Review

P2 receptors are involved in the mediation of motivation-related behavior

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Abstract

The importance of purinergic signaling in the intact mesolimbic–mesocortical circuit of the brain of freely moving rats is reviewed. In the rat, an endogenous ADP/ATPergic tone reinforces the release of dopamine from the axon terminals in the nucleus accumbens as well as from the somatodendritic region of these neurons in the ventral tegmental area, as well as the release of glutamate, probably via $P2Y_1$ receptor stimulation. Similar mechanisms may regulate the release of glutamate in both areas of the brain. Dopamine and glutamate determine in concert the activity of the accumbal GABAergic, medium-size spiny neurons thought to act as an interface between the limbic cortex and the extrapyramidal motor system. These neurons project to the pallidal and mesencephalic areas, thereby mediating the behavioral reaction of the animal in response to a motivation-related stimulus. There is evidence that extracellular ADP/ATP promotes goal-directed behavior, e.g., intention and feeding, via dopamine, probably via $P2Y_1$ receptor stimulation. Accumbal P2 receptor-mediated glutamatergic mechanisms seem to counteract the dopaminergic effects on behavior. Furthermore, adaptive changes of motivation-related behavior, e.g., by chronic succession of starvation and feeding or by repeated amphetamine administration, are accompanied by changes in the expression of the $P2Y_1$ receptor, thought to modulate the sensitivity of the animal to respond to certain stimuli.

Introduction

Extracellular nucleotides and nucleosides have been identified as important classes of signaling molecules participating in diverse functions of neuronal and non-neuronal tissues, e.g., fast excitatory neurotransmission, nociception, platelet aggregation and astroglial cell function [1, 2]. However, the sources of extracellular adenosine 5′-triphosphate (ATP) and adenosine, and the mechanisms regulating their concentration outside the cell are not well understood. The vesicular release of ATP which is co-stored with classic transmitters such as acetylcholine, norepinephrine, and serotonine [3–5], the outflow of endogenous purines in response to neuronal activity [6–8], e.g., after activation of postsynaptic NMDA receptors [9, 10], and the stimulation of adenylate cyclase [11, 12] are all potential sources of extracellular adenine nucleotides. Further, the release from glial cells, activated immune cells or dying cells under physiological and pathophysiological conditions contribute to the extracellular occurrence of purines [13–15]. Once adenine nucleotides reach the extracellular space, they are subsequently converted to adenosine through the actions of

a multiplicity of ecto-nucleotidase enzymes [16]. Otherwise, adenosine can be released via bi-directional nucleoside transporters in response to different physiological stimuli (e.g., depolarization) or directly after metabolic stress from various cells [17, 18]. Nucleosides and nucleotides exert their biological function by activation of cell surface P1 and P2 receptors. The P1 receptors (A_1, A_{2A}, A_{2B} and A_3 subtypes) exhibit relatively high affinity for adenosine in contrast to ATP [19] and couple to G proteins, while P2 receptors have a high affinity for ADP/ATP in contrast to adenosine [20, 21]. The P2 receptors belong either to the $P2X_{1,2,3,4,6,7}$ subclasses (ligand-gated cationic channels) or to the $P2Y_{1,2,4,6,11,12,13,14}$ subclasses (G protein-coupled receptors) [20, 22, 23] both widely distributed in peripheral tissues and the nervous system [24–26]. Increasing evidence has been provided by *in vitro* experiments in the peripheral and central nervous system that the extracellular signaling molecules ATP as well as adenosine contribute to the modulation of the release of, e.g., acetylcholine [27], noradrenaline [28–31], serotonine [32, 33] and dopamine [34]. ATP, co-released with noradrenaline from sympathetic axon terminals, may exert a presynaptic feedback mechanism in which released ATP modulates the subsequent co-transmitter release [35].

Electrophysiological investigations also proved a P2 receptor-mediated release of excitatory amino acids. Inhibitory effects of ATP on the release of glutamate were

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described for hippocampal slices and cultured neurons [36, 37]. In cultured Schwann cells [38], trigeminal mesencephalic nerve terminals in the brain stem [39], and primary afferent terminals in spinal cord slice preparations [40], ATP caused a facilitation of glutamate release. In these *in vitro* studies, the non-selective P2 receptor antagonists PPADS and suramin both antagonized the ATP-evoked effects.

The negative feedback regulation of transmitter release is an inherent property of ATP [41]. The metabolic breakdown of adenine nucleotides to adenosine, shown to inhibit the release of various transmitters [42], may interfere with the effects of the nucleotides themselves on neuronal transmitter release.

Modulation of neurotransmitter release by P2 receptors *in vivo*

Data on the consequences of P2 receptor stimulation on the release of neurotransmitters in the brain *in vivo*, the involvement of specific P2 receptor subtypes and especially on the subsequent functional outcome, e.g., on behavior, are rare. Different reasons may contribute to this situation. One cause may be the lack of agonists or antagonists that discriminate with sufficient selectivity between the P2X and P2Y receptor family or between their respective subtypes [20]. Further, there are no compounds selective for particular P2 receptors which are able to pass the blood–brain barrier and therefore can be applied systemically. Additionally, ligands at P2 receptors have different stability in the presence of degrading ecto-nucleotidases [16, 43, 44]. This highlights the problems conflicting the identification of P2 receptor subtypes involved in purinergic signaling *in vivo*.

First studies on cerebral transmitter release used ATP as the natural agonist and compared the potency order of related structural analogs by microdialysis.

Using this approach, Zhang and co-workers [45] have shown in the awake rat that ATP dose-dependently increases the striatal extracellular level of dopamine. Structural analogs of ATP increased the striatal dopamine concentration in the rank order of potency, 2-methylthio ATP (2-MeSATP) > ATP ≥ α,β-methylene ATP > ADP > AMP > adenosine. The most potent agonist 2-MeSATP clearly prefers $P2Y_1$ and $P2Y_{12}$ receptors compared to ATP but has a similar potency at the $P2X_1$ receptor; the enzymatically stable analog α,β-methylene ATP does not act at P2Y receptors and is most effective at $P2X_1$ and $P2X_3$ receptors [46]. Hence, these data suggest that receptors of the P2Y rather than of the P2X subtype may be involved in the increase of extracellular dopamine release in the striatum. The ectonucleotidase-resistant adenosine 5′-[β,γ, imido]-triphosphate caused a more prolonged increase of the dopamine level than ATP itself. Therefore, by their actual local activity, the different ectonucleotidases may be strong inhibitors of effects evoked by a rise of extracellular nucleotides. The ATP-induced release of dopamine [45] was depressed by

the non-selective P2 receptor antagonist suramin and the P2Y receptor antagonist reactive blue 2, but not by xanthine amine congener, a non-selective adenosine receptor antagonist. Suramin and PPADS are non-selective, but non-universal, P2 receptor antagonists. Whereas suramin interacts also with a large range of other proteins, including glutamate, GABA and 5-hydroxytryptamine receptors as well as various enzymes, PPADS is highly specific for P2 receptors [47], e.g., without affecting glutamate receptors [48, 49]. Further, Zhang and colleagues [45] have shown that the ATP-induced dopamine release was sensitive to pertussis toxin, suggesting that a G-protein coupled receptor is involved. The dopamine release could be abolished by tetrodotoxin, Ca^{2+}-depletion and ω-conotoxin GVIA, indicating that the opening of voltage-sensitive Na^+ channels and the Ca^{2+} influx through the N-type voltage-dependent calcium channel are both required for these P2 receptor mediated effects. In another study, the perfusion of the hippocampus with ATP produced concentration-dependent changes in hippocampal extracellular serotonin levels, likewise, which consisted of an initial rise phase, followed by a later rebound reduction phase [50]. 2-MeSATP increased the extracellular serotonine level dramatically, while α,β-methylene ATP produced only a slight increase.

Under special focus are studies on the physiological importance of P2 receptor-mediated effects on the release of various transmitters in the mesolimbic–mesocortical system, which is relevant for the generation and expression of motivation-related behavior. The central part of this circuit consists of inhibitory dopaminergic projections from neurons in the ventral tegmental area (VTA), activated by, e.g., environmental stimuli, as well as of excitatory corticofugal glutamatergic terminals from the prefrontal cortex [51, 52] which target the GABAergic medium size spiny projection neurons, the main cell type of the nucleus accumbens (NAc) [53]. These neurons are thought to act as an interface between the limbic cortex and the extrapyramidal motor system, and project to pallidal and mesencephalic regions including the VTA [54, 55]. Therefore, the modulation of accumbal activity by changing the balance of neurotransmitters or neuromodulators could result in altered behavioral responses. In this self-contained neuronal circuit, P2 receptors are involved in the release of transmitters.

The administration of 2-MeSATP into the somatic or axon terminal area of dopaminergic neurons in the VTA and NAc, respectively, caused a dose-dependent increase in the extracellular dopamine concentration [56–58]. In both regions, the infusion of the P2 receptor antagonists reactive blue 2 or PPADS not only abolished the 2-MeSATP-induced effects but also reduced the basal extracellular concentration of dopamine, indicating that endogenous extracellular ATP tonically modulates the somatodendritic dopamine release as well as that from axon terminals.

In addition to the inhibition of various P2X receptors, the non-selective P2 receptor antagonist PPADS acts efficiently at the $P2Y_1$ receptor subtype, whereas it is completely

ineffective at the $P2Y_{11}$ and the $P2Y_{12}$ receptors [59, 60]. Other P2Y receptors are not or only slightly affected by concentrations of PPADS higher than those used in the *in vivo* studies [22, 61, 62]. It has been shown that P2Y receptors, which are positively coupled to phospholipase C, but not those negatively coupled to adenylate cyclase, were inhibited by PPADS [63]. Therefore, it is tentatively suggested that the effects of endogenous ADP/ATP on transmitter release in the NAc may be mediated by the activation of $P2Y_1$ receptors leading to a stimulation of phospholipase C, the production of inositol-1,4,5-trisphosphate and the mobilization of intracellular Ca^{2+} resulting in a stimulation of neuronal exocytosis. This assumption was supported by microdialysis experiments using compounds with higher selectivities. Since, the $P2Y_1$ receptor is more sensitive to adenosine nucleotide diphosphates than to triphosphates, the $P2Y_{1,11,12,13}$ receptor-selective agonist ADPβS was infused into the NAc. ADPβS caused a concentration-dependent enhancement of dopamine in the extracellular space of the NAc shell region. This effect was abolished by co-perfusion with the selective $P2Y_1$ receptor antagonist MRS 2179 [64, 65], which also reduced the basal dopamine concentration when given alone. ADPβS also affects the $P2Y_{12}$ receptor [66]. However, when the selective $P2Y_{12}$ receptor antagonist AR-C 69931MX [67] was perfused together with ADPβS to eliminate the involvement of $P2Y_{12}$ receptor-mediated effects, the amount of dopamine evoked by AR-C 69931MX alone was additive to that evoked by ADPβS alone [68]. Therefore, a facilitatory effect of ADP/ATP on the accumbal dopamine level by stimulation of $P2Y_1$ receptors can be assumed. Further, effects at of $P2Y_{12}$ receptors negatively coupled to adenylyl cyclase also seem to inhibit the accumbal dopamine level; cyclic AMP was shown to facilitate the release of transmitters by either protein kinase A-dependent [69, 70] or independent pathways [71]. The localization of $P2Y_1$ receptor immunoreactivity within the NAc [72] and the demonstration of mRNA expression for the $P2Y_1$ receptor by RT-PCR in this region [73, 74] provide further support for the involvement of $P2Y_1$ receptors in purinergic signaling in the mesolimbic system. Note, that on the typical tyrosine hydroxylase-positive cells in the VTA, an intensive $P2Y_1$ immunoreactivity on cell bodies as well as on their processes was found [57]. The dopamine released from the dendrites [75, 76] might interact with dopamine D_2 receptors localized on somata and/or dendrites of VTA neurons. This interaction may decrease the responsiveness to afferent inputs originating from neighboring cells [77, 78]. The modulation of the somatodendritic dopamine release by stimulation of P2 receptors may therefore provide an indirect local inhibitory control and fine adjustment of dopamine release. The administration of 2-MeSATP into the VTA also caused an increased release of dopamine from the terminals of mesolimbic dopaminergic neurons in the NAc indicating a stimulated firing pattern of the respective cell bodies [57]. Therefore, P2 receptor agonists may modulate the activity of dopaminergic cell bodies in the VTA both by local direct stimulation and/or indirect inhibitory control

via dopamine release and subsequent dopamine D_2 receptor stimulation in the NAc.

Granted that ATP is co-released together with dopamine [79–83], ATP may exert a reinforcing function on its own effects in response to neuronal stimulation.

Electrophysiological investigations provide further evidence for the facilitation of ATP effects in the mesolimbic system. The enhanced activity of dopaminergic afferents to the NAc induced by P2 receptor stimulation is accompanied by an enhancement of the electrical activity of this area – an elevation of the absolute EEG power and the power in the alpha-frequency band (8–13 Hz) [84, 85]. It has been documented that the power in the alpha-band was also enhanced by mesolimbic neuronal and subsequent behavioral stimulation in response to the novelty of an open field system [84] corresponding to increased attentiveness or vigilance [86]. The selective enhancement of the power in the alpha-band was also observed after systemic administration of apomorphine, quinpirole, D-amphetamine and cocaine leading to the stimulation of dopamine D_2 receptors [87, 88]. In the human EEG, the increase in the alpha-activity is associated with enhanced intentional behavior and hedonistic emotions [89].

In contrast to the effects of ATP in the NAc, adenosine decreased the extracellular dopamine concentration [90, 91] and simultaneously shifted the power in the EEG spectrum to the low frequencies, mainly to the delta band (0.6–4.0 Hz) [85]. Increasing amounts in the delta and theta activity correlating with a slowing in the EEG are usually taken as a sign of either sedation [92], drowsiness and sleep, or abnormality of brain function [93].

The functionally converse effects of 2-MeSATP and adenosine on the extracellular concentration of dopamine as well as on the changes in the EEG pattern in the NAc turned into their opposite by the local administration of the P2 receptor antagonist PPADS or the nonselective adenosine receptor antagonist 8-(*p*-sulfophenyl) theophylline (8-SPT), respectively. In addition to its local accumbal effects, adenosine also slowed down the firing pattern in the VTA neuronal population, probably by activating the inhibitory GABAergic feedback projection from the NAc to the VTA via stimulation of accumbal A_{2A} receptors [94–98]. In conclusion, the local balance between the stimulation of adenosine- and ADP/ATP-sensitive receptors seems to be determinative for the net functional outcome at the level of transmitter release and consequently at the level of behavior [85, 99].

The idea of a purinergic modulation of the inhibitory feedback projection is supported by the effects of low doses of NMDA or glutamate which were shown to reduce the basal and also the 2-MeSATP-induced dopamine release under *in vivo* conditions, when the cross-talk between NAc and VTA is intact [58]. The same study showed that in the presence of ionotropic and metabotropic glutamate receptor antagonists, the dopamine release induced by 2-MeSATP was potentiated, suggesting that P2 receptor stimulation also facilitates the release of glutamate which, under basal conditions, inhibits the facilitated dopamine release.

It is known that mesolimbic dopamine modulates the processing of concurrent glutamate inputs to dorsal and ventral striatal target regions [100] and that the mesolimbic dopamine release underlies the control of purinergic activity (see above). In the unilaterally dopamine-depleted rat NAc, the increase of extracellular glutamate levels evoked by 2-MeSATP exceeded that of the native NAc fivefold. As found for dopamine, the effect of P2 receptor stimulation on the extracellular concentration of glutamate was dose-dependent for 2-MeSATP and ADPβS and could be inhibited by PPADS as well as by MRS 2179 [101, 102]. Furthermore, the activation of dopamine D_2 receptors in the dopamine-depleted NAc by quinpirole decreased the 2-MeSATP-evoked glutamate level [101]. Therefore, the P2 receptor-mediated glutamatergic transmission in the NAc may be processed depending on the dopaminergic activity, likewise. However, extracellular levels of glutamate monitored by microdialysis reflect the net flux between neuronal and non-neuronal release as well as uptake into surrounding nerve terminals and glial elements [103, 104]. Furthermore, an ATP-evoked glutamate outflow by cultured astrocytes was demonstrated and has therefore to be taken into account in this *in vivo* approach [105, 106].

Modulatory role of P2 receptors in explorative locomotor behavior

The dopaminergic and glutamatergic transmission in the mesolimbic–mesocortical system plays a fundamental role in facilitating the ability of motivational stimuli to elicit behavioral activation [107]. The NAc is generally accepted to be the main link between the limbic cortex and the extrapyramidal motor system modulating motivation and goal-directed locomotor activity [108]. The NAc receives a dense mesolimbic inhibitory dopaminergic innervation from the VTA [109] and excitatory glutamatergic afferents from the medial prefrontal cortex [110, 111], the amygdala and subcortical structures [53, 112, 113]. Depending on the strength of input, the activity of the main accumbal cell type, the GABAergic medium spiny neurons, and subsequently the generated motor behavior are differentially influenced [100, 114]. Based on the above-mentioned studies on P2 receptor-mediated transmitter release, it may be assumed that extracellular ATP alters the behavioral pattern via modulation of the neuronal activity of the NAc and VTA. The stimulation of P2 receptors by microinjection of 2-MeSATP into the rat NAc was characterized by a prolonged, more consistent and stronger directed locomotor activity in response to the stimulus of a novel environment [84, 115], an effect similar to that of ATP in frog embryos [116].

Hence, the 2-MeSATP effect was indistinguishable from the action of dopamine itself [117] or of other drugs known to release dopamine in the NAc like amphetamine [118]. Further, the time of increased power of the alpha-frequency band of the EEG corresponding to enhanced dopamine release as a response to the new environment

[119] was extended by 2-MeSATP. An inhibitory glutamatergic tone may be facilitated by 2-MeSATP as well, because this substance caused a reduction rather than a stimulation of the behavioral activity and the EEG in the dopamine-depleted rat. This assumption is supported by the stimulatory effects of NMDA receptor-antagonists on locomotor behavior after systemic and striatal administration [120]. The 2-MeSATP-induced effects on the locomotor behavior could be abolished by PPADS and reactive blue 2 excluding non-receptor mediated actions. In these experiments, only reactive blue 2, but not PPADS, enhanced the locomotor activity in the 5th to the 10th-min interval, possibly because of the affinity of reactive blue 2 to NMDA and APMA/kainate receptors [48, 121]. Further, the effect of 2-MeSATP was also abolished by the concomitant inhibition of dopamine D_1 and $D_{2/3}$ receptors, suggesting that the enhanced intentional behavior after accumbal injection of an ATP analog may be due to a P2 receptor-mediated dopamine release. Recent studies have shown that dopamine in the NAc may be involved in the processes that enable organisms to overcome work-related response costs in instrumental responding [122]. By facilitation of accumbal dopamine, probably via $P2Y_1$ receptors, extracellular ADP/ATP may be involved therefore in the drive of motivation-directed behavior.

However, the activity of the GABAergic projection neurons terminating in the ventral pallidum [123] may be modulated not only by activation of dopamine D_1 and/or $D_{2/3}$ receptors causing a behavioral stimulation but also by activation of glutamatergic receptors leading to an enhanced excitation of the GABAergic neurons with a subsequent behavioral inhibition. Therefore, it can be expected that the stimulation of P2 receptors by ADP/ATP or respective agonists as well as their inhibition by PPADS and subsequent decrease of glutamate levels may both enhance the locomotor activity in the open-field situation [115]. However, the pattern of movement caused by the two pharmacological manipulations was different. In contrast to the more goal-directed locomotor activity evoked by 2-MeSATP or the D_2 dopamine receptor agonist quinpirole, the locomotion induced by PPADS was characterized by a high number of direction changes, many disruptions of movement and a higher running speed, similar to effects caused by antagonists at the glutamatergic NMDA and AMPA/kainate receptors. In view of the above-mentioned decrease of extracellular glutamate levels [101], a reduced activation of the GABAergic projection may be one possible pathway involved. In another study, it has been shown that the stimulation of P2Y receptors can facilitate NMDA-induced currents in layer V pyramidal neurons of the rat prefrontal cortex [124]. Therefore, it is also conceivable that PPADS in the NAc led to an attenuation of NMDA-evoked effects and in this way to a stimulation of the locomotor activity comparable to the effects of glutamate receptor antagonists.

The above data show that the stimulation of P2 receptors in the NAc may not mediate motivational behavior per se. The administration of ADPβS into the NAc not only

elevated the level of accumbal dopamine but also enhanced the locomotor activity in the open field which can be blocked by the P2Y$_1$ receptor antagonist MRS 2179 (U. Krügel, unpublished observation). Therefore, it can be suggest, that the degree of stimulation of P2 receptors, probably mainly of the P2Y$_1$ receptors, mediating the dopaminergic and glutamatergic accumbal neurotransmission, generate different patterns of locomotion with consequences are relevant for the drive of motivation, goal direction and possibly to overcome anxiety. In this context, it has to be mentioned that after application of ADPβS into the lateral ventricle, rats showed an enhanced explorative activity in the open arms of an elevated plus maze indicating anxiolytic-like effects whereas MRS 2179 caused anxiogenic effects [125].

P2 receptors are also involved in adaptive changes of motivation-related behavior after chronic stimulation of the mesolimbic system. A repeated intra-tegmental injection of 2-MeSATP into the VTA, evoking the release of somatodendritic dopamine, followed by exposure to the open-field situation and a challenge with D-amphetamine after a priming phase-induced hypersensitivity to the behavioral effects caused by the novel environment [57]. Systemic application of D-amphetamine caused an enhanced locomotor activity, which could be blocked by pre-application of PPADS into the lateral ventricle. In addition, when PPADS was applied every time before repeated D-amphetamine treatment, the known behavioral sensitization was prevented, indicating the involvement of P2 receptors in the development and expression of behavioral sensitization to D-amphetamine [126]. It has been shown that after systemic application of D-amphetamine, diadenosine polyphosphates Ap4A and Ap5A and probably co-stored ATP were released together with dopamine [127]. These polyphosphates are agonists at P2 receptors, and the response on behavior subsequent to their stimulation may be prevented by the given P2 receptor antagonist.

An immunohistochemical study has shown that behaviorally amphetamine-sensitized rats expressed an enhancement of P2Y$_1$ receptor immunoreactivity on astrocytes and neurons as well as and astrogliosis. Both reactions were prevented by pretreatment with PPADS, suggesting a crucial involvement of P2Y$_1$ receptors also in behaviorally sensitizing processes [72].

Feeding is facilitated by endogenous ADP/ATP in the NAc

The different pathways in the P2 receptor-mediated regulation of the finally expressed pattern of locomotor activity caused by endogenous or exogenous stimuli may have consequences for feeding behavior. A decreased anxiety and enhanced goal-direction of movements may be suitable for the forage of the animal. For this background that accumbal dopamine may be involved in the threshold to spend cost in response to a rewarding stimulus [128], ADP/ATP are thought to promote the decision of the animal to feed via an enhancement of

accumbal extracellular dopamine. Previous studies [129, 130] have shown that dopamine release is increasing during feeding. This finding agrees with changes in the simultaneously recorded EEG to an enhanced power in the alpha-frequency band in the initial phase of the feeding both in the NAc and VTA [131]. The EEG changes in the VTA correlate with the somatodendritic dopamine release in the cell body region during feeding, showing that the mesolimbic pathway is activated by ingestive behavior [132].

Our group [133] could show by microdialysis experiments that the inhibition of accumbal P2 receptors by PPADS suppressed not only the feeding-evoked release of dopamine but also the amount and duration of food intake. It is suggested that in a situation when the stimulation of dopamine D$_1$ and D$_{2/3}$ receptors is low because of a PPADS-induced decrease in basal dopamine release, the impetus to take the presented food is reduced. Therefore, the efficiency of stimulation of P2 receptors has to be considered as an important trigger for feeding. The inhibition of P2 receptors by PPADS may lead to a predominance of adenosinergic mechanisms possibly responsible for the suppression of feeding-induced dopamine release and neuronal activity. Both effects were eliminated by the non-selective adenosine receptor antagonist 8-STP [131]. The inhibition of the multisynaptic inhibitory feedback projection to the VTA [134] by 8-SPT was shown to enhance extracellular dopamine levels [135] and to enhance feeding by inhibiting adenosine A$_{2A}$ receptors [136]. P2 receptors of the P2Y$_1$ receptor-subtype may represent the main pathway in the purinergic facilitation of feeding. Whereas the P2X receptor preferring agonist α,β-methylene ATP had no effect on the amount of food intake and duration of ingestion behavior, both parameters were enhanced by administration of ADPβS into the lateral ventricle of rats and could be abolished by co-application of PPADS [137].

However, the expression of a certain motivation-related behavior seems to be also determined by the balance of the dopaminergic and glutamatergic input to accumbal GABA neurons [100] as was reported for intentional locomotion. It has been repeatedly shown [138, 139] that glutamate antagonists elicit intense feeding in satiated rats mainly by inhibiting NMDA and non-NMDA receptors in the NAc region. Therefore, the local reduction of extracellular glutamate levels by PPADS [101] may have direct effects on the ingestive behavior. Using P2Y receptor antagonists as pharmacological tools in intact neuronal circuits, functional glutamate antagonistic effects, probably depending on the used dose and the basic level of 'mood', may facilitate the behavioral response to a motivation-related stimulus.

It has been shown that repeated food restriction – the succession of malnutrition ('hunger') and re-feeding – leads to sensitization [140, 141]. These experimental conditions imitate the sequence of events occurring during different human eating disorders [142]. In such a restricted feeding model for rats the P2Y$_1$ receptor mRNA was initially reduced, possibly related to the aversive or stressor effects

of the impaired energy balance [73]. However, a long-lasting food deprivation caused an increase of the P2Y$_1$ receptor mRNA in the accumbal region, thought to be a reflection of reduced stimulation of P2 receptors, in correlation to the reported reduced accumbal release of dopamine in food deprived rats [140]. It can be assumed that these adaptive changes in the P2 receptor expression are aimed to enhance the sensitivity of these receptors to respond to motivation-related stimuli like food.

Conclusions

In conclusion, P2 receptors of the mesolimbic–mesocortical system, probably of the P2Y$_1$ subtype, are involved in the release of various neurotransmitters, e.g., of dopamine and glutamate. Changes in the extracellular concentrations of these neurotransmitters are at least partly responsible for the generation and pattern of the behavioral outcome after motivation-related stimuli. Summarizing the presented data, ADP/ATP-evoked glutamate release in the NAc may contribute to a limitation of the urge to respond to a dopamine-supported stimulation of goal-directed behavior.

Furthermore, there is a functionally relevant balance between extracellular ADP/ATP and adenosine levels. Changes in the degree of the stimulation of their respective receptors cause a predominance of motivationally stimulatory (e.g., via P2Y$_1$ receptors) or depressant effects (via adenosine receptors) effects. Adaptive changes of motivation-related behavior, e.g., by chronic succession of starvation and feeding or by repeated amphetamine administration, are accompanied by changes in the expression of the P2Y$_1$ receptor, thought to modulate the sensitivity of the animal to respond to certain stimuli.

Acknowledgements

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