response and maintain a stable level of dopamine release, despite the decrease in its endogenous production after an external administration.[46] One of the studies supporting these data is the evaluation of dopamine releasing peaks in hypoxia in different species. In rats, the peak is given prior to any observable increase in catecholamines and about 60 seconds before the highest peak of recorded catecholamines.[47] In the cat, the delay can be up to 3 minutes.[47] Its inhibitory action, consensual in several species, is related to a post-synaptic action at D2 receptors and may be a target mechanism of desensitization if subjected to high and repeated doses of dopamine, culminating in an excitatory activity similar to that of D2 blockage. Therefore, the exogenous application of dopamine may be complicated, and the role of D1 receptor as a compensatory mechanism should be studied. It is also known that nanomolar DA concentrations can selectively and reversibly attenuate the influx of Ca^{2+}, creating an inhibitory feedback mechanism in neurotransmitter release. In short, it is not possible to affirm an excitatory role

of DA in all species, but rather a modulating role that will be responsible for controlling the response to other neurotransmitters. Regarding other catecholamines, the presence of NE is very residual compared to that of DA, and the highest concentration of NE is mainly in the sympathetic neurons.

Dopamine has receptors organized in two families, dependent on the action of adenylate cyclase and consequently on cAMP values. D1-like receptors (such as D1 and D5) are linked to Gαs by stimulating adenylate cyclase. In D1-like receptors there are other pathways that modulate their behavior, namely calcium and potassium channels and even arachidonic acid. Its release is dependent on the influx of Ca^{2+}, which raises some doubts in the literature because in different species were obtained results not very similar. In the rats CB this response is not only misleading, it is also minimal and in the CB of rabbits, although there is a response; this is not mediated by dihydropyridine-sensitive channels, the prevalent in type I cells.[45] Thus, their release may be due to inhibitory factors that act in exocytosis and Ca^{2+} flow. The D2-like (notably D2, D3 and D4) are bound to Gio proteins with opposite function to the former. However, in these receptors their short isoform, the presynaptic form, ends up having the counter-regulatory effects on the D1-like receptors.

The detection of high dopamine values in all mammals leads to a set of effects and it is necessary to distinguish peripheral effects from central effects. The first studies conducted are based on its excitatory effect on the ventilation of dogs, which was later confirmed in many other mammals, such as cats, rats and goats, where a dopamine-dependent ventilatory depression was present after CSN ressection. The use of a D2 receptor antagonist, incapable of passing the blood-brain barrier but through the pre and post-synaptic receptors of CB can lead to increased ventilation and an overexcitation of CB. Low doses of dopamine can block a response of CSN, in contrast to high doses that have an excitatory effect and this difference is important in the response of different receptors. In rats where dopamine D2 receptor agonists

were administered, via an intracerebroventricular route rather than via a peripheral route, ventilation was increased.[48] On the other hand, the administration of D2 antagonists such as domperidone, which cannot cross the blood-brain barrier, inhibits normal ventilation, which may help to understand a dopamine response in acute and chronic hypoxia situations, with an increase in CB stimulation.[49] It is already known that this ventilatory stimulus has CB activity with a dopamine release proportional to the intensity of the stimulus, the increase in CSN activity and the available extracellular Ca^{2+}.

Adenosine A2a receptors and dopamine D2 receptors stimulation in CB potentiates the dopamine inhibitory effects on CSN releases in cats. This interaction was also observed in rats *in vivo*, with adenosine enhancing dopamine inhibition on CB-receptor-mediated ventilation while its antagonist DPSPX decreased its inhibitory effects. In cases of CB ischemia where endogenous levels of adenosine are high, the effects of D2 agonists can be attenuated by A2a receptor blockage, suggesting a dopaminergic effect that undergoes an increase in ventilation.[45]

Adenosine is a product of ATP metabolism and can be recycled. It is an ubiquitous substance released in almost all major cell pathways, not being stored or released like the classical neurotransmitters. Its regulatory activity may be presynaptic, post-synaptic or unrelated to the synapse, such as modulation of the bloodstream. Extracellular adenosine results from the extracellular production of ATP by catabolism of the 5'ectonucleotidases pathway, as well as from intracellular production with release by the transport systems. More, cAMP can be released by secretory cells, converted by extracellular phosphodiesterases into AMP and then into 5'-ectonucleotidase which gives rise to adenosine of extracellular origin. Its intracellular production is mediated by a 5'-nucleotidase that defosphoreses AMP or hydrolyzes s-adenosyl-L-homocysteine (SAH). There are two transport systems, dependent and not dependent on Na^{+}, bidirectional that mediate the exchanges. The dependent

concentrates the nucleosides against a concentration gradient, having five subfamilies consistent with the substrate selectivity. Independent transport systems have two groups, distinguished by sensitivity to nitrobenzylthioinosine (NBTI). The transport pathways allow a conversion of small changes in intracellular ATP into large and disproportionate changes in the extracellular concentrations of adenosine.

Their receptors are divided into high affinity (A1 and A2a) and low affinity (A2b and A3), being activated by different endogenous concentrations of adenosine. Activation of its receptors, in addition to their direct activity in cells, may influence other neurotransmitters. A2a and A2b activate Gs proteins, leading to an increase of cAMP and intracellular Ca^{2+} mobilization. Both A1 and A3 activate Gio proteins, decreasing cAMP values and Ca^{2+} channels, while activating K^{+} channels and phospholipase C. Thus, A1 receptor agonists will play a more inhibitory role, while A2 receptors will mediate the excitatory processes.

Adenosine regulates excitatory processes in tissues such as the heart and the brain, leading to a decrease in heart rate for example. It also leads to vasodilation and helps maintaining cellular metabolism by balancing energy expenses with available substrates. It also helps to promote and/or maintain sleep, regulates libido and regulates blood brain circulation in case of need. In pathological situations it is neuroprotective, especially in situations of hypoxia, ischemia or brain injury by convulsion. It is also released by CB in hypoxic settings, contributing to the feedback mechanism.

Adenosine is capable of stimulating CSN activity which has been demonstrated after an exogenous application of adenosine in cats, leading to increased CSN activity both *in vivo* and *in vitro*. In rats, intra-carotid injection of adenosine activates ventilation, an effect which is mediated by A2 receptors and absent after CSN ressection. Taken together, all these data reinforce the results obtained in healthy humans subjected to an intravenous infusion of adenosine that led to hyperventilation, dyspnea and thoracalgia. Its effect on ventilation is

proportional to the proximity of CB to bolus site administration and adenosine and its antagonists modify the peripheral hyperventilatory response to hypoxia since it does not cross the blood brain barrier. Dipyridamole, an inhibitor of adenosine uptake, increases extracellular adenosine concentration and potentiates an increase in cAMP produced by hypoxia, a response blocked by A2 antagonists.[50] The presence of A1 receptors in rabbit CB chemoreceptor cells was demonstrated because their antagonists and agonists can modulate Ca^{2+} currents, although in rats these receptors are almost null. *Fitzgerald et al.* suggested that the effects of adenosine on ventilation result from a modulatory activity on other neurotransmitters.[49] However, this is an extracellular Ca^{2+} dependent process and in normoxia Ca^{2+} levels are not altered by adenosine. More recent studies have shown a small increase in intracellular Ca^{2+} levels triggered by activation of A2a adenosine receptors. Thus, the role of adenosine is present only in responses to an acute or chronic hypoxia.

ATP has a fundamental intracellular role in all cells, being a neurotransmitter in the central and peripheral nerves. Its release is usually by exocytosis, however in some nerve cells, it may be also mediated by carriers, involving ABCs carriers. The activity of ATP goes through the activation of 2 large groups of receptors: P2X, with seven subtypes described, if the receptor is ionotropic, and P2Y, with eight subtypes described if the receptor is metabotropic. The first study by McQueen and Ribeiro reported an increase in CSN activity by ATP in a dose-response response.[51] However, this response may be related to adenosine formed in the catabolism of ATP, since an ATP agonist (ß-MethyleneATP) decreased CSN activity. The role of adenosine in CSN activity cannot be excluded and the co-application of hexamethonium and suramine, nicotinic and P2X receptor blockers respectively, was demonstrated. In cococultures of chemoreceptor cells and juxtaposed petrosal neurons, hypoxia post synaptic activity was abolished in the presence of such inhibitors, which may indicate that ATP is also related to acetylcholine in chemoreceptor cells.[52] Rats with P2X

deficiency have a very attenuated ventilatory response in hypoxia and the fact that ATP is released proportionally to the intensity of hypoxia supports the excitatory role of ATP in CB. Adenosine is a product of catabolism but also a precursor of ATP, which forces us to understand whether the effects under hypoxia are linked to adenosine or to an ATP release with subsequent hydrolysis.

Acetylcholine (Ach) was the first neurotransmitter identified in the SNS. It delivers sodium that subsquently depolarized and stimulate muscle contractions. It is synthesized from coline and acetyl-CoA by an acetyltransferase. After release it is quickly removed so a re-polarization can occur, a process mediated by acetyltransferases that are bound to the plasmatic membrane. Acetylcholine is responsible for the electrical transmission that creates skeletal muscles tone in the neuromuscular junctions; interferes in cognition, learning and memory functions and motor control. It also intervenes in analgesia in autonomic basal ganglia and cholinergic neurons and regulates their synaptic release, as well as for other neurotransmitters. It has two groups of receptors: muscarinic and nicotinic, both abundant in the human brain. For pharmacology there are important differences although they have two common stimulants: acetylcholine and carbachol. Nicotinic receptors are ion channels with pentameric subunits, with muscle or neuronal subunits and muscarinids are coupled to G proteins, having five subtypes described where G protein coupled changes and where antagonist sensitivity is not always the same. They are also distinguished in terms of function where the nicotinic mediates the synaptic transmissions in the neuromuscular junctions, being mainly post-synaptic. However, there are also presynaptic receptors that regulate the release of other catecholamines such as dopamine or norepinephrine. Muscarinic agents play a role in the motor ganglion basal activity, analgesia and hypothalamic function. Their agonists excite or inhibit autonomic effector cells, innervated by postganglionic parasympathetic neurons, acting as a parasympathomimetic agent.

Ach action in the CB goes back to the 20th century where Cholinergic Hypothesis was formulated. Fidone *et al.* (1976) demonstrated that, after a CSN section, Ach concentration in the CB did not decrease, suggesting that ACh is present almost exclusively in the CB.[53] Also Shirahata *et al.* (1996) observed that ACh is released from both cat and pig chemoreceptor cells in culture, although the release from other cell types cannot be excluded.[54] It was the first neurotransmitter recognized in the CB synapse. However, an incomplete obstruction of nicotin antagonists in the CB response in hypoxia and the absence of expression of key enzymes in the acetylcholine biosynthesis in CB, question the first theory. However, the excitatory effects of acetylcholine on CB may involve other excitatory neurotransmitters, enhancing the role of adenosine/ATP in CB chemotransduction. Furthermore, recent studies have demonstrated the existence of all the necessary enzymes for the formation and inactivation of acetylcholine in the chemoreceptor cells of the CB. In samples from rat and rabbit CB, hypoxia inhibits the basal release of acetylcholine by the activation of muscarinic and dopaminergic receptors present in CB. Nicotinic receptors are also present in CB and nerve fibers, especially with the presence of α3, α4 and β2 subunits in chemoreceptor cells, but their physiological activity is not yet well studied and a mechanism of exogenous nicotine hyperapnea can be assumed. An application of nicotinic antagonists, such as hexamethonium, in co-cultures of type I cells only partially inhibits hypoxia-induced stimulation. Nonetheless, nicotinic receptors for acetylcholine in these same cells are known to be modulators, increasing Ca^{2+} and leading to the release of other neurotransmitters such as dopamine.

Adrenaline may also increase the sensitivity of CB, both at the glucose and pCO_2 levels. An *in vitro* study using cat CBs showed that after 12 minutes of 0 glucose there is no change in Ach or ATP release, hypothesising that hypoglycemia may not be an acute CB stimulus.[55]

A superinfusion with glucose or low values could stimulate 1900% the secretion of catecholamines (in an ATP-independent intracellular form) by type I cells, starting one minute after glucose administration, comparable to a state of anoxia.[56] In addition, a decrease in glucose may excite the cococultured petrosal neurons of mice and can induce the presynaptic co-release of ATP and Ach on type I cells. All this information is consistent with electrophysiological data of ion channel activity in isolated type I cells, which also demonstrated the presence of GLUT 1/3/4 facilitating glucose transporters in these cells, although GLUT2 has not been found.[57]

THERAPEUTIC IMPLICATIONS

The last decade has been fulfilled with several studies where the increase of adrenergic activity is present in pathologies like essential hypertension, sleep apnea and cardiovascular diseases. The first studies go back to 1992 where increased CB activity was demonstrated to be associated with the progression of essential hypertension induced by intermittent hypoxia. In this study, a bilateral denervation of the CB was performed and for 25 days the HT has decreased. Since then numerous studies have also shown the functional activity of CB and its denervation in sleep apnea. In 2012 and 2013 animal studies proved the role of the CB on the mechanism that leads to HT.[58,16] After a bilateral denervation of the CSN, a decrease on the development and maintenance of a hypertensive state was observed, with the reduction of vasomotor tonus and sympathetic renal activity. In an unilateral resection performed in rats with spontaneous hypertension the decrease was less effective but more than the reduction noted after renal denervation. Nevertheless, these results are not only detected in animal models. In humans a unilateral resection of the CB in hypertensive patients, after tumor removal, induced a decrease of arterial tension in short periods.[59] In the *International Congress of the Autonomic Neuroscience* these results were shown: the unilateral ablation of the CB diminished arterial pressure during 12 month and after that the effect was attenuated, suggesting that there is an attempt of compensation from the remaining CB.

Cardiovascular diseases and their relation with the CB goes back to 1999 and 2001 studies. In more recent studies animals with HF a CB ablation, by cryogenic destruction, have shown in a hyperventilation decrease, an oscillatory ventilation with sympathetic tonus diminished, improving their cardiac function and survival.[58] In humans, it was shown that the CB removal leads to a diminished peripheral chemosensibility with improved cardiac function, physical ability and ventilation at rest. The same team in 2014 has shown a decrease of ventilatory and arterial pressures in response to hypoxia, in patients with HF were bilateral

removal of the CB was performed, with a consequent decrease of the sympathetic response.[60] Changes in the cardiac rhythm were not found, which may indicate aortic bodies actitivity, peripheral chemoreceptors also sensitive to hypoxia.

The CB is also a good therapeutic target in diseases where hypoxia is present and its removal can ameliorate HT and HF not-pharmacologically controlled. In situations with hyperoxia it is registered a decrease of the response to hypoglycemia, although after a bilateral resection of the CB the response to hypoglycemia in normoxia stayed relatively normal, suggesting that hyperoxia can have a limited effect on counter-regulated activity.

Recently, the role of chronic excitation of the CB in patients with insulin resistance induced by diet has been described and a surgical removal of the CB in rats submitted to hypercaloric diets can prevent pathological metabolic changes, what shows once more the importance and need of CB studies regarding the control of metabolic diseases.[16]

It was demonstrated that chronic administration of caffeine can prevent hypertension, glucose intolerance and insulin resistance in pre-diabetic animals[60]. This protective effect is allied to a weight maintenance and decrease in visceral fat in obese animals, although the benefits regarding the insulin resistance and hypertension do not need to be accompanied by weight lost. Besides that, a study (Conde *et al.*, 2012) has shown blockage of adenosine receptors in the CB, and its consequent inhibition of sympathetic overstimulation, through chronical caffeine administration.[60]

The application of this method to humans through clinical trials is still a mirage, although caffeine consumption in a balanced diet is defended since several years ago, with positive results on type 2 diabetes and obesity incidence in risk groups.

It is also possible to prevent overstimulation of the CB using an old pharmacological ally: caffeine.[60] This xanthine is consumed all over the world through coffee, tea and even chocolate. Daily Coffee consumption differs from country to country, with an increase in

countries like EUA, UK or the Nordic countries. Caffeine acts through different cells mechanisms: release of Ca^{2+} from intracellular reservoirs by receptors of ryanodine; inhibition of cAMP phosphodiesterases; inhibition of adenosine receptors and antagonist of adenosine, being this the predominant mechanism on regular consumption caffeine concentration. In the subtypes of adenosine receptors, caffeine and theophylline have a bigger impact at A2a, followed by A1 and A2b, being the antagonist activity of the A3 almost null in humans. Caffeine may inhibit A2 receptors in physiological conditions because the activation of such receptors depends on adenosine concentration but also on the number of receptors on the action site. A chronical treatment with caffeine can lead to changes in adenosine receptors but others effects were reported. In fact, a positive regulation of A1 serotonin receptors, GABA, muscarinics and δ-opioids and a negative regulation of β-adrenergic with desensitization of nicotinic receptors were described in the cortex of mice exposed to chronic caffeine.[60]

As the activity of the CB passes by SNS stimulation, the SNS is also a possible therapeutic target, in order to reduce insulin-resistance. A bioelectronic medicine could be applied and could be the path, allowing to connect the peripheral nerve fibers to a sensor that recognizes certain types of typical electrical patterns of homeostasis disruption. It is a growing area and a strong candidate to CB therapeutic applications.

Thus, the CB as a glucose and hypoxia sensor can trigger response mechanism to metabolic changes induced by such states. Therefore, besides the mechanism of hyperventilation and rise of arterial pressure in hypoxic states, paradoxically the CB can also induce liver glucogenolysis and create insulin resistance on peripheral tissues in hypoglycemic states[16]. A correct modulation of its behavior can be a therapeutic target in a group of diseases where glucose homeostasis is disturbed, namely type 2 diabetes.

FIGURES

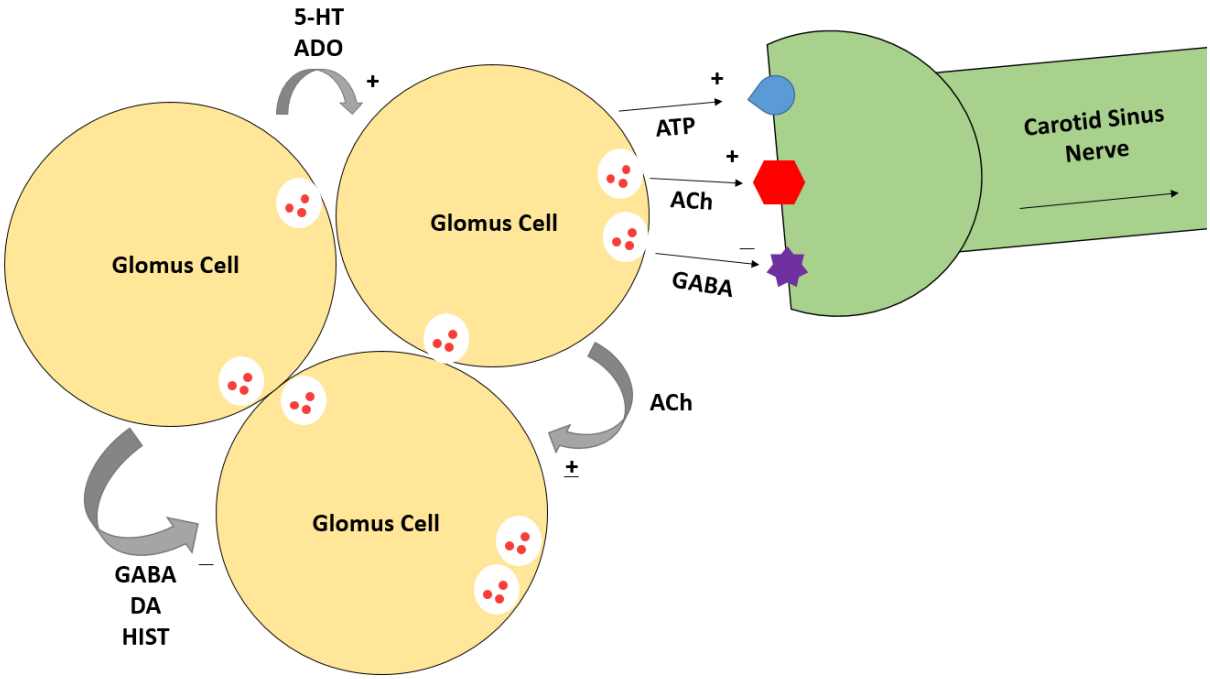

Figure 1: The glomus cells contain catecholamines such as dopamine and norepinephrine, serotonin, acetylcholine, neuropeptides, adenosine and ATP and all of them can modify, inhibit or stimulate CB actitivty. Ach – Acetylcholine; Ado – Adenosine; ATP – Adenosine Triphosphate; DA – Dopamine; GABA – Gamma Aminobutyric Acid; HIST – Histamine; 5-HT – 5-Hydrotryptamine Receptor.

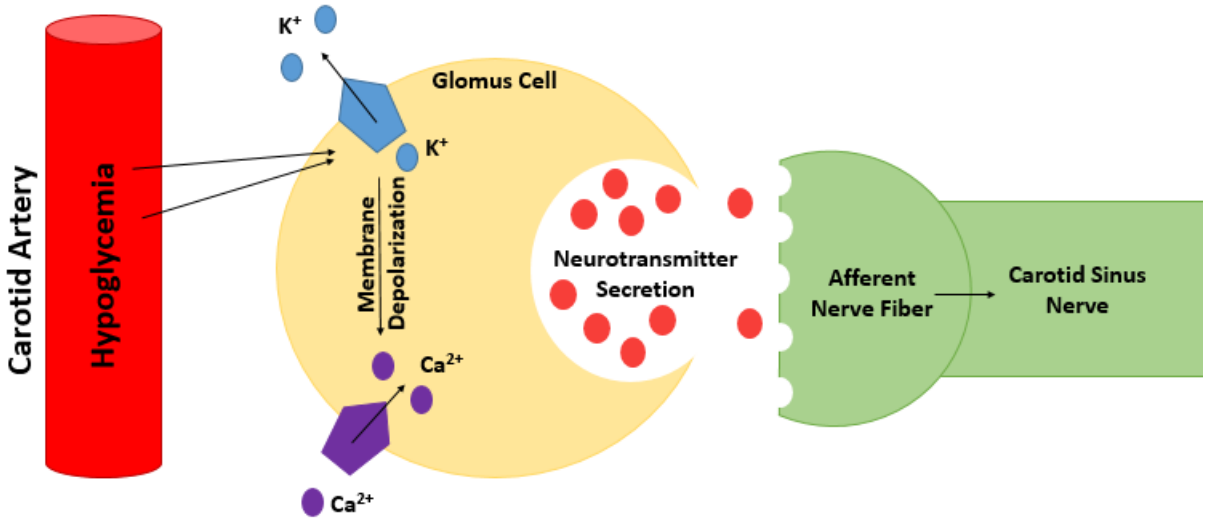

Figure 2: Glomus cells can identify hypoglycemia and activate a reversible mechanism,

similar to hypoxia, that will be proportional to the current glycopenia. Therefore, there is membrane depolarization, influx of extracellular Ca^{2+} and secretion of neurotransmitters with consequent stimulation of the afferent fibers and activation of the sympathetic-adrenal system through the carotid sinus nerve.

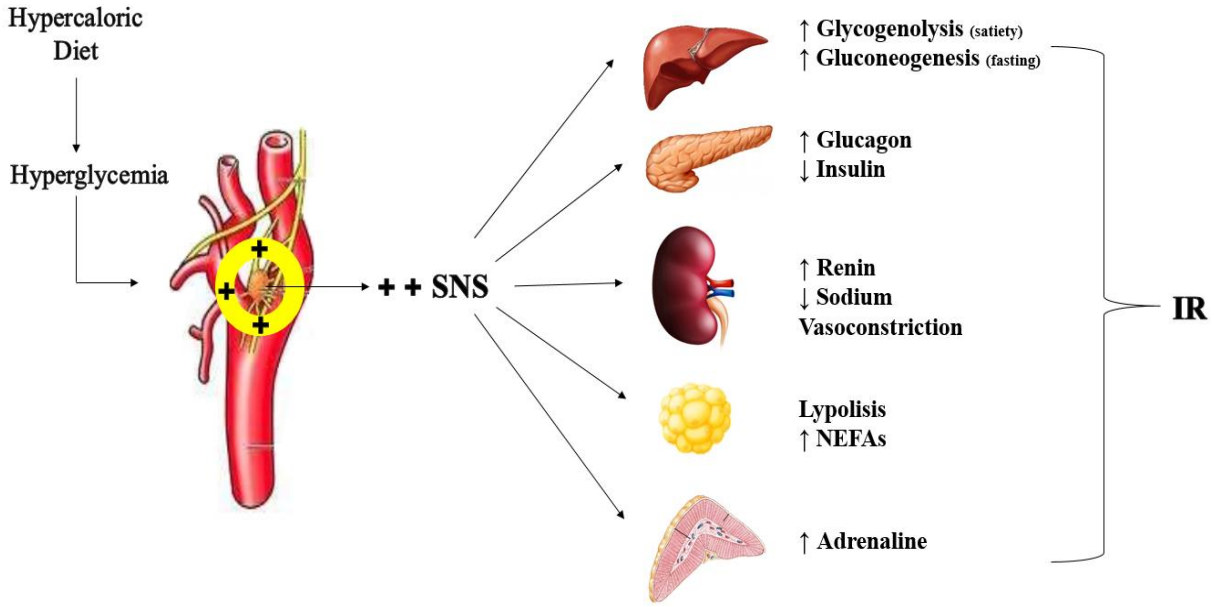

Figure 3: It is established that insulin can activate the CB and mediate its sympathetic activation. In hypercaloric diets, there is a chronic stimulation of the CB that leads to an increase of circulating catecholamines with stimulation of adrenergic receptors and also by sympathetic nerve release of NE. This sets off a set of systemic responses dependent on the expressed adrenoreceptor. In acute stimulation, there is activation of the hepatic sympathetic nerves with increased glycogenolysis in states of satiety and gluconeogenesis in fasting states. In the pancreas, this stimulation contributes to increased release of glucagon into the portal vein with moderate inhibition of insulin. In adipose tissue, there is lipolysis and release of NEFAs in the circulation. The kidney releases renin with sodium retention and vasoconstriction and adrenal medulla releases adrenaline. In chronic conditions insulin resistance and metabolic imbalance are observed.

CONCLUSION

Carotid bodies are chemoreceptors, highly vascularized, sensible to changes of partial pressure of oxygen and carbon dioxide, pH, blood flow and changes in glucose and insulin. CB afferent stimulation starts in the CSN, where signal transduction mediated by Ca^{2+} leads to neurotransmitters release. Dopamine is the most prevalent and such release can lead to a set of direct inhibitory factors on exocytosis and flow of Ca^{2+} with consequent inhibition of the CB activity. The CB can also respond to hypoxia and to hypoglycemia, because they are potentiators with different discharges of catecholamines and afferent activation.

Insulin is also a modulator of CB activity, resembling its activity to intermittent hypoxia on CB control when developing a sympathetic excitation response. The CB has insulin receptors and they respond to insulin increase with sympathetic activation; combining the insulin regulation with the sensibility to glucose, may help exogenous insulin-induced hypoglycemias in diabetic patients. However, in hyperinsulinemia the CB is overstimulated, conducting to a sympathoadrenal overexcitation that leads to insulin resistance. The insulin acts on the insulin receptors present on the CB leading to an increase of Ca^{2+} concentration and consequent release of catecholamines which aggravates the SNS stimulation, leading to pathologies like type 2 diabetes or metabolic syndrome. These diseases have associated mechanisms that intensify the hyperinsulinemia, creating a vicious cycle of cause-effect. Dysregulation on insulin secretion and consequent exposure of different tissues to chronic hyperinsulinemia lead to a multiplicity of diseases where the common ground is a insulin-resistance.

Thus, glucose homeostasis can be achieved by modulating CB activity and consequently sympathetic excitation creating a therapeutics targeting. More, *in vitro* and *in vivo* studies should be performed to completely understand such mechanisms. Availabe data supports the ideia the CB overactivity is involved in the development of metabolic diseases.

Different animal studies support a role of counter-regulation on hypoglycemia, with the suppression of its activity when denervation of the CSN is performed. In human studies is necessary to ensure a correct stratification of the therapy with the identification of the most chemosensitivity cells of the CB, allowing a more efficient approach. The identification of such mechanisms may open new treatment opportunities/options for insulin resistance and metabolic syndrome, and thus likely to have major impact on cardiovascular diseases and type 2 diabetes associated health burden.

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