Impaired ATP Release from Brain Astrocytes May be a Cause of Major Depression

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Received: 23 November 2019 / Accepted: 9 January 2020 / Published online: 11 April 2020
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Major depressive disease (MDD) is one of the most common mental disorders and a leading cause of disability [1]. Core symptoms include depressed mood, increased apathy, and a general loss of interest. In consequence, it represents not only a personal hardship for the patients themselves, but also a major socio-economic burden for society as a whole. In view of the low efficiency of the classic antidepressant drugs, it is most important to learn which pathophysiological events initiate MDD; this will make it possible to identify new therapeutic targets and eventually adequate pharmacological means to combat this disease. One of the difficulties in developing new anti-depressive pharmaceuticals is the inadequacy of the animal models used for this purpose. In humans, stressful life events including acute and chronic stress increase the risk for MDD [2]. Because a laboratory animal correlate of MDD is not known, mice/rats are exposed to acute stress (*e.g.*, tail suspension test, forced swim test, unavoidable foot shock test, social defeat test, or restraint stress) or chronic stress (chronic unpredictable stress) to induce depressive-like states [3, 4].

In place of the former neuro-centric hypothesis, depression has recently been suggested to constitute a glia-based synaptic dysfunction [5]. Thus, defective glial cells appear to be the primary cause of the disease, leading to secondary changes in neuronal functions. In fact, neuroglia (especially astrocytes) shape synaptic circuits by the release of

gliotransmitters, *e.g.* the extracellular signaling molecule ATP and its enzymatic degradation product adenosine [6, 7]. In mammals, ATP activates membrane receptors of two major types, the ligand-gated cationic channels P2X (P2X1–7) and the G-protein-coupled P2Y receptors (P2Y1, 2, 4, 6, 11, 12, 13, and 14). Adenosine occupies its own receptors of the A1, 2A, 2B, and 3 subtypes.

It is broadly accepted that overstimulation/upregulation of P2X7 receptors (P2X7Rs) on microglia (the resident macrophages of the central nervous system [CNS]) is a major cause of MDD; other possible reasons have largely been neglected [8, 9]. A complex interacting network of relevant brain structures has been identified, consisting of neuronal circuitries causally related to depressive-like behavior [1, 10]. The most relevant structures are the medial prefrontal cortex (PFC), hippocampus, anterior cingulate cortex, amygdala, nucleus accumbens, ventral tegmental area, lateral habenula, and raphe nucleus, with the PFC and hippocampus being especially important.

Here, we present evidence suggesting that in addition to the upregulation of P2X7Rs, the decreased astrocytic release of ATP in the PFC and hippocampus is also important for the development of MDD. Of course these two etiological factors are almost certainly not mutually exclusive but rather complementary, because a low local concentration of ATP may lead to the up-regulation of P2X7Rs on microglia/neuroglia in relevant brain areas. It has been shown that inescapable foot shock delivered to wild-type mice decreases the spine synapse density in the dentate gyrus of their hippocampi and causes depressive-like behavior (increased latency and number of escape failures to subsequent escapable foot shocks) [11]. Both the depressive-like behavior and remodeling of hippocampal spine synapses are absent in P2X7R-deficient animals.

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ATP can be released from astrocytes not only by Ca^{2+} -dependent exocytosis, but also by a range of non-exocytotic mechanisms [7]. Interestingly, both neurons and astrocytes concentrate ATP *via* a vesicular nucleotide transporter (VNUT) into synaptic-like vesicles (and into lysosomes only in astrocytes), from where they may be released by means of similar but not identical proteins of the exocytotic machinery. In both cases, the release from these storage sites involves *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE)-dependent merger of the vesicle membrane with the plasmalemma [12]. Although it is clear that the vesicular proteins involved in exocytosis are relatively similar in astrocytes and neurons, the astrocytic release of ATP is much slower than that of its neuronal counterpart.

The non-exocytotic release pathways of astrocytes include connexin hemichannels and pannexin channels to allow the outward passage of small molecules (< 1.5 kDa) such as glutamate or ATP from the cell interior. Glial cells and especially astrocytes are organized into networks and communicate *via* channels called gap junctions (composed of two opposed connexin hemichannels) that link the cytosol of adjacent cells [13]. In astrocytes, connexin-30 and -43 (Cx30, Cx43) are the predominant channel constituents, operating also in the hemichannel mode. Pannexins (in astrocytes, Panx-1) exist only as hemichannels.

As noted above, numerous lines of evidence support the contention that the modification of astrocytes in fronto-limbic regions is associated with depression [5]. This includes morphometric changes of astrocytes in post-mortem brain samples from individuals with depressive disorder or suicide completion [14]. Further, animal studies have shown a causal relationship between the selective destruction of fronto-cortical astrocytes and depressive-like behavioral changes [5]. Of course, astrocytes may alter the homeostasis of the CNS in many ways, by ensuring K^+ buffering, neurotransmitter uptake, the maintenance and regulation of synaptic transmission, and glucose metabolism. Thus, dysfunctional astrocytes may indirectly modify neuronal circuits and in consequence lead to the manifestation of pathological/depressive reactions. Nonetheless, a core mechanism in this process is thought to be the decreased release of ATP from astrocytes in the PFC and hippocampus (see below).

In mice susceptible to chronic social defeat stress, an animal model of the depressive-like state, the concentration of ATP is lower in the artificial cerebrospinal fluid collected from PFC or hippocampal slices than that from such slices from non-defeated control mice [15]. In addition, in another model of depressive-like behavior, the forced swim test, the duration of immobility can be reversed by the injection of ATP into the lateral ventricle.

The enzymatic degradation product of ATP, adenosine, is not responsible for this effect, because a slowly-degradable ATP analogue, ATP- γ -S, had the same effect as ATP.

Several lines of evidence indicate that ATP is released mainly by exocytotic mechanisms [15]. In perfect correlation with this assumption, the inositol 1,4,5-triphosphate (IP3) type-2 receptor, which is known to supply Ca^{2+} for exocytosis in astrocytes, has been shown to be essential for the non-depressed, normal behavior of mice. ATP concentrations are lower in the artificial cerebrospinal fluid of PFC and hippocampal slices from *Itpr2*^{-/-} mice in which the IP3 type-2 receptor is genetically deleted as compared with wild-type mice. Further, the astrocyte-specific transgenic expression of a dominant-negative (dn) form of SNARE inhibits the exocytotic release of ATP from astrocytes [6, 7]. In dnSNARE mice, astrocytic ATP release is dysfunctional, and depressive-like behavior in the forced swim test is evident. Most importantly, ATP administration completely reverses the increased immobility of dnSNARE mice in this model of depression.

Concordant with these findings, fluoxetine, a clinically applicable anti-depressant drug, increases the gliotransmission of ATP in the mouse hippocampus [16]. Chronic treatment of wild-type and VNUT-deficient mice with fluoxetine induces an increased release of ATP from the hippocampal slices of wild-type animals only. Furthermore, in mice with selective genetic deletion of VNUT in astrocytes, the fluoxetine-induced anti-depressive behavior in the tail suspension test is impeded, suggesting that VNUT-dependent ATP exocytosis plays a critical role in the therapeutic effect of fluoxetine. Another report, however, indicated that not vesicular exocytosis but Calhm2, a transmembrane channel protein, is the exit pathway for the release of ATP [17]. Conventional knockout and conditional astrocyte knockout of Calhm2 both lead to a significantly reduced ATP concentration, loss of hippocampal dendritic spine number, neuronal dysfunction, and depressive-like behavior (tail suspension test and forced swim test) in mice.

Disturbance of the intercellular connection between astrocytes due to defective gap junction channels is also increasingly considered as a cause of depression [18, 19]. The expression of Cx43-immunoreactivity is reduced in postmortem brains of patients suffering from MDD [19]. Conversely, treatment of mice with antidepressants from diverse therapeutic classes increases Cx43 expression at both the mRNA and protein levels. In good correlation with these findings, gap junction blockade by carbenoxolone or the Cx43-mimetic blocking peptides Gap26 and Gap27 infused into the PFC of mice cause anhedonia in the sucrose preference test [20]. Further, mice exposed to chronic unpredictable stress also develop the expected anhedonia, and exhibit decreases in diffusion of the gap

Fig. 1 Decreased release of ATP from prefrontocortical and hippocampal astrocytes causes “learned helplessness” in mice. Learned helplessness is a response to an unpleasant situation that the mice cannot actively avoid. In the tail suspension test (TST), mice are suspended by their tails with tape in such a position that they are unable to escape or hold on to nearby surfaces. Then, the sum of the time periods during which they stop escape reactions, *i.e.* they become immobile, is measured. The duration of the test is maximized usually at 6 min, in order to avoid unnecessary suffering of the animals. The release of the extracellular signaling molecule ATP from astrocytes occurs *via* exocytotic (from astrocytic synaptic vesicles and astrocytic lysosomal vesicles) and non-exocytotic pathways (P2X7 receptors, connexin/pannexin [hemi]channels, and Calhm2 channels). P2X7 receptors are ligand-gated cationic channels opened by large concentrations of ATP and may in addition become membrane pores after their long-lasting activation, allowing the outward passage of ATP. Despite decreasing the release of ATP *via* P2X7 receptors, P2X7 antagonists have been shown to act as anti-depressive agents, probably because of a preponderance of the microglial anti-inflammatory effects over the astrocytic effects. An unavoidable stressful stimulus reduces the basal release of ATP from astrocytes located in the prefrontal cortex and hippocampus. It is assumed that this change in ATP release induces “learned helplessness” in laboratory rodents. ASV, astrocytic synaptic vesicle; ASL, astrocytic lysosomal vesicle; P2X7, P2X7 receptor; Cx43, connexin-43 channel; Panx-1, pannexin-1 hemichannel; Calhm2, calcium homeostasis modulator 2 channel.

junction channel-permeable dye Lucifer Yellow, as well as in the expression of Cx43.

A malfunction of Cx43 may on the one hand alter the connectivity of astrocytes *via* gap junctions, but on the other hand it may also modify the hemichannel function of connexins. However, pannexins exist only in the hemichannel mode and therefore their blockade should interrupt the bidirectional contact with the extracellular space rather

than that with the cytoplasm of neighboring cells. In the social defeat stress model, a decrease in the expression of Panx-1 occurs in the medial PFC of susceptible mice [20]. Furthermore, pharmacological blockade of this Panx-1 with carbenoxolone or $^{10}\text{Panx}$ infusion induces depressive-like behavior in the chronic social defeat stress model, which is prevented by injection of ATP into the medial PFC. Hence, a reduction of the Panx-1 hemichannel-mediated basal release of ATP by carbenoxolone or social defeat stress may cause a depressive-like condition.

In conclusion, a plethora of experimental evidence shows that when the exocytotic or non-exocytotic release of ATP in the PFC and hippocampus is impeded in response to various stressful stimuli, a depressive-like state develops which is considered in animal studies as “learned helplessness” and anhedonia (Fig. 1). However, it has to be pointed out that there is no evidence for the assumption that astrocytes in depression-relevant areas of the brain other than the PFC/hippocampus fail to respond to stressful stimuli by a decrease in ATP release. Eventually, it appears that unavoidable stress in humans is probably one of the etiological factors contributing to the development of MDD.

Acknowledgements This insight article was supported by The Project First-Class Disciplines Development of Chengdu University of TCM (CZYHW1901), the National Natural Science Foundation of China (81774437, 81973969) and the Science and Technology Program of Sichuan Province, China (2019YFH0108, 2018SZ0257).

Conflict of interest The authors declare that they have no conflict of interest.

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