Perspective

Synaptic mechanisms of cadmium neurotoxicity

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Cadmium (Cd) is a toxic heavy metal ubiquitously distributed in the environment (water, air, food, smoke) with extreme ability to accumulate in the human body due to its delayed clearance (half-life time 15–30 years). Consequently, prolonged exposure to low doses of Cd causes multi-organ toxicity. Remarkably, the central and peripheral nervous systems are considered as one of the most vulnerable targets. Excessive Cd exposure can profoundly aggravate common neurodegenerative diseases and peripheral polyneuropathies as well as lead to mental deficits in children (Branca et al., 2020). Conceivably, that Cd-induced defects in communication between neurons could be triggering events in Cd neurotoxicity. Numerous studies have discovered the disturbances at the synaptic levels in response to both acute and chronic Cd administration. Furthermore, release of Cd, captured by neuronal tissue, into extracellular space is increased by stimulation of synaptic vesicle (SV) exocytosis (Minami et al., 2001), pointing to Cd accumulation within the SVs in presynaptic terminals. Being a divalent cation, Cd can enter cells through various ways (such as active transporters, carriers, channels, and endocytosis), which serve to transport physiologically essential cations (Ca, Mg, Cu, Mn, Zn). An important route for Cd penetration into neuronal cells relies on zinc transporters (ZnTs). Among them, ZnT3 is highly abundant in the membranes of the SVs and responsible for maintaining the vesicular Zn pool in brain (McAllister and Dyck, 2017). Presumably, presynaptic terminals containing from hundreds to thousands of SVs could be reservoirs for Cd accumulating in the SVs due to ZnT3 activity. Furthermore, SV membranes are enriched with anionic negatively-charged lipids that can electrostatically attract bivalent cations, including Cd. Likewise, voltage-gated Ca^{2+} channels (VGCCs), which are reversibly blocked by Cd, reside densely at the presynaptic site can concentrate Cd, facilitating its uptake. Moreover, Cd may slowly pass into the cytosol through some of the VGCCs. Inside the nerve terminals Cd could affect a plethora of processes, consequently disturbing various presynaptic functions, notably neurotransmitter release. The resulting synaptic defects can produce "devastating signals" which are propagated to the neuronal bodies. Such retrograde pattern of pathology spreading is observed in some neurodegenerative disorders. Recently, we have found that at very low concentrations Cd can desynchronize neurotransmitter release from motor nerve terminals (Tsentsevitsky et al., 2020). A focus on the mechanism behind this phenomenon (**Figure 1**) can delineate the early events in Cd neurotoxicity and reveal a bridge between Cd action and neurodegeneration.

Synchrony (timing) of neurotransmitter release is a substantial factor that determines the efficacy and plasticity of synaptic communication. The neurotransmitter release occurs shortly (within hundreds of microseconds) after action potential (AP) to maintain precise transfer of frequency-coded information. This synchronous mode of neurotransmitter release allows fast and flawless exchange of information between neurons, establishing the basis of proper neuronal network activity and delivery of instructions to effectors (e.g., muscles, visceral organs). Although a synchronous release usually dominates, a neurotransmitter may be released asynchronously during tens to hundreds of milliseconds after an AP. This asynchronous release is an essential modulator of neurotransmission by affecting: the duration of postsynaptic inhibition and activation; neuronal excitability and network activity; and coincide detection by neurons. Meaningfully, a prominent increase in asynchronous release was found in models of Alzheimer disease, epilepsy and spinal muscular atrophy characterized by loss of motor neurons. Also, IgGs from sporadic amyotrophic lateral sclerosis patients selectively bind to presynaptic membrane of motor neurons and enhance asynchronous release (Pagani et al., 2006). Accordingly, excessive Cd can aggravate neurodegenerative diseases and epileptic seizures via an increase in asynchronous release. It should be noted that SVs which mediate synchronous and asynchronous exocytosis can use separate endocytic routes. Particularly, adaptor protein-3 dependent endocytic recycling is utilized for the replenishment of the SV pool responsible for the asynchronous release. The same pathway generates SVs and endosomes enriched with Zn/Cd-translocating ZnT3 and the vesicular Zn facilitates the participation of these SVs in the neurotransmitter release. It is tempting to suggest that accumulation of Cd and Zn in the subpopulation of the SVs contributes to the enhancement of asynchronous release. Supporting this notion is that both Zn and Cd desynchronized neurotransmitter release in the motor nerve terminals (Tsentsevitsky et al., 2020). Like Cd poisoning, excess Zn might exacerbate neurodegenerative disorders as well as epilepsy. Accordingly, the severity of Cd neurotoxicity can be interconnected with alterations in Zn homeostasis. Indeed, we found that Zn enhanced Cd-induced desynchronization of neurotransmitter release (Tsentsevitsky et al., 2020).

Asynchronous release is determined by influx of extracellular Ca^{2+} and its utilization inside the nerve terminal. Mitochondria occupy ~1/5–1/3 volume of presynaptic compartment and they are present in close proximity to the SVs (**Figure 1**). Mitochondrial Ca^{2+} uptake markedly restrains a time frame for neurotransmitter release after arriving an AP, thus the compromised mitochondrial function leads to an increase in asynchronous release. Additionally, mitochondria damage at synapses leads to an overproduction of reactive oxygen species (ROS) (Zakyrjanova et al., 2020), which can enhance Ca^{2+} flux into nerve terminal through redox-sensitive TRPV1 channels. Remarkably, these channels serve as a main source of Ca^{2+} triggering the asynchronous release in solitary tract afferents. Moreover, TRPV1 channels reside on both presynaptic surface and membrane of SVs which mediate the asynchronous release (**Figure 1**). It is well-known that Cd is a redox inert metal, but it can indirectly induce oxidative stress and, hence, apoptosis in numerous cell types, including neurons (Branca et al., 2020). A growing body of evidence suggests that the mitochondrial dysfunction followed by ROS generation is a central causative event in Cd toxicity. Indeed, Cd strongly and directly inhibits the mitochondrial electron transport chain at the levels of complexes I, II and III (Branca et al., 2020). Along similar lines, we revealed that low concentration of Cd significantly increased mitochondrial ROS levels in motor nerve terminals. Antioxidants, including mitochondrial specific, as well as inhibition of TRPV1 channels, effectively suppressed Cd-induced enhancement of asynchronous neurotransmitter release. Accordingly, Cd can augment asynchronous release via increasing mitochondrial ROS production. In this scenario, the generated ROS can facilitate TRPV1 channel activity and, hence, asynchronous exocytosis (**Figure 1**). The ability of Zn to amplify Cd action on both mitochondrial ROS production and the asynchronous release in the motor nerve terminal emphasizes the link between Cd effect on mitochondria and timing of neurotransmitter release (Tsentsevitsky et al., 2020). Zn is known to have dual actions by acting as either an antioxidant or prooxidant (Branca et al., 2018; Lee, 2018). One explanation to this paradoxical action of Zn is that additional factors, such as Cd levels, may determine the prooxidant properties of Zn. As an oxidant Zn, can inhibit mitochondrial function at levels of the electron transport chain complex I, III and IV as well as α-ketoglutarate dehydrogenase complex of tricarboxylic acid cycle (Lee, 2018).

In neuronal cell lines, only higher concentrations (10–20 μM) of Cd 12–48 hours after administration disturbed mitochondrial function and significantly enhanced ROS levels (Branca et al., 2020). A plausible explanation for this is that exposure of neurons to low concentrations of Cd (1–10 μM) can increase the expression and activity of antioxidant enzymes, thereby protecting neuronal cell bodies against ROS overproduction (Branca et al., 2018). Presynaptic nerve terminals are distantly located from the soma and, hence, Cd-induced changes in the gene expression have no influence on the antioxidant capacity of the presynaptic compartment, which faces to a stronger oxidative stress in response to Cd application. Furthermore, presynaptic membranes have a specific lipid composition and are enriched with poly-unsaturated fatty acids and cholesterol (Krivoi and Petrov, 2019). These lipids are highly susceptible to free radical oxidation and the resulted products could affect TRPV1 channel activity directly or indirectly by acting via alterations in lipid raft integrity (Ciardo and Ferrer-Montiel, 2017). Additionally, a strong lipid peroxidation perturbs the membrane permeability thereby causing cell death. In many cell types, organs and brain regions, Cd-induced damages were associated with a prominent lipid peroxidation (Branca et al., 2020). We also detected lipid peroxidation of the synaptic membranes brought about by low concentration of Cd. Probably, lipid peroxidation could also contribute to an increase in TRPV1 channel activity and, hence, desynchronization of neurotransmitter release (**Figure 1**). Besides, Cd-mediated disturbance of the autophagic flux (Zou et al., 2020) can block the utilization of the impaired membranes and, consequently, facilitate the spreading of pathological signals. For instance, oxidized lipids, such as oxysterols, can easily escape from the affected membranes and modulate neurotransmitter release (Krivoi and Petrov,

2019) in nearby synapses.

There are some functionally distinct compartments in large nerve terminals. Indeed, in the frog motor nerve terminals proximal and distal parts are characterized by a higher and lower probability of SV exocytosis, respectively. The effects of Cd on timing of neurotransmitter release as well as lipid peroxidation were more pronounced at the distal part of the frog motor nerve terminal, while Cd increased the mitochondrial ROS production to the similar degree in both the distal and proximal regions. These results suggest that the proximal parts can have a higher antioxidant levels compared to the distal regions (Tsentsevitsky et al., 2020). Along the same lines, overnight exposure of SH-SY5Y cells to 10 µM Cd significantly decreased the levels of presynaptic protein GAP-43, abundantly expressed at the axonal tip (distal part of axon) and essential for neurite outgrowth (Branca et al., 2020). This supports our suggestion of a higher sensitivity of the distal axonal region to Cd. In this compartment ROS actively regulate axonal growth and retraction which implies maintaining of the intracellular antioxidant pool at low levels. Exogenous antioxidants can impair axonal remodeling depending on the distal part of axon (Olguin-Albuerne and Moran, 2018). Given the intensive axonal growth during development, low antioxidant capacity inherent to neurogenic regions (Olguin-Albuerne and Moran, 2018) and immature brain blood barrier, Cd poisoning can be devastating for the developing brain (Branca et al., 2018). In general, the brain has a relatively lower antioxidant guard and, additionally, Cd itself can deplete neuronal and glial glutathione, a key player in the first line of antioxidant protection (Branca et al., 2020). Accordingly, the antioxidant capacity can be a main limiting factor of Cd-neurotoxicity and decreased antioxidant defense during aging and in neurodegenerative diseases could unmask the detrimental effects of Cd accumulation.

In many electrophysiological studies, Cd at a broad concentration range (from 1 µM to 1 mM) is used as a non-specific VGCC antagonist, which suppresses AP-evoked fast neurotransmitter exocytosis. However, the abilities of Cd at lower concentrations (0.1–0.5 µM) to desynchronize the transmitter release and provoke oxidative changes in synapses suggest a more complex nature of Cd synaptic action. Only higher concentrations (2.5–100 µM) of Cd can exhibit toxicity and oxidative damage in numerous cell studies. Accordingly, the observed synaptic effects of Cd at the ultra-low doses point to the presynaptic site as a primary target. Given that levels of Cd in blood are normally low (nanomolar range) and concentrations above 0.05 µM can lead to signs of toxicity (Branca et al., 2018), Cd-induced disruption in synchrony of neurotransmitter release and function of synaptic mitochondria can be considered as early and (or) as triggering events in Cd poisoning. There are numerous open questions in the synaptic mechanism of Cd action. First, the precise pathways for Cd penetration into the synapses need to be revealed. Secondly, understanding how Cd can be retained in synapses and the role of SV pools in Cd deposition is still a work in progress. Next, the reasons for high susceptibility of synaptic mitochondria to Cd and molecular mechanism of Cd-mediated disruption of redox status in the nerve terminals are still to be identified. A promising direction for future studies is a detailed assessment of Cd-induced changes in synaptic membranes and the contribution of oxidized lipids to Cd toxicity. If initial events
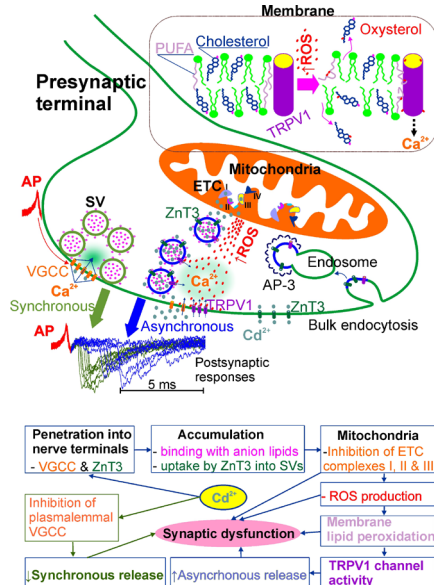
in the progression of Cd neurotoxicity occur in synapses then a hypothetic retrograde mechanism might deliver the pathological signal to the neuronal soma. Finally, *in vivo* studies connecting Cd-related changes in behavioral performance with aberrations in synaptic transmission can capitalize a relevance of the synaptic deficits in Cd poisoning. Noteworthy that developing target delivery of mitochondrial antioxidant to synapses may be promising strategy to therapy of Cd intoxication as well as synaptic dysfunction associated with desynchronization of the neurotransmitter release.

Figure 1 | Hypothetical mechanism of the cadmium-induced synaptic dysfunction.
Fast synaptic transmission mainly relies on synchronous neurotransmitter release time-locked with an arriving action potential (AP). Synchronous release is triggered by Ca^{2+} influx through voltage-gated Ca^{2+} channels (VGCCs) activated by an AP. Asynchronous release occurs with longer and variable delays after an AP and is dependent on Ca^{2+} entering into the cytoplasm via different channels, including TRPV1. Initially, cadmium (Cd) can interact with presynaptic membrane proteins, namely VGCCs and Zn transporters (ZnTs). This leads to a suppression of synchronous release due to partial inhibition of VGCCs and penetration of Cd into the intracellular space. Cd can be retained inside the nerve terminal due to an interaction with anionic lipids and deposition within subpopulation of synaptic vesicles (SVs) containing ZnTs. These SVs are formed via adaptor protein-3 dependent endocytic pathway; they contain TRPV1 channels and mediate the asynchronous release. Cd can inhibit complexes (I, II and III) of the electron transport chain (ETC) and the accumulation of Cd disturbs mitochondrial function causing an increase in reactive oxygen species (ROS) production. ROS can directly activate TRPV1 channels. Also, the elevation of ROS leads to membrane lipid peroxidation and oxidized lipids (e.g., oxysterols and derivatives of polyunsaturated fatty acids) can modulate TRPV1 channels (top scheme; in box). Increased TRPV1 channel activity augments asynchronous neurotransmitter release. Thus, Cd can cause synaptic dysfunction via affecting the timing of neurotransmitter release and redox status. Furthermore, generated oxidized lipids (particularly, oxysterols) can diffuse into extracellular space and exert an influence on neighboring synapses.

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