Effect of Okadaic Acid on Glucose Regulation

M.C. Louzao[1], M.R. Vieytes[2] and L.M. Botana[*,1]

[1]*Departamento de Farmacologia. Facultad de Veterinaria de Lugo. Universidad de Santiago de Compostela. 27002 Lugo. Spain*

[2]*Departamento de Fisiologia Animal. Facultad de Veterinaria de Lugo. Universidad de Santiago de Compostela. 27002 Lugo. Spain*

Abstract: Okadaic acid is the main toxin responsible for the natural phenomena known as diarrheic shellfish poisoning (DSP). This toxin is a tumor promoter C38 polyether fatty acid that contains acidic and hydrophobic moieties and is cyclic. Okadaic acid is a potent inhibitor of important classes of protein serine/threonine phosphatases such as protein phosphatase 1 and 2A. The toxin binds in a hydrophobic groove adjacent to the active site of the protein phosphatases and interacts with basic residues within the active site. Therefore okadaic acid causes increases in phosphorylation of proteins that affect a diverse array of cellular processes. For instance, this toxin modulates metabolic parameters in intact cells. In this sense it stimulates lipolysis, and inhibits fatty acid synthesis in adipocytes however increases glucose output and gluconeogenesis in hepatocytes. Additionally, okadaic acid reaches cytotoxic concentrations in the intestinal tissues in accordance with the diarrhea. Recent studies suggested that toxic effects of okadaic acid might be related to modification of nutrients, ionic and water absorption across the small intestine presumably by altering the transporter system. The subject of this review is limited to the effect of okadaic acid on glucose regulation and its cellular as well as clinical implications.

INTRODUCTION

In the 1970's, crude extracts of the black sponge *Halichondria okadai*, isolated off the coast of Japan, bore remarkable cytotoxic activity. The toxin okadaic acid (OA) was isolated from these extracts. Actually this toxin is produced by several species of marine dinoflagellates of the genus Dinophysis [1]. OA and other structurally related compounds (i.e. dinophysistoxins) concentrate in commercially valuable seafood and cause diarrhetic shellfish poisoning (DSP) having a dramatic impact upon human health and economics [2,3]. DSP toxins are potent and highly selective inhibitors of the catalytic subunits of serine/threonine protein phosphatases (PP) [1,4,5]. OA has subsequently emerged as a key laboratory tool for identifying and studying the myriad of events associated with the inhibition of protein serine/threonine phosphatases [6,7].

Phosphorylation level in cells results of a delicate balance between protein phosphatases and protein kinases. Kinases transfer a phosphate from ATP to a protein. Phosphatases remove the phosphate group, i.e. dephosphorylate the protein [8]. Regulation of the levels of phosphorylated proteins is fundamental to a large number of cellular processes. These include changes in gene expression, muscle contraction, protein synthesis, intracellular transport, phototransduction, and cell cycle progression, apoptosis or glycogen metabolism. Breakdown of endogenous glycogen stores provided glucose, which is a fundamental source of energy for all eukaryotic cells. These energy stores are replenished from glucose in the diet [9,10]. Certain tissues such as gut, muscle and adipose tissue, have acquired a highly specialized glucose-transport systems. The activity of transporters can be rapidly upregulated to allow these tissues to increase their rate of glucose transport by 10–40-fold in minutes. Those systems are crucial during the absorptive period (after a meal), to facilitate the rapid insulin-dependent storage of glucose in muscle and adipose tissue, so preventing large fluctuations in blood glucose levels. Dysfunctional glucose uptake into muscle and fat cells contributes to the onset of type II diabetes [10,11]. Understanding regulation of glucose is extremely important in the sense that involves several signal-transduction pathways that are superimposed on a complex series of transport processes. In this review, we focus on cell-biological effect of OA on glucose regulation.

OKADAIC ACID

The toxin ($C_{44}H_{44}O_{13}$) was named okadaic acid after the sponge from which it was isolated *Halichondria okadai* [1]. OA was characterized as the main toxin responsible for the natural phenomena known as diarrheic shellfish poisoning (DSP) which is a gastrointestinal disease [3,12,13]. OA and related compounds concentrate in commercially valuable shellfish. In general, mussels containing more than 2 μg OA per gram of hepatopancreas are considered unfit for human consumption [3]. The symptoms of DSP are usually self-limiting, subsiding in 1-3 days without significant medical intervention. Toxicokinetic studies have shown that it is largely distributed in animal body and mainly in intestinal tract [14]. Originally was classified as a potential anti-cancer agent, however it was found to have cancerous tumor promoting activity in the two-stage model of carcinogenesis on mouse skin [15].

*Address correspondence to this author at the Dept. Farmacologia, Fac. Veterinaria, 27002 Lugo, Spain; Tel/Fax: 34 982 252 242; E-mail: ffbotana@lugo.usc.es

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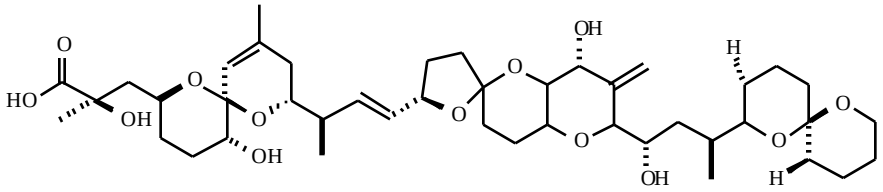

Fig. (1). Structure of okadaic acid [1].

Structure of Okadaic Acid

Complete structure of OA was determined by X-ray crystallography [6]. OA (Fig. (**1**)) is a polyether fatty acid and contains acidic and hydrophobic moieties [4,16]. OA exhibits a cyclic structure, which is maintained *via* an intramolecular hydrogen bond (Fig. (**2**)). This is reminiscent of other macrocyclic protein phosphatase inhibitors [16].

Initial total synthesis of OA by Isobe *et al* was first published in 1986 [6]. Total synthesis offers unique opportunities to tailor the details of the OA architecture with the aim of developing an empirical understanding of the relationship between structure, function and specificity. There are different approaches to the syntheses of OA such as Isobe, Ley and Forsyth synthesis, which can provide useful quantities of OA and its analogs [17-19]. Total syntheses of OA utilized carbon-nucleophilic species on the terminal spiroketal-containing domain to add to electrophilic C27 or C26 termini of the central core domain. Between 8-11 steps were used to advance central and terminal fragments into C15-C38 intermediates functionalized for coupling with the complementary C1-C14 fragments. Attachment of the C1-C14 intermediates to the C15-C38 intermediates was accomplished using aryl sulfonyl stabilized C14 carbanions and C16 aldehydes in the Isobe and Ley syntheses, whereas the Forsyth synthesis matched a C14 aldehyde with a C15 ketophosphonate [17-19]. Common to the Forsyth and Ley syntheses is the use of intact α-hydroxy carboxylate moieties jointly protected as acetals in the C1-C14 intermediates, which precludes the necessity for postcoupling oxidation to install the C1 carboxylate. The Forsyth synthesis uniquely requires a post-coupling formation of the C19 spiroketal, which adds two additional steps to the sequence. Further refinement of the synthetic routes coupled with the identification of structurally simplified phosphatase inhibitors based upon the OA architecture will provide much more practical sources of valuable tools for biochemical research and biomedical development.

INHIBITION OF PROTEIN PHOSPHATASES

In a landmark study, Bialojan and Takai demonstrated that OA acts as a potent inhibitor of the purified catalytic subunits of PP1, PP2A, with an IC50 for PP2A approximately two orders of magnitude lower than that for PP1 [20]. Structural information of the protein phosphatases targets of OA has been acquired, largely through X-ray crystallography (Fig. (**2**)) [16]. Today it is known that OA is a potent, competitive inhibitor of PP1, PP2A, PP4 and PP5, and a weaker inhibitor of PP2B [1,6,21]. Like PP2C, PP7 is not sensitive to inhibition by OA at μM

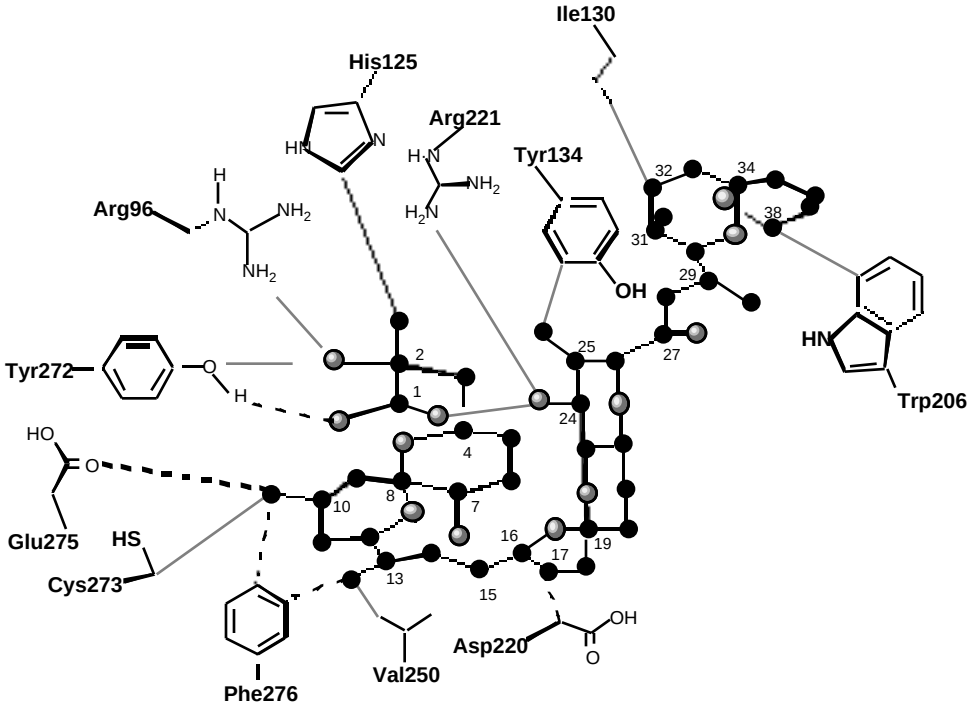

Fig. (2). Important PP1γ-OA interactions implicated by X-ray crystallography [6,16].

concentrations (table **1**) [1,22]. By inhibiting PP this toxin causes increases in phosphorylation of proteins that affect a diverse array of cellular processes. For instance, OA modulates metabolic parameters in intact cells. In this sense it stimulates lipolysis, and inhibits fatty acid synthesis in adipocytes however increases glucose output and gluconeogenesis in hepatocytes. Treatment with µM concentrations of OA, which is sufficient to completely inhibit the activity of PP1-PP5, results in a rapid cell death. At high concentrations cell death has the characteristics of necrosis, while treatment with 20-100 nM OA produces a classic apoptotic response [1].

Table 1. Inhibition of Serine/Threonine Protein Phosphatases Activity by Okadaic Acid (IC50, nM)

PP1	PP2A	PP2B	PP4	PP5	PP7
20-50	0.1-0.3	4 µM	0.1	3.5	> 1µM

Structure-Activity Relationship

A structure function study with OA revealed that modification of the carboxylic acid group of carbon-1 causes significant decreases in the ability of the toxin to inhibit phosphatase activity such that esterification of the carboxyl group (C-1) to produce 1-methyl okadate greatly reduces the inhibitory effect of OA [1,4]. Reduction of OA to produce okadaol, results in a similar decrease in inhibitory activity, and oxidation to produce 1-nor-okadaone completely abolishes the inhibitory effects. Similarly, acetylation of C-2, C-7, C-24 and C-27 producing OA tretraacetate causes severe decreases in toxin effectiveness. In 2001 Maynes *et al*. reported the co-crystal structure of PP1γ-OA [16]. In the PP1γ-OA crystal structure both the protein and the ligand adopt conformations that are nearly identical to their individual solid-state structures (Fig. (**2**)). Even the intramolecular hydrogen bond between the carboxylate and C24 hydroxyl moieties of OA is conserved in the PP1γ-OA structure. The X-ray structure shows that a subset of okadaic acid's extensive functionality is involved in direct protein contacts with PP1γ. These include the C-1 carboxylate (which interacts with Tyr272 of PP1γ and the C24 hydroxyl of OA), C2-hydroxyl (interacts with Arg 96), C2-methyl (His125), C10-methyl (Cys273, Glu275, Phe276) C13-methyl (Phe276, Val250), C17 methylene (Asp220), C24 hydroxyl (Arg221 and the C1-carboxylate of OA), C25 exomethylene (Tyr134), C32 methylene (Ile130) and the terminal C38 methylene (Trp206) (Fig. (**2**)) [6]. The importance of the hydrogen bond between Tyr-272 and the acid group of OA is exemplified by the observation that esterification or removal of the acidic moiety in OA results in elimination of its inhibitory activity and by the fact that mutation of Tyr-272 to Phe results in a 50-fold increase in the *Ki* value. Despite the intimate interaction of the C-2 hydroxyl group of OA with the Arg-96 side chain, removal of the hydroxyl group results in only a 7-fold increase in the *Ki* value. In contrast, mutation of Arg-221 to Ser confers resistance to inhibition by OA underlining the importance of the interaction between the Arg and the C-24 hydroxyl of OA [16]. Structural studies suggest that the hydrophobic domain facilitates the binding of OA to sensitive PPases [4]. Hydrophobic interactions occur between the C-4 to C-16 region of OA and PP1 residues Phe-276 and Val-250 (Fig. (**2**)) [16]. The hydrophobic ring of Phe-276 may inhibit entry of OA into the active site, overshadowing other favorable hydrophobic interactions. A cysteine (Cys-269) is present at the equivalent position in PP2A and may be the reason that PP2A has a higher affinity for OA than does PP1. Hence, hydrophobic-hydrophobic interactions may contribute not only to binding affinity, but also to specificity [6].

OA exhibits different inhibitory potential on the structurally similar PP family members. The structural basis for OA's selectivity for PP2A, PP3, and PP4 over PP1, and the relative inertness of PP2B to OA remain to be definitively determined. The structural similarities at the active sites of each of these enzymes is striking, whereas substantial differences external yet proximal to the active site may be dominant in defining inhibitor sensitivity. These may include the ß12- ß13 loop and the region represented by the hydrophobic surface groove of PP1. Although the catalytic domains of PP1 and the PP2A family share considerable sequence homology, there are important differences in structure between the two enzymes, as manifested in the differential binding of ligands and substrates [23]. The lipophilic side chains lining the hydrophobic surface groove proximal to the active site of PP1 are also largely conserved in PP2A, with the notable exceptions of a pair of cysteine moieties in PP1 (Cys127 and Cys202) that are absent from this region of PP2A. However, many of the acidic residues found on the surface of PP1 are absent from PP2A; Asp197, Glu256, and Glu275 of PP1 are replaced by His, Lys, and Arg, respectively, in PP2A. Also, Glu218, Asp220, Glu252 and Asp277 of PP1 are represented by neutral side chains in PP2A. Therefore, the surface electrostatic potential of PP2A is significantly less negative than that of PP1. Differences in substrate, subunit, and inhibitor recognition by PP2A may be rationalized on the basis of such key variations in surface function and local topology [6,24]. The side chain of Tyr-267 in PP2A should point into solvent, away from the active site, if it is in the same position as the equivalent Gly-274 in PP1, so this change should not be expected to be very significant. However, the allowed torsion angles of a tyrosine would constrain the 12- 13 loop to a larger degree and perhaps the loop is brought into a position to allow for a hydrogen bond to occur between the tyrosine and a hydroxyl group on OA (C-7-OH). The tyrosine could also produce a favorable hydrophobic interaction with the C-10 methyl group of OA. Further interactions in PP2A may result from substitution of Glu-275 in PP1 with Arg-268 in PP2A. Hydrogen bonding interactions may occur between this arginine and the C-7-OH group or hydrophobic interactions with the C-10 methyl group. Tyr272, which interacts directly with OA, has been shown *via* mutagenesis experiments to be important for toxin sensitivity [25]. The enhanced inhibitory potency of OA with PP2A is likely a combination of these effects [16].

The decreased inhibition of PP2B with OA is more difficult to interpret. Major active site differences between the two proteins are the replacement of Ile-133 in PP1 with Tyr-159 in PP2B, Tyr-134 in PP1 with Phe-160 in PP2B, Cys-273 in PP1 with Leu-312 in PP2B, and Phe-276 in

PP1 with Tyr-315 in PP2B. Of these, changing Phe-276 in PP1 to a tyrosine in PP2B may make the greatest impact on OA inhibition. The portion of the inhibitor that resides in this part of the active site is quite hydrophobic, interacting with Phe-276, the methylene carbon of Cys-273, the side chain of Val-250, and the aromatic ring of Tyr-272. The ring of Phe-276 points almost directly at one of the double ring systems of OA (carbons 4-12), and the introduction of a hydroxyl group in PP2B may be enough to disrupt the interaction between OA and the protein, thus reducing the inhibitory potency of OA for PP2B. The resistance of PP2B to OA may therefore arise from the combination of this residue change and subtle structural changes in key OA contact residues in the potential OA binding site [16]. It has also been examined the sensitivity of some of the novel PP members to inhibition by the marine toxin. PP-4 was found to possess toxin sensitivity very similar to that of PP-2A [26]. PP-6 sensitivity to the toxin inhibitors has not been determined [27]. However, based on its similarity to PP-2A it might be predicted that PP-6 will have inhibition characteristics much like PP-2A [4]. PP-5 was shown to be more PP-1-like in its sensitivity to OA [28]. The newly characterized PP-7 is not inhibited by levels of marine toxins that inhibit other PP enzymes. The mechanism for this insensitivity is unknown, but, like PP-2B, interactions within the molecule between the catalytic core and peripheral structures may be responsible.

GLUCOSE REGULATION

Sugar is a fundamental source of energy for all eukaryotic cells [10]. Levels of glucose are regulated to provide sugar to all cells especially to neuronal tissues that require free glucose to function and to avoid hyperglycemia which may lead diseases such as retinopathy.

Effect of Okadaic Acid on Regulation of Glucose Production

Glucose can be produced from three sources: from fat, from proteins *via* gluconeogenesis and from liver and muscle *via* glycogenolysis. Most of the glucose is stored as fat, which represents the major energy reserve in the body. The proteins could be broken down and processed into glucose *via* gluconeogenesis by the liver and kidney. Glucose is also provided by the breakdown of endogenous glycogen stores (Fig. (**3**)) that are primarily in the liver and muscle [10]. During fasting, glycogen phosphorylase initiates hepatic glycogen breakdown by cleaving a single glucose-1-phosphate, which is converted to glucose-6-phosphate. Glucose-6-phosphatase then converts glucose-6-phosphate to free glucose. In glycogen metabolism PP1 plays a key role (see Fig. (**3**)). In this sense Mabrouk *et al* reported that OA, as a PP1 inhibitor, causes a significant increase in glycogenolysis by increasing cytosolic protein phosphorylation [29]. Protein phosphorylation/dephosphorylation play an important functional role in regulation of the stimulus-secretion coupling in the beta cell. For instance, short-treatment of beta cells or permeabilized rat pancreatic islets with OA promotes Ca2+ entry and insulin exocytosis possibly through hyperphosphorylation (and thereby activation) of voltage-activated L-type Ca2+ channels. [30].

Insulin decreases gluconeogenesis by decreasing intracellular cyclic adenosine monophosphate (cAMP) levels, which inhibits phosphorylation of enzymes (Fig. (**3**)). It is well known that after insulin stimulation, insulin receptor substrate-1 and -2 (IRS-1 and IRS-2) are phosphorylated on several tyrosine residues [31-34]. OA pretreatment before insulin stimulation decreased IRS-1 and IRS-2 tyrosine phosphorylation. Among these, the activation of

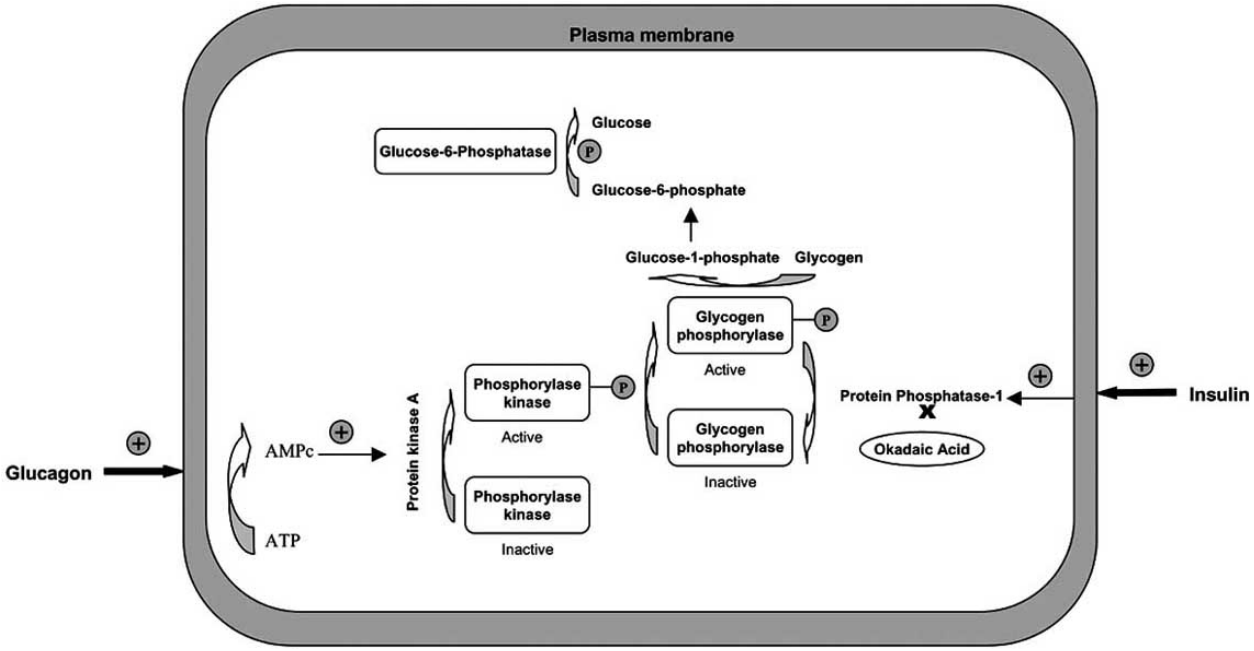

Fig. (3). Glycogen breakdown initiated by binding of glucagons and activation of adenylate cyclase. This launches a cascade of reactions leading to the phosphorylation of glycogen phosphorylase, its activation, and breakdown of glycogen and finally production of glucose. These effects are reversed with insulin. OA inhibits Protein phosphatase-1 and avoids inactivation of glycogen phosphorylase. [71].

phosphatidylinositol 3-kinase (PI 3-kinase) may be essential for insulin-induced Glut4 translocation and glucose uptake. In this sense, PI 3-kinase enzymatic activity was partly reduced in brown adipocytes pretreated with OA upon stimulation with insulin. Downstream of PI 3-kinase, insulin signaling diverges into at least two independent pathways through Akt/PKB and PKC. This was demonstrated by the fact that insulin-induced glucose uptake was totally abolished by the inhibitor in parallel with a total inhibition of insulin-induced PKC activity. However, activation of Akt/PKB by insulin remained unaltered [35]. Tanti *et al* in 1991 found that OA increase the glycolytic pathway with a dose-response relationship [36]. Those authors suggested also that OA might increase glucose metabolism by promoting hexose transport without stimulating any other intracellular step of glucose metabolism.

Stimulation of glycogen synthase by insulin is mediated through the PI-3 kinase pathway by activating PKB and PP-1 but inactivating glycogen synthase kinase-3beta (GSK-3beta) [37]. GSK-3 is probably the major regulatory kinase of glycogen synthase. It phosphorylates the metabolic enzyme at three sites, causing marked inhibition, although phosphorylation by other kinases may also play a role. Therefore, inhibition of GSK-3, together with activation of PP1, may explain the increased synthesis of glycogen from glucose in response to insulin [38]. There are reports that OA abolishes the effect of insulin on glycogen synthase kinase 3 beta (GSK-3beta) and activation of AKT [37], but other studies have shown that OA mimics the effects of insulin by upregulation PI3 kinase associated phosphoproteins [39,40]. Although there are numerous reports of OA being used as a cellular treatment to promote phosphorylation of signaling proteins, many of the published studies showed opposite results about the effect of OA on glucose production and metabolism.

Okadaic Acid and Regulation of Glucose Transport

OA reaches cytotoxic concentrations in the intestinal tissues in accordance with the diarrhea. Recent studies suggested that toxic effects of OA might be related to modification of nutrients, ionic and water absorption across the small intestine presumably by altering the transporter system. Some studies demonstrated that OA increases glucose uptake in many mammalian cells. Robinson *et al*. in 1993 showed a stimulation of glucose transport in isolated rat hemidiaphragms by OA [41]. The uptake of 2-deoxyglucose, an index of glucose transport and phosphorylation, was increased by OA in a concentration-dependent manner in muscles from lean mice [36]. However, glucose uptake induced by insulin is totally abolished by OA in parallel with a total inhibition of insulin-induced protein kinase C (PKC) activity [35].

Uptake of glucose involves: (i) the facilitative glucose transporters (GLUT family) (ii) the sodium-dependent glucose cotransporters (SGLT) [9,10,42,43].

The facilitative glucose transporters can only support the flux of glucose along an existing concentration gradient for the sugar [10]. 12 members of the family of facilitative glucose transporters (GLUT1-GLUT12) are now recognized (table **2**) [43]. Insulin finally acts by stimulating the translocation of GLUT4 containing vesicles from their

Table 2. Facilitative Glucose Transporters

Isoform	Functional characteristics (transport)	Tissue localization	Insulin sensitive
GLUT1	Glucose	Erythrocytes, brain, ubiquitous	No
GLUT2	Glucose; fructose	Liver, pancreas, intestine, kidney	No
GLUT3	Glucose	Brain	No
GLUT4	Glucose	Heart, muscle, white adipose tissue, brown adipose tissue, brain	YES
GLUT5	Fructose; glucose	Intestine, testes, kidney, skeletal muscle, adipose tissue	No
GLUT6	Glucose	Brain, spleen, leucocytes	No
GLUT7	n.d.	n.d.	n.d.
GLUT8	Glucose	Testes, brain, skeletal muscle, adipose tissue	No
GLUT9	n.d.	Liver, kidney	n.d.
GLUT10	Glucose	Liver, pancreas, skeletal muscle	No
GLUT11	Glucose; fructose	Heart, muscle	No
GLUT12	n.d.	Heart, prostate, muscle, small intestine, white adipose tissue	YES

n.d: not determined.

Table 3. Sodium-Glucose Cotransporters

Protein Name	Predominant substrates	Functional characteristics (transport)	Tissue localization	Link to disease
SGLT1	Glucose and Galactose	Cotransporter Na+ Uniporter Na+ Channel: urea and water	Small intestine>>trachea, kidney and heart	Glucose galactose malabsorption
SGLT2	Glucose	Cotransporter Na+	Kidney cortex	Familial renal glycosuria
SGLT3	Na+	Glucose activated Na+ channel	Small intestine, skeletal muscle, kidney, uterus and testis, plasma membranes	n.d.
SGLT4	n.d.	n.d.	Small intestine, kidney, liver, lung and brain	n.d.
SGLT5	n.d.	n.d.	Kidney	n.d.
SGLT6	myo-inositol, glucose and xylose	Cotransporter Na+	Small intestine, brain, kidney, liver, heart and lung	n.d.

n.d: not determined.

intracellular storage sites to the plasma membrane, also augments GLUT4 activity and increases glucose transport [10,43]. There are controversial results of the role of OA on insulin-induced glucose transport in adipose cells [35]. Some studies showed that OA exerts a full insulin-like effect on translocation of GLUT4 proteins to the plasma membrane in rat adipocytes [36,39,44]. OA produced a greater increase than insulin in the rate of glucose transport, and both agents together had a partial additive effect suggesting the presence of different pathways to activate glucose transport and GLUT4 translocation [39]. However, pretreatment of brown adipocytes with OA totally inhibited insulin-induced glucose uptake in a dose-dependent manner.

The sodium-dependent isoforms mediate the transport of glucose against a concentration gradient in mammalian cells but also in invertebrate animals [45]. The driving force is the flux of sodium along an electrochemical gradient that is directed opposite to the transport of glucose [46]. Six proteins cotransporters have been identified (table **3**) [47]. Ishikawa in 1997 indicated that there was an increase in glucose transport in rat small intestine after treatment with 0.1 μM OA that was related with regulation of phosphorylation of SGLT1 [48]. However, the exposition of human lung fibroblasts to OA (0.1 nM-1μM) in a microplate cell assay at different time intervals causes a decrease in intracellular glucose and stimulation of glucose metabolism (40-50 % over controls) [49].

Cotransport of Na^+ and glucose is quantitatively the most important absorptive mechanism in the small intestine, as illustrated by the success of oral rehydration solutions in diarrhea. All the systems that provide a favorable Na^+ gradient will increase glucose transport (Fig. (**4**)). Na^+/K^+-ATPase actively extrudes Na^+ from cells in exchange for K^+ which provides the driving force for secondary transport of glucose. Inhibition of the enzyme by ouabain reduces glucose uptake in rabbit [50]. We have recently observed that protein kinase A PKA positively modulates glucose absorption in rabbit enterocytes (Fig. (**4**)), since glucose uptake was increased after incubation with choleric toxin and the PKA activator dibutyryl cAMP [50,51]. In agreement with that the PKA inhibitor H89 decreased glucose absorption. However, we found that activation of protein kinase C (PKC) by phorbol esters decrease the maximum rate of transport by rabbit (Fig. (**4**)) and rat SGLT1 [50]. We did no found a direct effect of OA on regulation of glucose transport in basal conditions. However this toxin modify the effect of kinases on glucose uptake. When PKA is inhibited, OA increased phosphorylation produced by PKC and consequently the glucose uptake decreased. If PKC is blocked, OA increased glucose uptake induced by PKA (Fig. (**4**)). This coordinated control of phosphorylation by PKC, PKA and phosphatases was already reported in other cells such as endothelial showing the influence of OA on glucose regulation [50,52].

OKADAIC ACID AND HUMAN DISEASE

Deregulation of protein phosphatases has been implicated in insulin resistance, associated with diabetes mellitus and other metabolic disorders. Hence, OA is being employed in basic studies directed towards understanding diverse human diseases such as cancer, AIDS, inflammation, osteoporosis, Alzheimer's, and diabetes [15,53-55].

OA is suspected to participate in causing digestive tumors in humans [56,57]. This toxin had a new pathway of cancerous tumor promotion without direct interaction with the phorbol ester receptor [58] but with activation of protein kinases [59]. It was shown that OA-induced inhibition of protein phosphatases produces activation of protein kinases other than protein kinase C [50] and this fact was also related to Alzheimer's disease [60]. Protein hyperphosphorylation due to inhibition of phosphatases *in vivo* by OA induces neuronal stress and subsequent neurodegeneration [61,62], similar to alterations observed in Alzheimer's disease brain [63,64]. Treatment with OA induced hyperphosphorylation the cytoskeletal protein tau and preferential dendritic damage, with subsequent accumulation of phosphorylated tau in cell bodies and dystrophic axon-like neurites. OA-induced neurodegeneration may provide a useful model to study AD [62,65]. Results obtained in experiments with OA suggest 1) that PP-2A down-regulates extracellular signal-regulated protein kinases

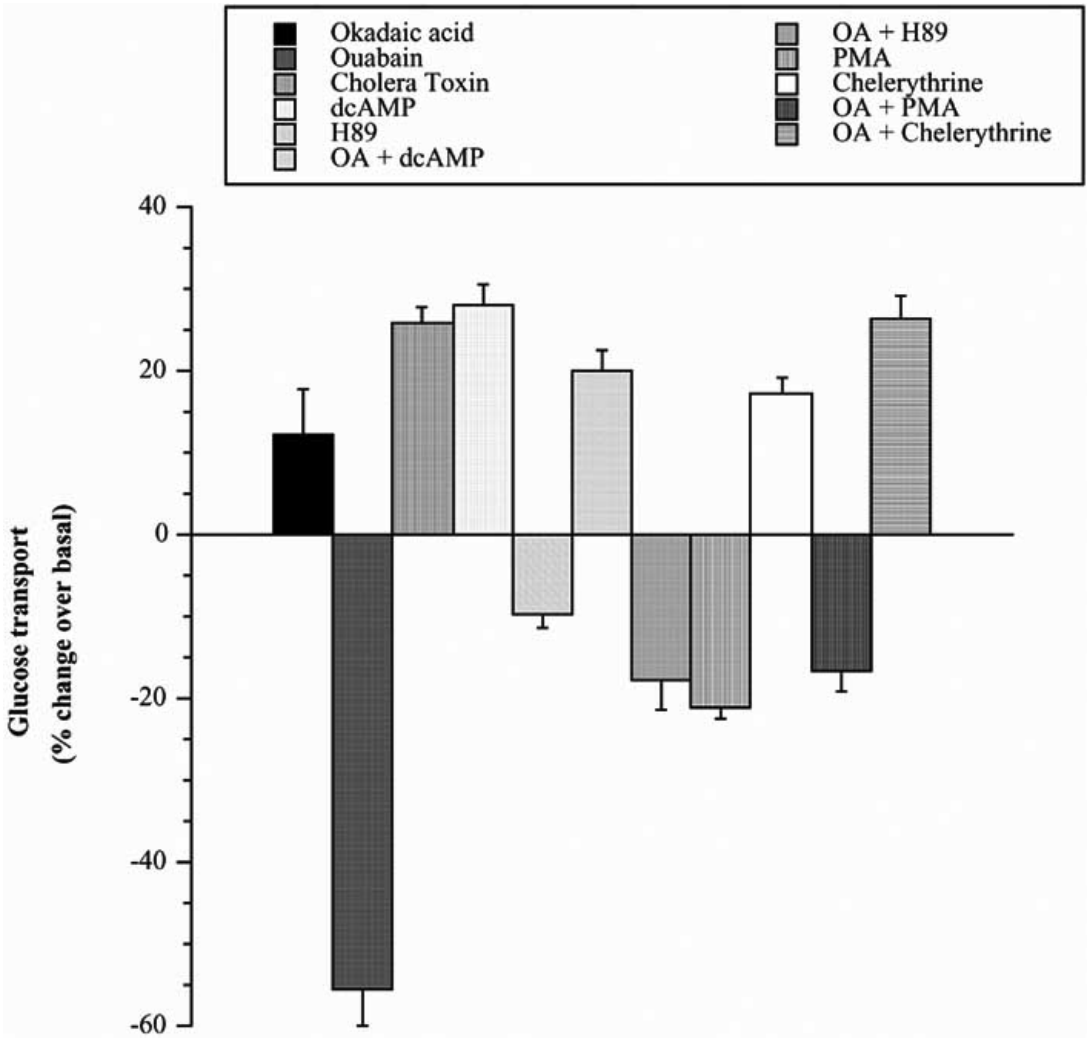

Fig. (4). Effect of OA on glucose uptake regulation in rabbit enterocytes. Freshly isolated rabbit enterocytes were incubated with the fluorescent derivative of D-glucose 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG) in Hank's solution at 37ºC. Fluorescence intensity was determined by fluorimetry. Enterocytes were preincubated for 10 min with 0.1 mM ouabain (Na^+-K^+ ATPase inhibitor) or 1 µM okadaic acid (OA), cholera toxin (100 ng/ml) was preincubated for 90 min. Experiments in the presence of a selective activator of PKA (400 µM dibutyryl cAMP) or inhibitor (100 µM H89) have enabled to study the effect of PKA on 2-NBDG absorption. The effect of OA on modulation of glucose uptake by PKA in rabbit enterocytes, was analyzed by giving the toxin before administration of dibutyryl cAMP or H89. The influence of OA on 2-NBDG absorption induced by 300 ng/ml PMA (PKC activator) and 1 µM chelerythrine (PKC inhibitor) was also registered. In all cases the effect of drugs on 2-NBDG absorption was evaluated from the initial fluorescence read and expressed in percentage of change in glucose transport over basal. Values are means ± SE from n=5.

(ERKs) activities through dephosphorylation at the serine/threonine residues of these kinases, and 2) that in AD brain the decrease in PP-2A activity could have caused the activation of ERKs and the abnormal hyperphosphorylation of tau both *via* an increase in its phosphorylation and a decrease in its dephosphorylation [60]. On the other hand exposure of cells to 50 nM OA for 2 h induced a reduction in cellular glutathione transferase, glutathione reductase and catalase activity. These results indicate that OA induces an oxidative stress imbalance very important in the development and course of neurodegenerative disease, especially Alzheimer's disease [66].

Regulation of kinases and phosphatases in early gene activation in monocytes it was recently investigated because these cells are implicated in the pathogenesis of acute inflammatory states, such as sepsis and acute lung injury. OA as an inhibitor of PP2A could be a regulator of activating protein (AP) family that can drive cytokine expression, which is a valid therapeutic target in the setting of acute inflammation [67].

1 nM OA elicited an increase in rate of O2 consumption in the porcine carotid artery similar to that by cerebrospinal fluid (CSF) from vasospastic patients. It was also observed that phosphatase inhibition with 1 nM OA significantly slowed relaxation after a stretch. Those results pointed out that the inhibition of smooth muscle phosphatase may be involved in the mechanisms associated with cerebral vasospasm after subarachnoid hemorrhage.[68].

Insulin promotes glucose uptake into muscle and adipose tissues through translocation of the Glut4 glucose transporter

from an intracellular pool to the plasma membrane. An impairment of the ability of insulin to stimulate glucose uptake in muscle and adipose tissues contributes to the development of type 2 diabetes, hypertension and cardiovascular disease. The ability of insulin to increase glucose transport and activate protein kinase B is reduced in fat cells from Type II diabetic subjects. Protein kinase B can be activated by OA, which bypass the upstream defects in the insulin signaling pathway in Type II diabetic cells and, thus, increase glucose uptake [69,70]. However, the biomedical potential of OA is limited by factors such as OA lacks sufficient specificity in its inhibition of a particular OA-sensitive PPase. Hence, may simultaneously affect a variety of important cellular processes, in addition to the targeted ones.

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