Wnt/β-Catenin Pathway on Cocaine-Induced Neuroadaptations: A Novel Target for Therapeutic Opportunities?

Santiago Cuesta1,*, Alejandro M. Pacchioni1

1 Área Toxicología, Departamento de Ciencias de los Alimentos y del Medioambiente, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario (U.N.R), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
2 Douglas Mental Health University Institute, Montreal, Quebec, Canada.

Copyright: © 2016 Alejandra M. Pacchioni. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Drug addiction is a chronic and enduring phenomenon that has been extensively investigated in the last decades. Over the years, different animal models have been designed to contribute to the elucidation of the neurobiological processes involved in relapse behavior and to evaluate potential pharmacotherapies that may prevent or reduce the risk of relapse [1]. Animal models such as behavioral sensitization, conditioned place preference and intravenous self-administration have been extensively used. The primary difference in these models is the way in which the drug is administered; while the importance of the impact of non-contingent vs contingent drug administration for the molecular basis of addiction is a subject of continuous debate. However, recently it has been described that animal models of addiction (behavioral sensitization vs self-administration) remarkably overlap in terms of neurocircuitry as well as in the molecular changes underlying their respective behavioral responses [2].

Behavioral sensitization is a progressive and enduring enhancement of the motor stimulant effects elicited by repeated administration of psychostimulants [3]. The development of sensitization can be examined as two distinct temporal and anatomical domains termed initiation or induction, and expression. Each one is characterized by specific molecular and neurochemical changes. It has been shown that initiation is associated with changes in the ventral tegmental area (VTA) and in the prefrontal cortex (PFC) [4-7], while expression is linked to changes in the nucleus accumbens (NAcc) [8,9]. A great deal of evidence shows that changes in synaptic plasticity underlie both initiation and expression of behavioral sensitization [2,10,11]. The initiation of sensitization has been shown to be disrupted by ibotenic acid lesions in both prelimbic and infralimbic regions of the PFC [7]. Moreover, several studies have suggested that cocaine induces a functional decrease of the D2R in the PFC that would serve to enhance excitatory transmission to subcortical regions [12-15]. The long-term changes, cocaine induces a decrease in basal glutamate levels in the NAcc that increase after a cocaine challenge [8,16]. These higher levels of glutamate will act on the AMPA receptors (AMPAR) promoting higher behavioral responses [8,17]. Moreover, withdrawal from drug exposure induces changes in the NAcc neurons which can be temporarily reversed by re-exposure to the drug. For instance, during cocaine withdrawal there is an increase in synaptic strength of AMPAR relative to NMDAR-mediated currents given by an increase in the surface expression of AMPAR [18-20], as well as changes in dendritic spine density [21-24]. While the mechanisms underlying these structural modifications are not completely clear, the regulation of the actin cytoskeleton, [25,26] together with the activity of small GTPases and the induction of different genes and their targets (e.g. ΔFosB, NFKB, Cdk5-MEF2, etc) [23] would be involved. Altogether, this evidence indicates that cocaine-induced sensitization is the result of an interaction between dopaminergic and glutamatergic neurotransmission (Figure 1A).

We used the behavioral sensitization paradigm to model addiction-like behavioral responses in order to investigate the role of Wnt (Wingless-related integration site) factors pathways. Wnt factors signal in axon pathfinding, dendritic development, and synapse assembly in both the central and peripheral nervous systems. Wnts also modulate the basal synaptic transmission, and the structural and functional plasticity of synapses in the central nervous system [27]. The Wnt growth factors belong to a large family of secreted proteins that can signal through different receptors including Frizzled (Fz) [28] and the atypical tyrosine kinase receptors Ror2 and Ryk [29,30]. The interaction between Wnt and Fz leads to the phosphorylation of Dishevelled (Dvl, first intracellular effector). Downstream of Dvl, the Wnt pathways diverge into three branches: the canonical or Wnt/β-catenin, the planar cell polarity and the Wnt/calcium pathways [31]. The activation of the canonical pathway results in the phosphorylation of GSK3β (Glycogen synthase kinase 3β) leading to β-catenin stabilization and subsequent entrance to the nucleus where it promotes gene expression [28,32]. While in the absence of Wnt, GSK3β phosphorylates β-catenin marking it for degradation by the proteasome [33]. Wnt signaling is also regulated by the presence of a physiological antagonist: Dickkopf-1 (Dkk-1), a secreted protein that specifically blocks the canonical Wnt pathway by binding to LRP6 [34].

In the past decade, mounting evidence has suggested a link between dysfunction of Wnt signaling and neurological disorders such as Alzheimer’s disease, bipolar disorder and schizophrenia [35,36]. For instance, Alimhamad et al [37] showed that amphetamine increases GSK3β activity and decreases β-catenin levels in the PFC and in the striatum, while D R antagonists produce the opposite effect. Despite the relevance to cocaine effects of dopamine and its neurotransmission (Figure 1A).

*Corresponding authors: Alejandra M. Pacchioni, Área Toxicología, Departamento de Ciencias de los Alimentos y del Medioambiente, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario. Suipacha 531, (2000) Rosario, Santa Fe, Argentina, Tel: 54-341-4804602; Fax: 54-341-4804598; Email: pacchioni.alejandra@conicet.gov.ar

Received: December 08, 2016; Accepted: December 26, 2016; Published: December 30, 2016
While no changes were found in the NAcc, when compared to saline treated animals, revealed that the animals that received chronic cocaine and developed sensitization, proposing a new role for this pathway.

When we focused on the expression of behavioral sensitization, we found that chronic cocaine induced an increase on β-catenin levels in the NAcc (both in total homogenates and in the nuclear fraction), a decrease in the CPu and no changes in the PFC when compared to saline treated animals. Once again, all these changes were only present in sensitized animals and entailed a cocaine-induced increase in the activity of the Wnt/β-catenin pathway in the NAcc.

Finally, we studied the importance of the changes of β-catenin levels induced by cocaine during development in the long-term neuroadaptations that cause the expression of sensitization. In other words, we evaluated the long-term consequences that the pharmacological prevention of β-catenin reduction has during the cocaine treatment. We found that not only the expression of sensitization was blocked 3 weeks after LiCl treatment, but also that the protein levels were modified. Specifically, we found that the LiCl pretreatment by itself induced an increase in β-catenin levels in the NAcc, regardless of the presence of cocaine. Therefore, there was no change on the activity of the pathway after the cocaine injection. These results suggest that it is not the actual level of β-catenin but the variation of the pathway activity what impact in the behavioral response, highlighting an important role of the pathway in the cocaine-induced long term neuroadaptations (i.e. expression of behavioral sensitization).

Taken together, these results proposed for the first time that changes in the Wnt/β-catenin pathway effectors are involved in short and long-term neuroadaptations required for cocaine-induced behavioral sensitization. In the past decade, a relationship between dopamine neurotransmission and intracellular effectors of the Wnt/β-catenin pathway has been shown [37,45-47]. Taking into account the mounting evidence about changes in dopamine and glutamate neurotransmission that underlies cocaine-induced short and long-term neuroadaptations [2,23,48], it is possible that cocaine-induced changes in Wnt/β-catenin pathway activity are linked to them. For instance, it has been shown that cocaine induces a functional decrease of D1 receptors in the PFC that would serve to enhance excitatory transmission to subcortical regions [12-15]. Therefore, it is possible that cocaine-induced inhibition in the Wnt/β-catenin pathway is related to a functional decrease in dopamine neurotransmission. In fact, Galli et al [49] have recently demonstrated that inducible expression of Dkk-1, a physiological inhibitor of the Wnt/β-catenin pathway [34], in adult mice striatum decreases D1R and D2R clusters, leading to deficits...
in dopaminergic transmission. Hence, another possibility that needs to be tested is whether chronic cocaine decreases Wnt synthesis or increases Dkk-1 levels. However, the fact that we found significantly lower levels of Wnt7b mRNA in the PFC points out to a decrease in Wnt synthesis. Interestingly, Wnt7-Dvl signaling has been associated to presynaptic assembly and neurotransmitter release [50]. Moreover, we showed that inhibition of the Wnt canonical pathway at the level of Dvl in the PFC exacerbates initiation of cocaine-induced sensitization. We therefore hypothesized that the inhibition in the Wnt/β-catenin pathway observed in the PFC of sensitized animals may have been associated with a functional decrease of Dvl leading to a disconnection of D R. However, when we evaluated β-catenin levels in the PFC after a cocaine challenge on day 28, we found similar levels compared to controls regardless of the behavioral measurement. Interestingly, when we measured levels of β-catenin before the challenge and after a period of abstinence, we found an increase when compared to the control group. In other words, after the abstinence, a cocaine challenge reduced β-catenin to the control level, despite the behavioral outcome (sensitized or non-sensitized). It is possible that D R are involved in this mechanism as well as during development, but more work needs to be done to establish the relevance of these changes in cocaine-induced long-term neuroadaptations.

As regards the long-term changes induced by cocaine in the Wnt/β-catenin pathway, it seems that there are two main modifications in the neurotransmission that could be associated with them: on the one side, the dopaminergic changes on receptor sensitivity and dopamine release in the NAcc [51-54], and on the other, the glutamatergic changes in this same area. In the case of the dopaminergic transmission, it is likely that the increased activation of the D R in the NAcc induced by cocaine causes the accumulation of β-catenin through inhibition of GSK3β [55]. This change could, in turn, influence the glutamate transmission. After 3 weeks of withdrawal from repeated cocaine, the surface expression of AMPAR is increased in the NAcc, while a cocaine challenge leads to a decrease in behavioral sensitized animals after the challenge [9,11,18]. Recently, it has been shown that over-expression of β-catenin in hippocampal cell cultures mimics the effect of increased neuronal activity increasing the total dendritic length and decreasing the density of surface synaptic AMPAR clusters [47]. Then, it is possible that the cocaine-induced increase in β-catenin levels mediated by dopamine in the NAcc activates the pathway as well as facilitates the removal of AMPAR from the surface after the cocaine challenge, giving rise to the expression of behavioral sensitization. On a side note, we emphasized on the long-term effect in this area because our results did not show an immediate influence on the pathway in the NAcc. However, it is possible that the fact that we did not sample the NAccs in core and shell could hide small changes.

Interestingly in the CPu, we found that both development and expression of sensitization are associated with a decrease of β-catenin levels in those animals that showed behavioral sensitization after a challenge, while it was significantly increased after 3 weeks of abstinence, similar to what happened in the PFC. Taken together, these results suggest that behavioral sensitization requires a reduction in β-catenin, below basal levels, in order to manifest. However, in the case of development of sensitization in the CPu we also found that nuclear levels of β-catenin were similar to the control ones and forcing the decrease (by infusing an inhibitor) was not enough to induce the behavioral sensitization. In other words, it seems that this decrease is necessary but not sufficient for the development of sensitization. In the case of the expression of sensitization, changes in β-catenin, and probably in the activity of the Wnt canonical pathway, might be more important and could be mediated by the dopaminergic transmission through D R. A reduction in striatal D R levels have shown after repeated drug exposure in non-human primates [56]. Then, and considering that the antagonism of D R is linked to β-catenin accumulation [37] while the activation is associated with β-catenin degradation [57], it is possible that repeated cocaine exposure could facilitate the accumulation of β-catenin found in the CPu of abstinent animals. Although the fact that β-catenin levels in CPu increase during abstinence while they decrease after a challenge may suggest that changes in the activity of the Wnt canonical pathway could be characteristic of the long-term neuroadaptations, further work must be done to fully clarify the role of this pathway in the CPu.

We also demonstrated that the activation of the pathway mediated by LiCl administration before each cocaine injection not only prevented the development of sensitization by restoring β-catenin levels in the PFC, CPu and Amyg, but also prevented the expression of behavioral sensitization by keeping the levels of β-catenin increased in the NAcc. Previously, we proposed that the increase in β-catenin levels in the NAcc, together with it decrease in the CPu, are correlated to the behavioral changes. In this scenario, the LiCl results seemed contradictory at first glance. However, if we take into account that it is the fold-change of β-catenin that dictates Wnt pathway activity and not the absolute level [58], then the LiCl results strengthen our previous assumptions that it is the change in β-catenin and the consequent activation of the canonical pathway what matters for the expression of sensitization. To our knowledge, this is the first time behavior [2], our findings suggest that the Wnt canonical pathway may be involved in the early stages as well as in relapse of substance abuse. Although one must always be wary of extrapolating clinical relevance.
The authors thank Florencia Cerchiara for her English technical assistance.

References


