ISSN 0006-2979, Biochemistry (Moscow), 2021, Vol. 86, No. 4, pp. 471-479. © Pleiades Publishing, Ltd., 2021.
Published in Russian in Biokhimiya, 2021, Vol. 86, No. 4, pp. 554-563.

MINI-REVIEW

Normalization of Calcium Balance in Striatal Neurons in Huntington's Disease: Sigma 1 Receptor as a Potential Target for Therapy

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Received October 16, 2020
Revised December 24, 2020
Accepted December 24, 2020

Abstract—Huntington's disease (HD) is a neurodegenerative, dominantly inherited genetic disease caused by expansion of the polyglutamine tract in the huntingtin gene. At the cellular level, HD is characterized by the accumulation of mutant huntingtin protein in brain cells, resulting in the development of the HD phenotype, which includes mental disorders, decreased cognitive abilities, and progressive motor impairments in the form of chorea. Despite numerous studies, no unambigous connection between the accumulation of mutant protein and selective death of striatal neurons has yet been established. Recent studies have shown impairments in the calcium homeostasis in striatal neurons in HD. These cells are extremely sensitive to changes in the cytoplasmic concentration of calcium and its excessive increase leads to their death. One of the possible ways to normalize the balance of calcium in striatal neurons is through the sigma 1 receptor (S1R), which act as a calcium sensor that also exhibits modulating chaperone activity upon the cell stress observed during the development of many neurodegenerative diseases. The fact that S1R is a ligand-operated protein makes it a new promising molecular target for the development of drug therapy of HD based on the agonists of this receptor.

DOI: 10.1134/S0006297921040076

Keywords: sigma 1 receptor, Huntington's disease, dendritic spines, Ca^{2+}, store-operated calcium entry, endoplasmic reticulum

INTRODUCTION

Huntington's disease (HD) is a neurodegenerative, dominantly inherited genetic disorder characterized by three main clinical signs: mental disorders, cognitive decline, and extrapyramidal symptoms. All these pathological features rapidly progress and eventually lead to dementia and cachexia [1, 2]. The primarily affected brain region in HD is the striatum because it contains GABAergic medium spiny neurons (MSNs), which constitute 95% of striatal neurons and are most susceptible to cell death. The striatum is a part of the brain basal ganglia that play a key role in the movement and behavior control. Degeneration of striatal cells leads to the impaired motor activity resulting in the emergence of distinctive involuntary, disorderly, abrupt movements (semantically united by the term *chorea*), which are the main clinical symptom of the disease.

At the molecular level, an increase in the number of CAG repeats in the first exon of the huntingtin gene above a threshold of 36 codons leads to the pathological extension of the polyglutamine tract in the protein and development of the HD phenotype [3]. The product of this gene is a soluble huntingtin (Htt) protein with a molecular weight of 348 kDa. Despite the established genetic nature of HD, the molecular and biochemical pathways disrupted in the cells due to the presence of mutant huntingtin (mHtt) are still not fully understood. It is known

Abbreviation: ER, endoplasmic reticulum; HD, Huntington's disease; IP_3, inositol trisphosphate; IP_3R1, inositol trisphosphate receptor type 1; Htt, huntingtin; mHtt, mutant huntingtin; MSN, medium spiny neuron; NMDAR, N-methyl-D-aspartate receptor; S1R, sigma 1 receptor; SOCE, store operated calcium entry; STIM, stromal interaction molecule; VGCC, voltage-gated calcium channel.
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that Htt is transcribed in various tissues and has many interaction partners [4]. It is involved in important cellular processes, such as gene expression, intracellular transport of proteins and vesicles, signal transduction, and anti-apoptotic biochemical cascades. As shown in animal models, the absence of Htt leads to the embryonic death. According to the Htt crystal structure, the polyglutamine tract is an alpha-helix involved in various protein–protein interactions [5]. It can also take on alternative folding patterns, depending on the protein environment. The extension of this region increases the probability of its interaction with protein partners atypical for the wild-type Htt and, most likely, is the reason for the accumulation of mHtt aggregates in the nucleus, cytoplasm, and neuronal processes. The main consequence of the polyglutamine tract expansion in mHtt is changes in the protein structure, followed by a hypothetical loss of normal function and acquisition of new toxic properties, which in any case leads to the disruption of cell processes.

In recent years, the aggregation hypothesis of HD pathogenesis, which assumes direct relation between mHtt aggregation and toxicity, has been questioned. The toxic effect of mHtt is currently associated with the aberrant interactions of its monomeric or oligomeric forms [6, 7]. The latest data indicate that the disaggregated forms of mHtt are more toxic; moreover, the first biochemical signs of pathology can be detected in the cells even before the appearance of mHtt aggregates [7-9]. Therefore, the search for new alternative theories that could explain the toxicity of mHtt at the molecular level remains relevant. It was shown that the Htt protein with an expanded polyglutamine tract is characterized by several new pathological properties that affect the key aspects of neuronal functioning, such as axonal transport [10], endocytosis [11], synaptic transmission [12], and Ca^{2+} signaling [13]. Disruptions in the mechanisms of Ca^{2+} regulation in the MSNs are associated with selective degeneration of these cells during the HD progression [14]. This review summarizes the literature data on the impaired Ca^{2+} signaling in HD, especially in the endoplasmic reticulum (ER), and its contribution to the development of synaptic dysfunction – one of the earliest signs of neuropathological processes at the cellular level. The role of the sigma 1 receptor (S1R) in the development of HD pathogenesis and the prospects of using its agonists (e.g., pridopidine) to normalize the Ca^{2+} balance in neurons and to maintain the functional activity of synapses at the earliest stages of neuropathological changes are also discussed.

ROLE OF CALCIUM IN THE PATHOGENESIS OF HUNTINGTON'S DISEASE

Ca^{2+} is one of the most important secondary messengers in neurons that converts incoming signals from outside of the cell into the activation of effector enzymes. It launches Ca^{2+}-mediated cascades of biochemical reactions that form a specific cellular response that affects the structure and the function of neurons. Various stimuli trigger the mechanisms of Ca^{2+} regulation via inward Ca^{2+} currents into the cytoplasm of neuronal cells through the voltage-gated (VGCC) and ligand-gated Ca^{2+} channels, as well as through transient receptor potential canonical (TRPC) channels. The intracellular Ca^{2+} concentration also increases when Ca^{2+} enters the cytoplasm from the intracellular stores, mainly from the smooth ER, after activation of signaling cascades mediated by other secondary messengers, such as inositol 3-phosphate (IP_3) or after activation of ryanodine receptors. Since ER is the main dynamic Ca^{2+} in the cells, there is a mechanism that ensures Ca^{2+} influx from the extracellular matrix in order to maintain a stable level of Ca^{2+} in the ER in the absence of influx through the VGCCs and ligand-gated Ca^{2+} channels.

Store-operated Ca^{2+} entry (SOCE) is a cascade of biochemical reactions activated when depletion of intracellular Ca^{2+} stores induces Ca^{2+} entry from the extracellular space to replenish these stores [15]. During this process, Ca^{2+} sensors of the stromal interaction molecule (STIM) protein family become activated. STIM proteins, in turn, activate highly selective Ca^{2+} channels from the ORAI and TRPC families located on the plasma membrane, through which Ca^{2+} enters the cytosol and then is transported into the ER through sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA).

In non-excitable cells, SOCE is the main mechanism for the replenishment of intracellular Ca^{2+} stores. For a long time, it had been believed that SOCE was absent in neurons, until it was shown that proteins of the ORAI family, as well as STIM1 and its homologue STIM2, are expressed in the CNS [16-18]. STIM2 protein is a more sensitive Ca^{2+} sensor than its homologue STIM1, since it is activated by small changes in the ER Ca^{2+} concentration [18]. STIM2 has lower oligomerization kinetics and, therefore, binds less efficiently to the ORAI family proteins [19]. It is assumed that this provides the neuroprotective effect which prevents an excessive increase in the Ca^{2+} concentration in the neurons.

The role of Ca^{2+} as a secondary messenger is difficult to overestimate, since the most important neuronal functions, such as changes in the excitability of neurons (through modulation of the activity and expression of ion channels), synaptic transmission, and synaptic plasticity, as well as changes in gene expression, are based on numerous Ca^{2+}-dependent processes, including activation of Ca^{2+}-dependent effector proteins involved in Ca^{2+} signaling. Therefore, due to the extreme sensitivity of neurons to changes in the Ca^{2+} concentration, even small alterations can disrupt the fine mechanisms of Ca^{2+} regulation and ultimately lead to the neuronal death [20].

Molecular biology studies have shown that HD development is accompanied by changes in the concentration of Ca^{2+} in striatal neurons [21], as well as alterations in the expression levels of many Ca^{2+} signaling proteins. The earliest studies of HD pathogenesis revealed the neurotoxic effect of glutamate, which causes degeneration of striatal neurons. In particular, intrastriatal injection of neurotoxins, such as quinolinic acid, induces excessive Ca^{2+} influx into the neurons through ionotropic glutamate receptors and leads to pronounced cell death due to excitotoxicity [22]. As a result, the animals exhibit an HD-like phenotype. One of the hypotheses linking an increased vulnerability of MSNs and the toxic effect of glutamate is that mHtt increases the activity of extrasynaptic NMDA receptors (NMDARs, N-methyl-D-aspartate receptors) [23]. In the presence of mHtt in MSNs, the cells exhibit an increased density of Ca^{2+} currents through the N2R subunit-containing NMDARs (which are predominantly extrasynaptic in mature neurons). While activation of synaptic NMDARs promotes expression of the anti-apoptotic, antioxidant, and neuroprotective factors (such as the brain-derived neurotrophic factor, which supports neurite growth and dendritic spine formation), activation of extrasynaptic NMDARs produces the opposite effect associated with the activation of pro-apoptotic factors. Pharmacological inhibition of NMDARs by low doses of memantine, resulting in the blockade of the extrasynaptic receptor pool, has a neuroprotective effect, as demonstrated in a primary culture of striatal cells isolated from the HD mouse model [24], as well as in phase I clinical trials [25].

An important step in the development of the calcium hypothesis of HD pathogenesis was the discovery of a new toxic function of mHtt consisting in its direct association with the C-terminus of the type 1 inositol 3-phosphate receptor (IP_3R1) located on the membrane of ER, the main dynamic intracellular Ca^{2+} store. In a mouse model of HD, mHtt, Htt-associated protein type 1, and IP_3R1 form a ternary complex on the ER membrane, which mediates an excessive Ca^{2+} release from the intracellular store due to the increase in the IP3 affinity for its receptor [26]. A decrease in the Ca^{2+} content in the ER leads to the impaired protein folding, accumulation and aggregation of unprocessed proteins, thus causing the ER stress. To replenish Ca^{2+} reserves, ER launches a compensatory mechanism – SOCE [27]. Upregulation of this biochemical pathway was observed in various cellular models of HD [28-30], as well as an increase in the level of the STIM2 protein, which is responsible for the initiation of this biochemical pathway [30]. In addition, SOCE hyperactivation was demonstrated in induced pluripotent stem cells obtained from HD patients [31]. Over time, due to the excessive SOCE activation, the compensatory mechanism becomes pathological, since Ca^{2+} begins to accumulate in the cytoplasm, ultimately inducing apoptosis of the MSNs [32]. In addition, SOCE hyperactivation can lead to the impairment of specific functions of neuronal cells.

A number of studies have shown that SOCE activation negatively affects the functioning of VGCCs by initiating their internalization [33]. Electrophysiological analysis of striatal neurons revealed an initial increase in the VGCC density, which then decreased in the case of HD [34]. The decrease in the VGCC density may be a critical factor determining the inhibitory effect of MSNs, since VGCCs directly control the neurotransmitter release [35]. Long-term inhibition of VGCCs can lead to a reduction in the GABA (inhibitory neurotransmitter) release and impaired inhibition of the effector brain regions. At the same time, it was shown that in a culture of cortical neurons obtained from the HD mouse model, the entry of Ca^{2+} into the presynaptic terminal through the N-type VGCCs is upregulated, leading to the activation of glutamate release [35]. In addition, both upregulation of the L-type VGCC expression and increase in the total density of Ca^{2+} currents have been found in cortical neurons [36]. An increased glutamate release from the axon terminals of cortical neurons is observed at rather early stages of neuropathology, long before the onset of the first clinical symptoms [36, 37], which is consistent with the hypothesis that an increased glutamate release causes excitotoxic damage of the MSNs. At later stages, the release of glutamate from the cortical neurons decreases, which contributes to the development of corticostriatal synaptic dysfunction [37]. Since mHtt is expressed in all types of brain cells, changes in Ca^{2+} homeostasis can disrupt Ca^{2+}-dependent mechanisms of synaptic transmission both in the pre- and postsynaptic regions partly due to the activation of SOCE.

Disruptions in the functional activity of mitochondria play a significant role in the development of HD pathogenesis. Mitochondria have a critical role in the neuronal maintenance by generating ATP and biosynthetic substrates, maintaining Ca^{2+} homeostasis, and initiating apoptosis. Mitochondrial fusion and fission are two important mechanisms that directly affect the activity and functioning of mitochondria [38]. Fusion helps to alleviate the stress by mixing the contents of partially damaged mitochondria as a form of complementation. Fission is necessary for the generation of new mitochondria; it also contributes to the quality control by allowing removal of damaged mitochondria and may promote apoptosis upon high levels of cellular stress. Histopathological examination of HD patients revealed a significant and progressive, depending on the stage, decrease in the number of mitochondria in the MSNs and noticeable changes in their size [39].

Combined with a significant upregulation of expression of the Drp1 fission protein and decrease in the content of mitofusin type 1 protein, these changes indicate a high level of cellular stress in neurons [40]. Mitochondria

are one of the intracellular Ca^{2+} stores that capture excessive cytosolic Ca^{2+} and support its tight intracellular regulation. Depletion of the buffering capacity of mitochondria results in a critical change in the membrane potential, causing the opening of the mitochondrial permeability transition pore and release of apoptotic mediators, such as cytochrome *c*, into the cytosol, which triggers the neuronal death. A large number of studies indicate a change in the membrane potential and a decrease in the Ca^{2+} buffering capacity in mitochondria [41-45]. Moreover, in patients with juvenile HD, a decrease in the buffering capacity was observed much earlier compared to its manifestation in adulthood [46].

Almost 90% of IP_3Rs localize to specialized areas of the ER membrane associated with the mitochondria (MAMs, mitochondria-associated ER membranes). The disturbances in the Ca^{2+} balance in the ER due to the IP_3R1 hyperactivation can critically affect the organization of MAMs, the synchrony of molecular processes, and the functional relationship between the two organelles, which ultimately leads to the disruption in the functioning of mitochondria and initiation of pro-apoptotic signaling cascades. In particular, a decrease in the colocation of the ER and the mitochondria was demonstrated recently in the culture of striatal neurons obtained from the HD mouse model [47]. A significant reduction in the levels of IP_3R1 and chaperone Grp75, a key protein that provides Ca^{2+} transfer from the ER to the mitochondria, has been shown in the striatum in two different HD mouse models and in the striatum of HD patients. A decreased level of mitofusin type 2 was also observed in the striatum of HD patients. Inhibition of the Drp1 protein not only prevented the loss of contacts between the ER and the mitochondria, but also restored Ca^{2+} transfer from the ER to the mitochondria, thereby restoring Ca^{2+} balance in neurons.

Synaptic contacts are most sensitive to changes in the intracellular Ca^{2+} concentration, especially at the postsynaptic side, since the functional activity of postsynaptic dendritic spines largely depends on the intracellular Ca^{2+} concentration. Changes in the Ca^{2+} regulation in striatal neurons at the earliest stages of HD development lead to the elimination of synaptic contacts and development of corticostriatal synaptic dysfunction, which is believed to further lead to motor impairments characteristic to this neuropathology. It is believed that elimination of dendritic spines in MSNs mostly depends on the expression of mHtt in these cells. However, some studies suggest a connection between the stability of dendritic spines and impaired Ca^{2+} signaling in the presynaptic zone [48-50]. In particular, recent studies demonstrated an increased frequency of miniature synaptic glutamate releases, mediated by spontaneous Ca^{2+} release from the ER, and reduction in the glutamate release upon the action potential generation [50] in the cortical neuronal culture in the presence of mHtt. A decrease in the number of mushroom dendritic spines, which are considered as stable, functionally active postsynaptic structures, was observed in the culture of cortical neurons isolated from the HD mouse model. A decrease in the number of mushroom spines on the pyramidal neurons is a result of impaired homeostatic synaptic plasticity resulting from the disturbed Ca^{2+} signaling [51]. The importance of the afferent innervation from the cortical neurons has also been demonstrated using the optogenetic approach. Prolonged suppression of the spontaneous activity of cortical neurons led to a significant decrease in the dendritic spine density in MSNs in the corticostriatal co-culture isolated from the HD mouse model compared to the wild type neurons [2]. Similar results were observed *ex vivo* in the optogenetic studies of the corticostriatal sections from HD mice. The peak amplitude and the area of AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor)- and NMDAR-mediated responses evoked by the stimulation of cortical neurons were reduced in the MSNs, which correlated well with a decrease in the density of dendritic spines in these cells [52].

The development of synaptic dysfunctions in the presence of mHtt appears to be a systemic pathological process, since functional changes in the thalamostriatal synapses are also observed in HD, and these changes precede the corticostriatal ones [53]. In particular, both thalamostriatal and corticostriatal synapses demonstrated an increase in the extrasynaptic Ca^{2+} currents through NMDARs, as well as the in ratio between the currents through AMPARs and NMDARs. Thus, the disturbances of Ca^{2+} homeostasis in striatal neurons in the presence of mHtt can affect a number of molecular mechanisms in HD MSNs. In this regard, drugs that contribute to the normalization of Ca^{2+} balance in striatal cells, e.g., substances that prevent IP_3R1 association with mHtt or SOCE inhibitors, may be potential therapeutic agents for the HD treatment.

ROLE OF SIGMA 1 RECEPTOR AS A CALCIUM BALANCE MODULATOR IN NEURONS IN HUNTINGTON'S DISEASE

Sigma receptors were originally described as a subtype of opioid receptors, but now they are attributed to a separate class, which is unique in the structure and array of its ligands. The most pharmacologically studied among these proteins is the type 1 receptor. Its activity and location depend on the functional state of the cell, stimulation with ligands, and Ca^{2+} level in the ER. In its inactive state, as well as upon stimulation with antagonists, S1R forms a stable complex with the resident ER chaperone, the binding immunoglobulin protein (BiP or GRP78), which acts as a Ca^{2+} sensor [54]. S1R is a ligand-gated molecular chaperone that participates in various biochemical pathways activated by cellular stress. For exam-

ple, upon stress initiation in the ER, S1R regulates the IP_3R function, providing Ca^{2+} transfer to the mitochondria, maintenance of ATP synthesis, and inhibition of the apoptotic cascade initiation [54, 55]. The formation of the S1R complex with IP_3R occurs in the MAMs. Several independent groups have shown that S1R inhibits SOCE in non-excitable cells [56, 57]. In particular, S1R activation by agonists decreases SOCE amplitude, while application of receptor antagonists promotes the activity of this biochemical pathway. The knockdown of S1R in the cell culture also increases the amplitude of SOCE. It was also demonstrated that S1R directly interacts with the STIM1 and ORAI1 proteins and prevents their association [56]. In addition, S1R plays an important role in maintaining the bioenergetic balance in neurons and acts as a modulator of multiple ion channels of various types, including Ca^{2+} channels and Ca^{2+}-activated channels [58-62]. The missense mutation E102Q in the receptor molecule leads to the development of the juvenile form of amyotrophic lateral sclerosis [63]. This mutation downregulates ATP production in neurons and causes the death of nerve cells [64]. S1R knockdown in the hippocampal neurons leads to a decrease in the size of mitochondria and to changes in the membrane potential [65]. In retinal ganglion cells, S1R prevents excitotoxicity and reduces cell apoptosis by regulating Ca^{2+} signaling and by suppressing activation of the pro-apoptotic factors, such as Bax and type 3 caspase [66]. The disturbances in the Ca^{2+} balance in the ER can directly affect mHtt aggregation during the HD development. In particular, it was demonstrated that IP_3R1 is an important molecular target in HD, because its knockdown or chemical inhibition reduce mHtt aggregation and prevent cell death [67]. It was also shown that intranuclear inclusions, consisting of mHtt aggregates in neurons, co-localize with S1R in the brain of patients affected by the polyglutamine expansion diseases, including HD [68]. The downregulation of S1R expression by the anti-sense RNA in the cellular model of HD increased the number of mHtt aggregates both in the cytoplasm and in the cell nucleus. Moreover, the proteasomal activity was also significantly reduced after the S1R knockdown [68].

Important results indicating the involvement of S1R in the modulation of Ca^{2+} signaling in neurons were obtained in the study of the neuroprotective properties of pridopidine, which is currently considered as a potential therapeutic agent for the treatment of HD [69-71]. Pridopidine was originally discovered as a “dopamine stabilizer” that binds to the D2 dopamine receptors. However, the affinity of pridopidine for the D2 dopamine receptors is low (dissociation constant, 60 μM). At the same time, the structural analogue of pridopidine, compound 3PPP (3-(3-hydroxyphenyl)-N-n-propylpiperidine), is a high-affinity S1R ligand (dissociation constant, 80 nM) [72]. The dose-dependent relationship for pridopidine has a bell shape, which is typical for most S1R agonists [73]. Recent studies by positron emission tomography showed that at a clinically relevant single dose, pridopidine acts as a selective S1R agonist, showing almost complete binding to S1R and negligible binding to the D2 and D3 dopamine receptors [74].

Pridopidine and 3-PPP in nanomolar concentrations exhibited a neuroprotective effect in the cellular model of HD. Both compounds stabilized synaptic connections between the cortical and striatal neurons in the primary corticostriatal co-cultures obtained from the YAC128 HD mice. Cas9-mediated S1R knockdown abolished the neuroprotective effect of pridopidine and 3-PPP. Interestingly, S1R knockdown led to a significant decrease in the dendritic spine density in the co-culture of cortical and striatal neurons isolated from the wild-type mice. This observation points to the important role of S1R in maintaining the stability of dendritic spines. The synaptoprotective effect of pridopidine is directly related to the Ca^{2+} regulation in neurons, which was confirmed in a series of Ca^{2+} imaging experiments. Earlier studies have shown that abnormal Ca^{2+} signaling in the postsynaptic spines is responsible for their destabilization during the HD development. Incubation of pridopidine to the corticostriatal culture isolated from the HD mouse model prevented IP_3R1 hyperactivity, restored the level of Ca^{2+} in the ER, and decreased the activity of SOCE. S1R knockdown in the cultured wild-type neurons led to the depletion of Ca^{2+} in the ER. This suggests that S1R stabilizes dendritic spines through the homeostatic control of Ca^{2+} levels. Pridopidine also exerted the neuroprotective effect in the culture of cortical neurons isolated from HD mice, thus normalizing defects in the homeostatic synaptic plasticity and restoring the density of dendritic spines [51].

CONCLUSIONS

To summarize all the above, disturbances in the Ca^{2+} signaling during the HD development impair many functional aspects of neuronal cells. At the early stages of the disease, MSNs are able to prevent Ca^{2+} imbalance due to a large number of compensatory mechanisms. However, with age, their neuroprotective potential decreases because of a general decrease in the metabolic activity and reduced expression of Ca^{2+}-buffering proteins. Continuing disturbances in the Ca^{2+} regulation ultimately lead to the depletion of the compensatory capacity of the cells and stable increase in the cytosolic Ca^{2+}, which eventually results in neuronal degeneration.

S1R is a promising therapeutic target for the treatment of HD since it is involved in the modulation of various cytosolic Ca^{2+}-dependent signaling pathways. Activation of S1R by selective agonists protects neurons from the glutamate excitotoxicity, reduces SOCE hyperactivation, and maintains structural integrity of MAMs

necessary for the synchronization of activities of the mitochondria and the ER to ensure the bioenergetics balance in the cells. Pridopidine, which is a highly selective S1R agonist, displays the neuroprotective effect in various cellular and animal models of HD, largely due to the normalization of Ca^{2+} signaling in neurons.

The synaptoprotective effect of pridopidine is particularly important, as it is observed both in the cortical and striatal neurons, indicating a systemic effect of pridopidine in HD therapy. Since the development of synaptic dysfunctions is one of the earliest signs of neuropathology at the cellular level, normalization of Ca^{2+} balance by pridopidine could prevent the disease development at the molecular level at the earliest stages. In this regard, it can be assumed that the most pronounced therapeutic effect of pridopidine will be in preventive therapy, even before the emergence of the first clinical symptoms, which will support the compensatory capabilities of neuronal cells and significantly delay the progression of HD.

Funding. This work was supported by the Russian Science Foundation (project no. 19-15-00184, "The role of calcium in the pathogenesis of Huntington's disease" section) and by the Russian Foundation for Basic Research (project no. 18-34-00994, "The role of the sigma 1 receptor as a modulator of calcium balance in neurons in Huntington's disease" section).

Ethics declarations. The authors declare no conflict of interest. This article does not describe any research involving humans or animals as objects.

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