

● PERSPECTIVE

Regulation of neural stem/progenitor cell functions by P2X and P2Y receptors

Neural stem/progenitor cells: Radial glial cells constitute multipotent cells in the ventricular zone, lining the wall of the lateral ventricle of the embryonic brain. They have the capacity to give rise to cells belonging to all three major lineages (neurons, astrocytes and oligodendrocytes) of the nervous system (Tang and Illes, 2017). Contrary to the long-held dogma, persistent neurogenesis throughout life occurs in two specific neurogenic niches of the adult brain: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the hippocampal dentate gyrus (Illes et al., 2013). Although there is no general consensus on terminology, we will call the embryonic radial glia and its progeny “neural stem cells” (NSCs) and their adult counterparts “neural progenitor cells” (NPCs).

The purinergic system: Adenosine triphosphate (ATP) is utilized not only for the intracellular storage of energy, but it functions also as a transmitter/cotransmitter in neuro-neuronal or neuron-effector synapses after vesicular secretion; further, ATP is a signaling molecule coordinating the activities of non-neuronal cells in the central and peripheral nervous systems. ATP is released from astrocytes by various pathways such as by pannexin and connexin hemichannels, the latter ones providing the substrate for gap junction formation. Additional exit pathways are the ATP-binding cassette (ABC) proteins, such as the multidrug resistance-associated protein, osmolytic transporters linked to anion channels, and also vesicular release mechanisms (Illes and Ribeiro, 2004). Calcium waves in astrocytes were shown to be initiated and supported by the release of ATP leading to the stimulation of P2Y_{1,2} receptors (Rs) at neighboring astrocytes. An alternative possibility is the diffusion of the Ca²⁺ secretory inositol-1,4,5-trisphosphate (InsP₃) from one astrocyte to the other one through the connecting gap junctions. Calcium waves are defined as oscillations of intracellular free Ca²⁺ that propagate in astrocytic networks and are considered to constitute an information-processing system operating in parallel with neuronal circuits. After having been released, ATP stimulates two types of purinoceptors, the ligand-gated cationic channel (P2X; seven mammalian subtypes: P2X_{1–7}) and the G protein coupled receptors (P2Y; eight mammalian subtypes: P2Y_{1,2,4,6,11–14}) (Köles et al., 2011). Subsequent to receptor-activation ATP is degraded by a family of ecto-ATPases to the active metabolite adenosine, which is eventually enzymatically inactivated to inosine. While P2XRs respond to ATP only, P2YRs respond also to adenosine diphosphate (ADP), uridine-5'-triphosphate (UTP) and uridine 5'-diphosphate (UDP).

Stimulation of proliferation, migration and differentiation by P2Y receptor activation: Cells isolated from the neurogenic zones of embryonic and adult rodents, can be cultured in

medium supplemented with growth factors, such as epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) to yield neurospheres (free floating clusters of NSCs), and after subsequent plating, adherent cultures of proliferating NSCs/NPCs (Tang and Illes, 2017). Fetal midbrain (m) tissue from humans (h) can also be used to generate hmNSCs in EGF and FGF-2 enriched culture medium (Rubini et al., 2009). Whole-cell patch clamp recordings from plated hmNSCs allowed their electrophysiological classification into two types: Whereas one of them did not fire action potentials on the injection of depolarizing current pulses, the other one fired under the same conditions a rudimentary, tetrodotoxin-sensitive spike. In spite of these differences in the endowment with voltage-dependent Na⁺ currents, neither cell type responded to the application of ATP, excluding the presence of P2XR-channels. However, Ca²⁺-imaging documented the presence of P2YRs. Superfusion with higher concentrations of ATP/ADP and UTP/UDP increased the level of intracellular Ca²⁺ ([Ca²⁺]_i), in a PPADS and MRS2179-antagonizable manner; these two substances are preferential and selective antagonists of P2Y₁Rs, respectively. It has also been shown that UTP releases ATP from hmNSCs by P2Y₂R stimulation; this ATP then acts at the P2Y₁Rs mentioned. Already low concentrations of ATP caused oscillations of [Ca²⁺]_i, which are supposed to enhance cell proliferation, migration and differentiation. Further, UTP, but not ATP induced the dopaminergic differentiation of these hmNSCs. A profound increase in the number of tyrosine hydroxylase (rate limiting enzyme in the synthesis of dopamine) immunopositive cells was observed after incubation with UTP.

It has been shown that culturing of rat striatal neurons in neurobasal medium (NBM), but not in dulbecco's modified eagle medium (DMEM), led to the loss of their ability to fire trains of action potentials in response to depolarizing current injection (Rubini et al., 2006). ATP induced similar increases of [Ca²⁺]_i both in neurons and astrocytes; PPADS and MRS2179 blocked the effect of ATP, by identifying P2Y₁Rs as its site of action. The depletion of intracellular Ca²⁺ pools by cyclopiazonic acid markedly depressed the effect of ATP. The remaining component of the ATP-induced [Ca²⁺]_i transients was due to the influx of Ca²⁺ via storage-operated channels (SOCs) located in the plasma membrane. Interestingly, NBM-cultured neurons co-stained for the stem cell marker nestin, the astrocyte/stem cell marker glial fibrillary acidic protein (GFAP), and for the neuronal marker microtubule-associated protein 2 (MAP2). These experiments show that brain neurons in culture might de-differentiate and adopt stem cell/astrocyte-like properties.

Induction of necrosis/apoptosis by P2X₇ receptors: Cultured NPCs prepared from the SVZ of mice responded to ATP and dibenzoyl-ATP (Bz-ATP) with inward currents, which largely increased in the presence of a low Ca²⁺/no Mg²⁺ (low X²⁺)-containing external medium (Messemer et al., 2013). Moreover, this current response was abolished by the selective P2X₇R antagonist A-438079, and was missing in NPCs obtained from P2X₇^{-/-} animals. All these findings, combined with the higher potency of Bz-ATP when compared with that of ATP, unequivocally identify the involved receptor as belonging to the P2X₇ subtype. The stimulation of P2X₇Rs is known to immediately

open a non-selective cationic channel; a long-lasting or repetitive application of ATP/Bz-ATP leads to the formation of a wide membrane pore of about 900 Da size, due to channel dilation or the recruitment of pannexin-1 hemichannels. In consequence, certain fluorescent dyes such as Yo-Pro-1 or ethidium bromide, which have been shown to be unable in entering the cell *via* the cationic P2X7R channels, pass the membrane pore and become available in the intracellular space for DNA intercalation and fluorescence light emission. In fact, we found that after long-lasting ATP superfusion, both the development of a second slow current component following the early rapidly rising one, and the measured Yo-Pro-1 fluorescence proved the opening of wide-diameter membrane pores in the SVZ NPC cell membrane.

The opening of the membrane pore could cause osmotic shock and on a somewhat longer term the loss of cytoplasmic ingredients of vital importance. The MTT cell viability test documented the death of a large fraction of SVZ-NPCs after incubation with Bz-ATP. This effect was mediated by P2X7Rs, since NPCs taken from P2X7^{-/-} mice failed to decrease under the same conditions. Programmed cell-death (apoptosis) was also triggered by P2X7R stimulation; Bz-ATP incubation triggered the activation of active-Caspase-3, a key enzyme of the apoptotic signaling cascade (Ulrich and Illes, 2014; Oliveira et al., 2016).

The drawback of investigations on cultured NPCs is that these cells may alter their properties due to the culturing procedure and the presence of excessively high growth factor concentrations in the medium. Experiments carried out “*in situ*”, on brain slices prepared from Tg (nestin-EGFP) mice can be easily identified under a fluorescence microscope and appear to deliver more reliable results. For example, in the SGZ of the dentate gyrus, the NPCs remain in their natural environment, receive the normal neuronal and humoral inputs, and in consequence are expected to maintain their original state and reactivity.

With a similar approach as used for the experiments with SVZ-NPCs of Tg (nestin-EGFP) mice (agonist potency, selective antagonists, low X²⁺ medium), we proved the presence of P2X7Rs in SGZ-NPCs, as well (Rozmer et al., 2018). In addition, we found that pilocarpine-induced massive seizures (“status epilepticus”) *in vivo*, increased the sensitivity of NPCs against Bz-ATP, when measured *in situ* in hippocampal slice preparations. Incubation of the slices with pilocarpine for longer time periods also resulted in larger Bz-ATP current amplitudes, an effect which could be prevented by tetrodotoxin, a blocker of voltage-sensitive sodium channels. This finding suggested that seizures increase neuronal firing in the dentate gyrus with a resulting release of ATP and other neurotransmitters/mediators onto P2X7Rs of NPCs, resulting in facilitated receptor function. In addition to P2X7R currents also P2Y1R currents were functionally up-regulated by pilocarpine-induced seizures or incubation with pilocarpine. On the basis of our data we hypothesized that seizures cause excessive proliferation of NPCs, which are further facilitated by P2Y1R activation. This excessive proliferation leads to the migration of some NPCs to ectopic locations in the hilus hippocampi, where they generate granule cells orchestrating pathological neuronal firing; this scenario causes the transition of a one-time seizure activity into chronically persisting, recurrent epileptic fits. Our hypothesis explains the maturation of juvenile febrile convulsions to temporal lobe epilepsy in adult humans.

Conclusion: Purinoceptors may modulate NPC proliferation, migration, differentiation and necrosis/apoptosis, and thereby interfere with fundamental (patho)physiological processes in the brain and spinal cord. The pharmacological activation or blockade of purinoceptors could be a major therapeutic maneuver to favorably influence a great number of neurologic/psychiatric illnesses.

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