Buenos Aires, August 28th, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10998-review.doc).

Title: Renal dopaminergic system: pathophysiological implications and clinical perspectives

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Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 10998

The manuscript has been improved according to the suggestions of reviewers:
We would like to thank to the reviewers for their thoughts about our review and for their useful comments to improve the quality of the manuscript.

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer #1:

(1) Abstract lines 9-11. Please revise this sentence as it is confusing. Does the author means it is a potential therapeutic target?

Accordingly to the reviewer´s suggestion, we modified the sentence as follow:

"Given its properties on the regulation of renal blood flow and sodium excretion, exogenous dopamine has been postulated as a potential therapeutic strategy to prevent renal failure in critically ill patients."

(2) Abstract lines 11-16. Please revise this sentence. It is too long and confusing for general audience

In order to clarify the sentence for general audience, we replaced the sentence as follow:

"The aim of this review is to update and discuss on the most recent findings about renal dopaminergic system and its role in several diseases involving the kidneys and the potential use of dopamine as a nephroprotective agent."

(3) There are few grammatical errors such as please remove "the" at the start of the introduction. Please check the manuscript for similar errors.

We proceeded according to the reviewer´s request. The article "The" at the start of the introduction and at the beginning of other sentences were deleted throughout the review. We also have checked the spelling and grammar of the entire manuscript. Also, the revised version of the manuscript was reviewed by an independent colleague (Dr. Della Penna) in order to improve the language.

(4) Influence of dopamine on the glomerular podocytes has also been studied but authors have not mentioned this in the review. There should be some mention about this in the review.

We agree with the reviewer´s comment. Evidence about dopamine effects on glomerular podocytes has been included in the review (see section Introduction, page 5, lines 15 to 20 of the revised manuscript):

"At glomerular level, dopamine increases cAMP in messangial and podocyte cells via D1-like

receptor and inhibits angiotensin II-mediated contraction in mesangial cells[24,25]. Through this mechanism, dopamine induces depolarization of the podocyte that may lead to its relaxation[26]. These data suggest that dopamine can augment natriuresis and diuresis by increasing directly water and sodium filtration at glomerular level."

Reviewer #2:

(1) Manuscript is poorly written and unclear in its current form.

As we have previously indicated for comment #3 of reviewer #1, we have checked the spelling and grammar of the entire manuscript. Also, the revised version of the manuscript was reviewed by an independent colleague (Dr. Della Penna) in order to improve the language.

(2) The sections and headings appear forced without segues between them.

A brief paragraph was introduced between consecutive sections in order to provide a link (segue) between them.

After "Effects of intrarenal dopamine on oxidative stress and renal inflammation" section and before "Role of renal dopaminergic system in the pathophysiology of hypertension":

"Although these evidence indicate that alteration of the renal dopaminergic system may be associated with increased blood pressure via oxidative stress, this seems not to be the only mechanism by which impairment of renal dopaminergic system could lead to hypertension."

After "Role of renal dopaminergic system in the pathophysiology of hypertension" and before "Renal dopamine, hyperinsulinemia and pathophysiology of diabetic nephropathy":

"Given its participation on sodium and water excretion and blood pressure regulation as well as its antioxidant properties in the kidney, alteration in renal dopaminergic system should also be considered in the pathophysiology of other diseases associated with kidney damage such as diabetic nephropathy."

After "Renal dopamine, hyperinsulinemia and pathophysiology of diabetic nephropathy" and before "Dopamine as nephroprotective agent? Experimental and clinical evidences in renal disfunction":

"Despite its role in the pathophysiology of diabetic nephropathy, the potential clinical use of dopamine in this context is still matter of basic research. Nonetheless, the therapeutic use of dopamine is restricted to its dose-dependent actions on the cardiovascular system."

After "Dopamine as nephroprotective agent? Experimental and clinical evidences in renal disfunction" and before "Renal dopamine in the pathogenesis of edema formation":

"As it has been demonstrated in patients with heart failure, the interaction between two natriuretic hormones, such as ANP and dopamine, can also be present in other situations with increased extracellular fluid, such as nephrotic syndrome."

(3) There should be an overall message and more emphasis on possible clinical implications of the renal dopaminergic system. The introduction does a poor job introducing the readership to the topic.

In order to improve the introduction to the readership, we have shortened the physiology of renal dopamine to give an overall message and more emphasis on clinical aspects of renal dopaminergic system. To please comment #4 of reviewer #1 we have also introduced in this section, some mention about the effects of dopamine on the glomerular podocytes:

"Kidney has all the bioenzymatic machinery necessary to possess a local dopaminergic system. Renal dopamine production depends on the precursor L-dihydroxyphenylalanine (L-Dopa) and dopa decarboxylase activity. Although dopa decarboxylase is present in high concentrations in the proximal tubular cells, L-dopa uptake by sodium dependent and independent transporters represents the limiting step for intrarenal dopamine synthesis[1-4]. Intrarenal dopamine can leave the cell through the apical

border by a diffusional process, whereas plasma dopamine can be uptaken through the basal cell border by a saturable process[5]. The organic cationic transporters (OCTs and OCTNs) have been postulated as potential carriers for dopamine through the tubular cells[5-7]. Finally, dopamine can be eliminated with urine flow or degraded by methylation (via catechol-O-methyl transferase or COMT) to 3-methoxytyramine, and by deamination (via monoamine oxidase or MAO) to 3,4-dihydroxyphenylacetic acid[9].

Several organs and systems are involved in the regulation of blood pressure. In particular, the kidney plays an essential role in the etiology of hypertension, but also represents a target organ vulnerable to hypertensive tissue damage. Alterations in renal tubule transport may be linked to the onset of hypertension in which dopamine could play an important role by affecting sodium handling on the proximal tubule[10]. Dopamine, as a major regulator of proximal tubule salt and water reabsorption, exerts its physiological actions through two families of receptors located in the tubular cell surface: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3 and D4)[11-14]. Through activation of D1-like receptors, locally produced dopamine acts as an autocrine/paracrine natriuretic hormone by inhibiting the activity of both apical (e.g., Na^+/H^+ exchange, Cl^-/HCO_3^- exchange and Na^+/Pi cotransport) and basolateral (e.g., Na^+, K^+-ATPase and Na^+/HCO_3^- cotransport) transporters[15-17]. The D1-like receptors, coupled to the stimulatory G proteins Gαs and Golf, are characterized by their capacity to activate adenylate cyclase, while D2-like receptors, coupled to the inhibitory G proteins Gαi and Go, are characterized by their capacity to inhibit adenylate cyclase and modulate ion channels[18,19]. The classical signaling pathway for D1-like receptors leads to activation of adenylate cyclase and increases cyclic adenosine 3′,5′-monophosphate (cAMP) levels and protein kinase A (PKA) activation. PKA may either directly phosphorylate a target protein, such as a sodium transporting protein, or initiate a cascade of phosphorylation events by phosphorylation and activation of dopamine and cAMP-regulated phosphoprotein DARPP32[20]. D1 receptor can also stimulate phospholipase Cβ1 in renal tubules [21]. On the other hand, D2-like receptors can suppress Akt (protein kinase B) signaling pathway[22]. Both types of dopamine receptors are also linked to mitogen-activated protein kinase (MAPK) activation through different pathways and can interact with each other, resulting in new signaling pathways. In renal cortical cells the interaction between D1 and D2 receptors increases phospholipase C (PLC) stimulation[23].

At glomerular level, dopamine increases cAMP in messangial and podocyte cells via D1-like receptor and inhibits angiotensin II-mediated contraction in mesangial cells[24,25]. Through this mechanism, dopamine induces depolarization of the podocyte that may lead to its relaxation[26]. These data suggest that dopamine can augment natriuresis and diuresis by increasing directly water and sodium filtration at glomerular level. Besides these effects on sodium and water homeostasis, it has been demonstrated that dopamine could exert anti-inflammatory and anti-oxidants properties by activation of D1-like and D2-like receptors[27-30].

To date, several studies reported that an intact dopaminergic system is required to maintain renal hemodynamic, fluid and electrolyte balance, redox steady state and blood pressure within a normal range and to antagonize the renin-angiotensin system[31,32]. In this way, alterations in dopamine production and its receptor number, function and/or post-translational modification are associated with different pathological scenarios like oxidative stress, genesis and progression of renal dysfunction, edema formation and genetic or essential hypertension. In clinical practice, dopamine is used as a first line vasoactive agent in patients with hemodynamic instability unresponsive to fluid therapy[33]. However, despite its diuretic and natriuretic properties, its clinical use in patients with renal failure remains controversial."

(4) The two figures included in the manuscript are both well designed and helpful, but I would like to see more tables and figures in such a long review paper.

We thank the reviewer for the compliment. In agreement with the suggestion, we designed new tables and figures to improve the quality of the review. See tables 1 and 2, and figures 3, 4 and 5.

(5) *Specific comments: Abstract: as a 'therapeutic strategy' rather than 'therapeutic strategic'. The aim of this review is to update and comment _on_ the most recent. Usually 'consensus of' and not 'consensus about'.*

In addition to the comments #1 and #2 of reviewer #1, grammatical mistakes from the abstract section were corrected and replaced as follow:

"Given its properties on the regulation of renal blood flow and sodium excretion, exogenous dopamine has been postulated as a potential therapeutic strategy to prevent renal failure in critically ill patients. The aim of this review is to update and discuss on the most recent findings about renal dopaminergic system and its role in several diseases involving the kidneys and the potential use of dopamine as a nephroprotective agent."

(6) Core tip: perhaps remove 'levels' and rather state maintain the normal balance of sodium and water, blood pressure and renal redox steady state. Last sentence is unnecessary clunky - 'open the possibility to consider it as a potential'. Consider rewriting.

We proceed to remove "levels" as suggested by the reviewer and last sentence was re-written as follows:

"Recent findings from experimental and clinical studies allow us to understand the complexity of this system as well as its possible contribution for future therapeutic strategies to prevent renal diseases."

(7) Introduction: I do not like the opening sentence. I would go into less details in the introduction paragraph and rather focus on the clinical importance of the renal dopaminergic system. The authors should also address a rationale for the review in the introduction paragraphs. Moreover, there needs to be a better segue between the introduction and subsequent sections.

These comments were answered in items #2 and #3.

(8) Intrarenal dopamine against oxidative stress and inflammation: The heading is not very clear. Nice figure.

We thank the reviewer for the compliment. The heading was removed and replace by "Effects of intrarenal dopamine on oxidative stress and renal inflammation"

(9) Rol of renal dopaminergic system in the pathophysiology of hypertension: Typographic error in heading - should be 'role' not 'rol'. Again - very nice figure.

We thank the reviewer for the compliment. The typo was corrected.

(10) Renal dopamine, hyperinsulinemia and pathophysiology of diabetic nephropathy: Adults with type 1 and 2 diabetes demonstrate insulin resistance, and the reduced insulin sensitivity is associated with diabetic nephropathy (Bjornstad et al. Diabetes Care 2013). It is plausible that the regulatory mechanisms of the renal dopaminergic system are impaired in diabetic nephropathy due to insulin resistance? I would consider making the second paragraph the first paragraph of this section.

Based on the reviewer´s comment, we added the following sentence (see page 16, lines 6 to 9 and page 18, lines 7 to 9):

"Diabetic nephropathy is a major cause of mortality in both types diabetes. Adults with type 1 and 2 diabetes demonstrate insulin resistance, which is associated with diabetic nephropathy[105,106]."

"Based on these evidences, it is possible that the regulatory mechanisms of the renal dopaminergic system are impaired in diabetic nephropathy due to insulin resistance."

As the reviewer required, the second paragraph was placed before the first paragraph.

(11) Dopamine as nephroprotective agent? Experimental and clinical evidences in renal disfunction: Another typo - should be dysfunction and not disfunction. Please provide references to the first sentence / statement. I would also specify whether this is in pediatric or adult populations, as dopamine is used less frequently in pediatrics.

The typo was saved and the first sentence was provided with a reference (#33). We also specify in the text whether it is an adult or pediatric population according to the literature reference.

(12) Renal dopamine in the pathogenesis of edema formation: Please provide segues between the different sections. In its current form the manuscript is difficult to read.

This comment was answered in item #2.

(13) Future perspectives: I would provide some speculations on plausible triggers leading to defects in the renal dopaminergic system. This would be useful for the readership.

In addition with the comment #2 of reviewer #3 (see below), we replaced the text of section "Future perspectives" to improve the presentation and readability as follows (see page 29, lines 16 to 19):

"Some of these trigger factors could be an excess of sodium intake that could lead to an activation of intrarenal angiotensin II and increase in ROS, an increase in carbohydrate intake and a high fat diet, both factors that promotes an insulin resistance state."

(14) Conclusions: The last sentence is poorly written.

The sentence was removed and replaced by the following:

"The comprehension of the mechanisms by which renal dopaminergic system is involved in the pathogenesis and development of renal diseases may contribute to improve the diagnosis, evolution, prognosis and treatment of renal pathologies."

Reviewer #3:

(1) Although generally well written, there are a number of issues that the authors should consider to improve the overall readability of the review. Major comments - The authors should considerably shorten the text under the headings "Role of renal dopaminergic system in the pathophysiology of hypertension", "Renal dopamine, hyperinsulinemia and pathophysiology of diabetic nephropathy", "Dopamine as nephroprotective agent? Experimental and clinical evidences in renal disfunction", and "Renal dopamine in the pathogenesis of edema formation". A large part of data might be provided in tables.

We have tried to improve the manuscript according to the comments and requests of the three reviewers. Although at some points it's hard to please everyone, because whilst one reviewer recommends shortening the text, the same and/or another reviewer suggest the addition of new data and paragraphs. However, we proceed as requested by the reviewer and large part of data plus additional new data were placed in new tables to provide a shorten version (see tables 1 and 2).

(2) The authors should improve the presentation and the readability of the text under the heading "Future perspectives".

As we have previously indicated for comment #13 of reviewer #2, we replaced the text of section "Future perspectives" to improve the presentation and readability as follows:

"An intact renal dopaminergic tonus is required for the maintenance of sodium homeostasis and

normal blood pressure. By its anti-oxidative and anti-inflammatory properties, intrarenal dopamine plays a major role as a nephroprotective agent to prevent or ameliorate renal dysfunction. Oxidative stress or hyperinsulinemic states may decrease the number of functional dopaminergic receptors in the proximal tubules. In this way, it is worthwhile to test the effect of antioxidant drugs to enhance or restore the biodisponibility of these receptors. A recent observation that dopamine receptors availability in the plasma membrane may be regulated by other hormones, like ANP, could open up a possible therapeutic approach[194].

It has been emphasized the importance of endogenous dopamine and renal D1 receptor on the regulation of sodium and body fluid homeostasis. Although there is evidence that a defective renal dopaminergic system contributes to the development and maintenance of hypertension, it is still not clear what triggering factor causes the selective defect in the renal dopaminergic system. Some of these trigger factors could be an excess of sodium intake that could lead to an activation of intrarenal angiotensin II and increase in ROS, an increase in carbohydrate intake and a high fat diet, both factors that promotes a reduced insulin sensitivity state. Furthermore, the renal dopaminergic system is sensitized by a high salt intake and volume expansion, which opens the question about how intrarenal sodium sensors may influence on renal dopamine bioavailability. This approach may lead to the development of new pharmacological strategies in conditions of salt retention and hypertension. Moreover, identification of abnormalities in different steps of crucial importance for the regulation of the renal dopaminergic tonus should provide additional molecular biological tools for the early diagnosis and treatment of pre-hypertensive patients."

(3) In the conclusion, the authors could specify the "new strategies to improve the clinical management of these pathologies".

In accordance with the comment #14 of reviewer #1, the sentence was re-written as follows:

"The comprehension of the mechanisms by which renal dopaminergic system is involved in the pathogenesis and development of renal diseases may contribute to improve the diagnosis, evolution, prognosis and treatment of renal pathologies."

(4) The authors should address the role of lipid mediators (prostanoids, leukotrienes, epoxyeicosatrienoic acids, and hydroxy- and dihydroxyeicosatrienoic acids) in the mediation of dopamine's effects in the kidney. The interactions between dopamine 3 receptor and endothelin B receptor in the kidney could be mentioned. - The authors could mention general principles of renal dopamine receptor signal transduction and regulation (for example, adenylate cyclase/cAMP/protein kinase A; mitogen activated protein kinases; ion channels; protein kinase C; and phospholipases).

We proceed to mention in the text, section "Role of renal dopaminergic system in the pathophysiology of hypertension" the following sentences related to the role of lipid mediators and endothelin B (see page 11, lines 9 to 34):

"Another possible mechanism involved in the impaired natriuretic effect in SHRs could be related to impaired endothelin B and D3 receptor interaction[91]. The endothelin B and dopamine receptors can interact to regulate renal function and blood pressure[92]. It has been demonstrated that activation of renal D3 receptor induces natriuresis and diuresis, but this effect is reduced in the presence of an endothelin B receptor antagonist, demonstrating that dopamine effects depends partially on endothelin B receptors. Moreover, stimulation of endothelin B receptor increases D3 receptor protein expression and viceversa in renal proximal tubule from wistar Kyoto rats but not from SHRs[91,93]. Another study indicates that D3 receptors physically interact with proximal tubule endothelin B receptors and that the blunted natriuretic effect of dopamine in SHRs may be explained, in

part, by abnormal D3/endothelin B receptor heterodimerization[92].

The interaction between prostanoids and renal dopamine on sodium and water excretion must also be considered. It has been demonstrated that the natriuretic response to dopamine was lower in Dahl salt-sensitive rats but this effect was reversed when chromosome 5 was transfer into these rats, leading to an increase of the renal expression of CYP4A protein and the production of 20-HETE[94]. Moreover, the inhibition of Na^+, K^+-ATPase activity by dopamine in the proximal tubule may be the result of the synergism between 20-HETE and the D1 signaling pathway[95]. In addition, other metabolites of arachidonic acid produced in the proximal tubule are epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids. As 20-HETE, epoxyeicosatrienoic acids can also regulate Na^+, K^+-ATPase activity and serve as second messengers for the natriuretic effects of dopamine. Since renal production of cytochrome P450 metabolites of arachidonic acid is altered in hypertension, a lower prostanoid synthesis may be involved in the impaired response to dopamine in this context[96]."

We also proceed to mention in the text, section "Introduction" the following sentence related to general principles of renal dopamine receptor signal transduction and regulation (see page 4, lines 27 to 28 and page 5, lines 1 to 14):

"The D1-like receptors, coupled to the stimulatory G proteins Gαs and Golf, are characterized by their capacity to activate adenylate cyclase, while D2-like receptors, coupled to the inhibitory G proteins Gαi and Go, are characterized by their capacity to inhibit adenylate cyclase and modulate ion channels[18,19]. The classical signaling pathway for D1-like receptors leads to activation of adenylate cyclase and increases cyclic adenosine 3′,5′-monophosphate (cAMP) levels and protein kinase A (PKA) activation. PKA may either directly phosphorylate a target protein, such as a sodium transporting protein, or initiate a cascade of phosphorylation events by phosphorylation and activation of dopamine and cAMP-regulated phosphoprotein DARPP32[20]. D1 receptor can also stimulate phospholipase Cβ1 in renal tubules [21]. On the other hand, D2-like receptors can suppress Akt (protein kinase B) signaling pathway[22]. Both types of dopamine receptors are also linked to mitogen-activated protein kinase (MAPK) activation through different pathways and can interact with each other, resulting in new signaling pathways. In renal cortical cells the interaction between D1 and D2 receptors increases phospholipase C (PLC) stimulation[23]."

(5) Minor comments - The authors should explain all abbreviations. - Spelling in the text: p.11 Chun G, and p.18 Marik PE (G and PE are unnecessary); p.13: ciclooxigenase; p. 17: naranja. - The authors should check the spelling in the references 19, 22, and 94.

As a consequence of other comments and suggestions made by the reviewer #2 and#3, references from Chun and Marik were introduced in tables 1 and 2 respectively.

Ciclooxigenase was corrected by cyclooxygenase.

"naranja" was replaced by its corresponding reference (#33).

References #19, #22 and #94 were corrected.

We like to thank once again to the reviewers for comments and suggestions in order to improve our review.

Thank you again for considering our manuscript to be published in the *World Journal of Nephrology*.

Sincerely yours,

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