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The nigrostriatal DA pathway and Parkinson's disease

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Summary. The discovery of the nigrostriatal DA system in the rat was made possible by the highly specific and sensitive histochemical fluorescence method of Falck and Hillarp in combinations with electrolytic lesions in the substantia nigra and removal of major parts of the neostriatum. Recent work on DA neuron evolution shows that in the Bottlenose Dolphin the normal DA cell groups of the substantia nigra are very cell sparse, while there is a substantial expansion of the A9 medial and A10 lateral subdivisions forming an impressive ''ventral wing'' in the posterior substantia nigra. The nigrostriatal DA pathway mainly operates via Volume Transmission. Thus, DA diffuses along concentration gradients in the ECF to reach target cells with high affinity DA receptors. A novel feature of the DA receptor subtypes is their physical interaction in the plasma membrane of striatal neurons forming receptor mosaics (RM) with the existence of two types of RM. The ''functional decoding unit'' for DA is not the single receptor, but rather the RM that may affect not only the integration of signals in the DA neurons but also their trophic conditions. In 1991 A2A receptor antagonists were indicated to represent novel antiparkinsonian drugs based on the existence of A2A/D2 receptor–receptor interactions and here P2X receptor antagonists are postulated to be neuroprotective drugs in treatment of Parkinson's Disease.

Historical introduction

The development of the Falck-Hillarp method for the localization of catecholamines (CA) and serotonin (5-hydroxytryptamine, 5-HT) at the cellular level (see Carlsson et al., 1962) led to the demonstration of CA cell bodies in the substantia nigra (Dahlström and Fuxe, 1964a). These CA cell bodies most likely represented dopaminergic (DA) neurons, this statement being based on the biochemical correlates of this region of the brain (see Anden et al., 1964). Processes from these then putative DA cells were found to enter the zona reticulata and were mainly interpreted as DA axon bundles; however, they were later shown to mainly represent DA dendrites (Björklund and Lindvall, 1975). In the neostriatum, nucleus accumbens and olfactory tubercle, a densely packed punctate to diffuse CA fluorescence was found, this probably representing densely packed DA nerve terminals based on a pharmacological analysis and biochemical correlates (Fuxe, 1965a, b). With the development of a method for demonstration of monoamine containing nerve fibres based on axotomy with the accumulation of monoamines in the fibres on the

cell body side of the lesion (Dahlström and Fuxe, 1964b), it became possible, using strategically placed lesions, to trace the DA axons from the DA cells of the substantia nigra to the DA terminal regions of the striatum. The DA axons reached the crus cerebri, formed tracts in the capsula interna and entered the fibrae capsulae internae (Anden et al., 1964, 1965). These studies represented the first mapping of the nigrostriatal DA pathway in the rat, and subsequently it was shown that the monkey DA pathway was quite similar (Battista et al., 1972). The nigrostriatal DA pathway in the rat is schematically shown in Fuxe et al. (1985). The islandic striatal DA nerve terminal system was described in detail in 1972 (Olson et al., 1972; Tennyson et al., 1972), but was first reported by Fuxe in 1970 (see Fuxe et al., 1971). It mainly originates from the ventral tier of the substantia nigra, and contains calbindin negative nigral DA cells (see Gerfen, 2004), appears to be mainly involved in motivational learning (see Agnati et al., 2003a), and effects its action mainly via D1 and D4 receptors (Rivera et al., 2002; Agnati et al., 1988).

In the course of this early work plastic responses were found in the nigral DA cells after striatal ablation, which demonstrated early increases (2 days) in size and DA fluorescence intensity, followed in time (28 days) by shrinkage and disappearance of DA fluorescence in the DA cells of the zona compacta (Anden et al., 1965). These qualitative descriptions of plasticity in DA nerve cells were later on quantitatively described by Agnati et al. (1984) using morphometry and microdensitometery techniques (see also Janson et al., 1991). In the latter paper hypertrophy of DA cells could be observed in the vervet monkey after long term ventromedial tegmental lesions, representing compensatory responses to partial lesions of the nigrostriatal DA pathway. One of the most beautiful examples of plasticity in CA and 5-HT terminal systems are found in the cerebellum following endothelin 1 induced ischemic lesions causing hyperinnervation of the remaining granular cell islands in the lesioned cerebellar area (Fuxe et al., 1993). The microdensitometry and microfluorimetry of DA fluorescence disappearance after tyrosine hydroxylase inhibition in DA cell groups and DA terminal systems has had a strong impact on the discovery of discrete DA turnover changes in the brain in pharmacolcogical, physiological and pathophysiological experiments (Agnati et al., 1980a), providing a novel understanding of their function, and as targets for drug action.

The nigrostriatal DA pathway and brain evolution

Recently, Manger et al. (2004) have studied the distribution and characteristics of DA cells in the midbrain of the bottlenose dolphin revealed with tyrosine hydroxylase immunohistochemistry. It was observed that many components of the A9 DA nuclear complex were only weakly developed, with a minimal number of cells in the pars compacta, pars lateralis and pars ventralis. In contrast, the medial A9 and lateral A10 cell groups had merged and expanded to form a massive ventral wing of DA cells in the posterior substantia nigra, which may reflect the need for a DA modulation of whole body movements versus the specialized limb movements of other mammalian orders. As discussed already by Manger et al. (2004, see also Manger, 2005) it seems possible that the subdivision of CA cell groups and of other immunohistochemically identifiable transmitter cell groups may be the same within the same phylogenetic order of mammals. The above architecture of DA cell groups may therefore be typical for all cetaceans and represent an evolutionary trend that would be independent of brain size, phenotype and lifestyle. In support of this view Manger's group has recently found that the highveld molerat exhibits the same nuclear parcellation

of DA, 5-HT, and cholinergic cell groups as the laboratory rat, in spite of a significantly regressed visual system, an unusual circadian rhythm, and a subterranean behavioural phenotype (Da Silva et al., 2006). This line of investigation is beginning to allow us to understand how the DA system may be changing in the course of brain evolution and how this specifically relates to the emergence of novel phylogenetic orders of mammals. The next step will be to see how these changes affect the DA axonal trajectories and the DA terminal network, including the size and DA contents of their varicosities in various target regions and thus regional DA transmission. Understanding the evolutionary processes and occurrence of changes in the DA system is of importance when attempting to project results obtained in animal models to pathological conditions found in humans.

The nigrostriatal DA pathway and communication

Since the first indications of their existence (Fuxe, 1965a, b), the DA varicosities have been regarded as the sites for storage, synthesis and release of DA, all being originally regarded as representing synaptic terminals. However, after action of DA releasing compounds like amphetamine, a diffuse specific DA fluorescence, probably representing an extracellular fluorescence, appeared around DA cell bodies and dendrites in the midbrain (Fuxe and Ungerstedt, 1970), indicating that DA released by amphetamine action could reach the extracellular space. In 1975 Descarries et al. showed that the majority of the cortical 5-HT varicosities were asynaptic. In view of the indications that CA can be released from all varicosities (Malmfors, 1965; Fuxe, 1965a, b) it seemed likely that DA may not only operate via synapses but also via asynaptic varicosities, which seemed likely to be the major mode of communication in the partially DA denervated striatum (Fuxe, 1979). Based on a lack of correlation of the regional distribution of beta endorphin and enkephalin terminals and their opiate receptors, Agnati et al. (1986) introduced the concept of two principal modes of communication in the CNS: (1) volume; and (2) wiring, transmission (VT and WT). VT mainly takes place via the extracellular fluid and WT mainly via synapses. The electronmicroscopic observations that extrasynaptic striatal DA receptors and asynaptic striatal DA varicosities were in the majority made it clear that VT was the major mode of communication in the nigrostriatal DA pathway (Jansson et al., 2002), involving leaking DA synapses and asynaptic DA varicosities. These two transmission modes enables the DA filtering action on glutamate inputs to the striatal neurons, acting as a high pass filter (Agnati et al., 2005a).

In the nucleus accumbens shell, D1 receptor and TH immunoreactive (IR) terminal mismatches have been observed, where high densities of TH IR terminals, representing DA nerve terminals, surround D1 receptor rich rostrocaudal columns containing only few DA terminals, seen as patches in the transverse sections (Jansson et al., 1999). These results open up the possibility that DA may diffuse via concentration gradients into these patches to activate high affinity DA receptors involving distances of 100–200 μm. This migration process may be accelerated by the existence of pressure waves and temperature gradients causing movements of the ECF (Agnati et al., 2005b). Uncoupling protein 2, present in mitochondria, predict thermal synapses, since it produces a disappearance of the H^+ gradient with generation of heat (Horvath et al., 1999). It was therefore very significant that UCP2 rich terminal islands were in good register with the TH IR nerve terminals surrounding the D1 rich patches, with a high degree of overlap, but co-storage in the same DA terminal could not be determined (Rivera et al., 2006). It therefore seems possible that UCP2 may act to enhance the migration of DA into the D1 rich mismatch region. Strong UCP2 IR is

only located in discrete DA terminal systems in the ventral striatum and cerebral cortex which seem specialized for VT with large intensely TH IR varicosities (Rivera et al., 2006). These observations raise several novel aspects regarding the dynamics of DA VT in the brain.

By analyzing the migration of molecules in the brain with dual probe microdialysis, both probes in the striatum with an inter-probe distance of 1 mm, Hoistad et al. (2000) could not obtain evidence for long distance migration of intact 3H-DA, at least at physiological concentrations as studied in the out probe after chromatographic separation. Only indications for long distance migration of 3H-DOPAC and 3H-HVA were obtained. It is of interest that after DA denervation observations were obtained indicating an increased and specific clearance of 3DA derived compounds from the extracellular fluid into the brain circulation with changes in the blood-brain barrier, which is in line with the view that DA participates in the regulation of brain microcirculation (Iadecola, 1998).

The nigrostriatal DA pathway, receptor–receptor interactions and development of A2A antagonists for treatment of PD

The first indications for the existence of intramembrane receptor–receptor interactions were obtained in 1980 with substance P modulating the binding characteristics of high affinity 5-HT agonist binding sites in membrane preparations of the CNS, reflecting a possible increase in the number of high affinity 5-HT agonist binding sites (Agnati et al., 1980b). In 1981 it was possible to demonstrate that CCK-8 modulated the affinity and number of striatal D2 antagonist binding sites (Fuxe et al., 1981). In 1982 the receptor mosaic hypothesis of the engram was introduced (Agnati et al., 1982). Formation of supramolecular aggregates of receptors in the plasma membrane was postulated. This could affect the synaptic weight and be a mechanism for engram formation and thus represent the molecular basis for learning and memory. This work indicated that conformational changes in membrane receptors, induced via other receptors, could be produced not only via changes in membrane potential or via changes in their state of phosphorylation, but also via direct physical receptor–receptor interactions. The molecular basis was postulated to be heteromerization of the seven-transmembrane spanning G protein coupled receptors (Zoli et al., 1993). The first evidence came with the discovery of the GABA B heterodimer (see Marshall, 2001).

Two types of DA receptor mosaics (RM) can now be distinguished, namely DA RM1, formed by the same type or subtype of DA receptors and DA RM2, formed by DA receptors directly interacting with other types of transmitter receptors (GPCR or ion channel receptors) (see Agnati et al., 2003b, 2005a). DA receptor mosaics of a mixed type may also exist, these being formed by different receptors but with an RM1 present. RM1 can show cooperativity, since all receptors bind the same transmitter, in this case DA (Agnati et al., 2005a). These RM are located in the lipid rafts of the DA cells, where flottilin-1 seems to play an important role as an adapter protein (Jacobowitz and Kallarakal, 2004).

The A2A/D2 heteromeric receptor complex is of particular interest in relation to Parkinson's disease (PD), since it is located in the striato-pallidal GABA pathway, being the first neuron in the indirect pathway mediating motor inhibition when activated, and offers new treatment strategies for PD, as A2A inhibits D2 receptor recognition and signalling in the heteromeric complex (Ferre et al., 1991; Fuxe et al., 1998). Based on this work, A2A antagonists were postulated to be potential antiparkinsonian drugs acting by enhancement of D2 signaling. Unspecific adenosine receptor antagonists, like caffeine and theophyllamine, were in fact already shown in 1974 to enhance the motor activat-

ing effects of l-dopa and DA receptor agonists in rat models of PD (Fuxe and Ungerstedt, 1974), but the underlying mechanism was unknown. In view of the existence of the A1/D1 heteromeric complexes (Gines et al., 2000) in the direct GABA pathway inhibiting D1 recognition and signaling, the antiparkinsonian effects of these methylxanthines may be caused by a combined blockade of A1 and A2A receptors, enhancing D1 and D2 signaling, respectively. The evidence for the existence of A2A/D2 heteromers is based on coimmunoprecipitation experiments (Hillion et al., 2002), and by qualitative and quantitative assessment of fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET) (Canals et al., 2003; Kamiya et al., 2003). Zones of interaction for A2A/D2 heterodimers have been analyzed, and an involvement of epitope–epitope electrostatic interactions have been demonstrated. By means of mass spectrometry and pull-down experiments a positively charged Arg-rich epitope from the N terminal part of the third intracellular loop of the D2 receptor has been shown to electrostatically interact with two adjacent aspartate residues or a phosphorylated serine, both negatively charged in the C terminal part of the A2A receptor (Ciruela et al., 2004). It is of significance that Woods and coworkers (Woods et al., 2005) have found that the interface in the D1/NMDA heteromeric complex (Lee et al., 2002) may involve similar electrostatic interactions. Thus, the C terminus of the NR1-1 subunit contains an Arg-rich epitope that can interact with adjacent glutamates or a phophorylated serine in the C terminal part of the D1 receptor. This electrostatic interaction could therefore represent a fundamental mechanism for heteromerization and receptor–receptor interactions.

Apart from the intramembrane A2A/D2 interaction in the heteromeric receptor complex, with A2A reducing the D2 decoding mechanism inter alia over adenylate cyclase (AC) and L type voltage dependent Ca channels, there exists a crosstalk at the AC. At this level D2 inhibits via Gi/o the A2A activation of the AC and thus A2A signalling (Fuxe et al., 2001). In advanced PD, when D2 signaling is very low, the A2A antagonist may therefore still show certain antiparkinsonian actions by counteracting the exaggerated A2A signaling set free by the low D2 tone. However, A2A antagonists may not modulate the other D2 effectors, like the activation of inwardly rectifying potassium channels and inhibition of calcium channels. When discussing the A2A/D2 interaction at the membrane level it should be considered that the stoichiometry of the A2A/D2 heteromeric complex, as for the A2A/D3 heteromeric complex (Torvinen et al., 2005), is unknown in the striatum. However, even a DA receptor tetramer can become modulated by an A2A monomer or homodimer, since they may regulate the cooperativity in the tetramer (Agnati et al., 2005a; Torvinen et al., 2005). Finally, it may be that the integration of the adenosine and dopamine induced conformational changes in these RM may be different when geometry (spatial organization) is different, due to differential modulation of the cooperativity in the DA receptor tetramer (Fuxe et al., 2006).

The major antiparkinsonian action of A2A antagonists appears to be the increase in the therapeutic ratio of l-dopa and D2 agonists allowing, for example, a lowering of the l-dopa dose with reduced development of dyskinesias (see Fuxe et al., 2001, 2003a; Chase, 2004), which may be explained by the existence of the striatal A2A/D2 heteromeric complex, where A2A inhibits D2 signaling. However, it is difficult to understand why A2A antagonists alone can exert antidyskinetic actions in models of PD (Kanda et al., 1998), since D2 signalling will become increased. However, a hypothesis has been advanced (Antonelli et al., 2006) that l-dopa induced dyskinesias may substantially be produced by a change in the balance of A2A/D2 heteromers versus A2A homomers in the

plasma membrane due to l-dopa induced internalization of A2A/D2 heteromers. Thus A2A homomers become dominant and abnormal increases in A2A signalling will develop with increases in inhibition of protein phosphatase 1. The excessive phosphorylation of the abnormal RM formed by the l-dopa induced panorama of transcription factors will assist in their stabilization involving phosphorylated ionotropic glutamate receptors (Chase, 2004), and abnormal motor programs will be formed with the appearance of dyskinesias. In this way we can begin to understand the multiple actions of A2A antagonists responsible for its antiparkinsonian and antidyskinetic actions primarily involving the striato-pallidal GABA neurons in the indirect pathway.

The D1 enriched direct pathway overexpresses D3 receptors upon development of l-dopa induced dyskinesias (Bordet et al., 2000). It therefore seems possible that antidyskinetic drugs can be developed based on a D3/D1 receptor interaction in the direct GABA pathway (Fuxe et al., 2003b).

Increases in the understanding of DA RM and their interactions with DA receptor interacting proteins forming a local horizontal molecular network and acting as an integrated recognition-transducing system in the lipid rafts of the membrane (Agnati et al., 2005a), will open up new targets for treatment of PD. Thus, there can be drugs developed acting inter alia on the synthesis and release of the DA receptor oligomeric building blocks in the endoplasmic reticulum, on adapter and scaffolding proteins for DA receptors, on DA receptor cotrafficking, on the insertion of the DA RM building blocks into the membrane, and on DA induced receptor assemblies.

The nigrostriatal DA pathway and PD

In sporadic PD there is increasing evidence that mitochondrial dysfunction, with reduced formation of ATP and increased formation of reactive oxygen species (ROS) leading to oxidative damage, plays an important role in the pathogenesis (Dauer and Przedborski, 2003; Beal, 2000). Both genetic susceptibility factors and environmental factors such as toxins, virus infections and hypercaloric diet may be involved in the etiology (Barja, 2004; Fuente-Fernandez and Calne, 2002).

Braak et al. (2004) have made the discovery that PD may be a multisystem disorder, where projection neurons with unmyelinated or weakly myelinated axons are especially vulnerable. The nigrostriatal DA neurons represent such a type of neuron. In presymptomatic stages mainly the medulla oblongata with the dorsal motor nucleus of the vagus nerve and the olfactory bulb are affected shown inter alia with alfa synuclein immunoreactivity, indicating a possible neuro-invasion by an unknown pathogen (Braak et al., 2003). It also seems possible however that these vulnerable neurons may be especially susceptible to mitochondrial dysfunction since there is high demand for ATP to make it possible for the Na/K ATPase to restore the resting membrane potential after each action potential depolarizing the entire axon as it travels long distances down to the terminals. The deficiencies in complex I activity in PD may also enhance the misfolding of proteins and their aggregation, resulting in abnormal protein–protein interactions (Agnati et al., 2005a), contributing to the neurodegeneration (protein conformational disorders). The ubiquitin-proteosomal pathway cannot cope with this increased demand for protein degradation, especially since this process is ATP dependent. The misfolding of proteins also takes place in the DA axons and terminals that may lead to interference with axoplasma flow that also is highly ATP dependent, explaining, for example, the swollen alfa-synuclein IR neurites found early in PD (Braak et al., 2004). These protein aggregates give rise to the Lewy bodies in PD.

There may be several reasons why, for example, certain DA nerve cells in the zona compacta, like those in the ventral and lateral

parts, are more vulnerable to neurodegeneration in PD than other DA cells in the zona compacta. It may be that they belong to different types of trophic units (Agnati et al., 1995) and therefore can't receive the same trophic support from extracellular FGF-2 (Fuxe et al., 1996) and GDNF (Grondin et al., 2002). It may also be that the DA cells with highest vulnerability have lower amounts of, or different types of, ATP sensitive potassium channels (KATP) that are not as sensitive to metabolic stress. Therefore, they cannot spare sufficient amounts of neuronal energy as they cannot effectively shut down the firing of the DA cells in response to the reduced ATP/ADP ratio via opening of the K^+ channels leading to hyperpolarization (see Liss and Roepner, 2001).

It is of importance that hydrogen peroxide (H_2O_2) can activate the sulfonylurea receptor 1 that contains KATP channels, causing glutamate dependent inhibition of striatal dopamine release (Avshalumov and Rice, 2003). This may be an additional reason why DA cells with these types of KATP channels, activated also by ROS, may show increased protection against PD (Liss and Roepner, 2001).

Based on the mitochondrial hypothesis of PD, it must be emphasized that the reduction of ATP signalling may be a significant factor in causing the degeneration of the nerve cells in PD, but this has not been previously discussed. Thus, ATP is known to be an extracellular activity dependent signalling molecule in neuron/glia communication

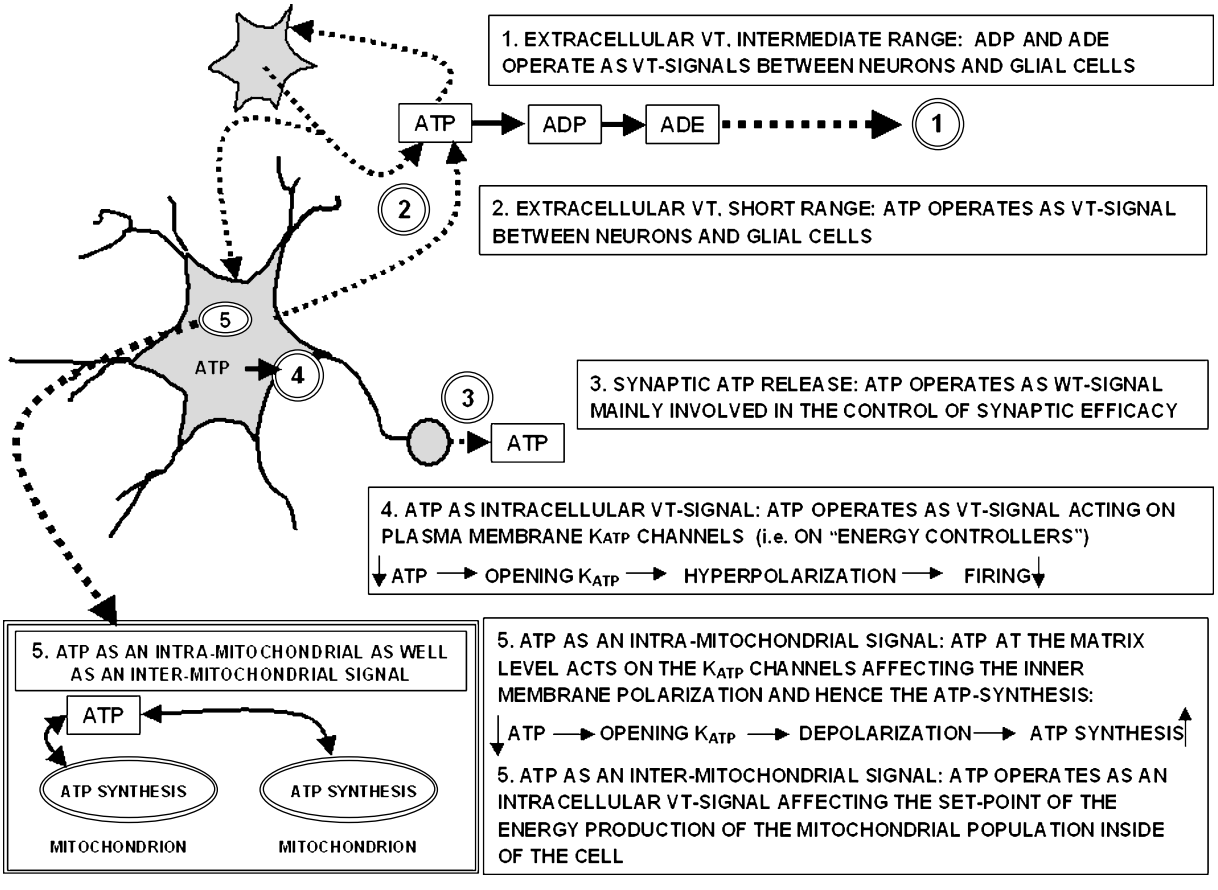

Fig. 1. Schematic illustration of ATP as a volume transmission (VT) and wiring transmission (WT) signal, and as an intracellular and intra-inter mitochondrial signal

acting via many subtypes of P2x (ion channel coupled) and P2y (G protein coupled) receptors and may exert trophic actions (Fields and Stevens, 2000; Burnstock and Knight, 2004). As seen in Fig. 1, ATP can operate as a short-range volume transmission (VT) signal between neurons and glia while its metabolites ADP and adenosine (ADE) can operate as intermediate VT signals. ATP may also operate as a synaptic signal. Thus, ATP may be released from DA cells and dendrites as a transmitter, like DA.

Reduction of ATP as an intracellular signal can, as discussed, lead to the opening of KATP channels on the plasma membrane with hyperpolarization and reduction of the firing rate. As also illustrated, ATP can act as an intramitochondrial and intermitochondrial signal. Its reduction here activates mitochondrial ATP sensitive potassium channels, leading to depolarization and an increase of ATP synthesis, in this way affecting the set point of energy production (Busija et al., 2004). Taken together the combined actions on KATP channels by ATP reductions will allow a balance to develop between firing and ATP synthesis (Fig. 2). If this energy balance is disrupted the nigrostriatal DA function wears off.

Such a disruption may be brought about if subtypes of ATP P2x receptors (see North, 2002) exist on the nigral DA nerve cells. These ATP receptors are permeable to small monovalent cations and certain subtypes to Ca ions. It is of significance that certain subtypes of P2x receptors may be activated by ROS, especially H_2O_2 and hydroxyl radicals, as demonstrated in vagal lung afferent fibres (Ruan et al., 2005) and such P2x receptors may be postulated to exist on DA nerve cells (Fig. 3). The resulting sodium influx will produce a depolarization of the DA cell since the Na/K-ATPase cannot maintain the ion gradient, and the KATP channels close upon depolarization (Bryan et al., 2004) and the inhibitory D2 autoreceptor activated inwardly rectifying K^+ current mainly operate at resting membrane potential (Fig. 3). The most interesting P2x receptor relating to this hypothesis would be the P2x7 receptor, as it fails to desensitize and larger currents are found upon repeated application. After several

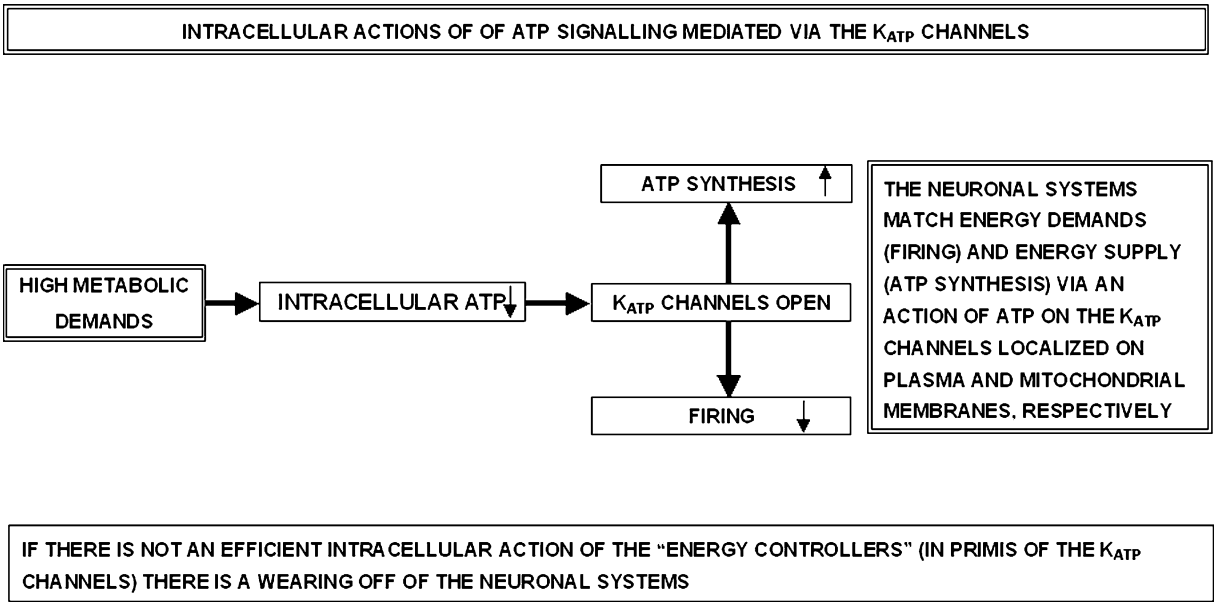

Fig. 2. Scheme of plasma membrane and mitochondrial KATP channel activation by ATP depletion leading to energy balance in conditions with high metabolic demands

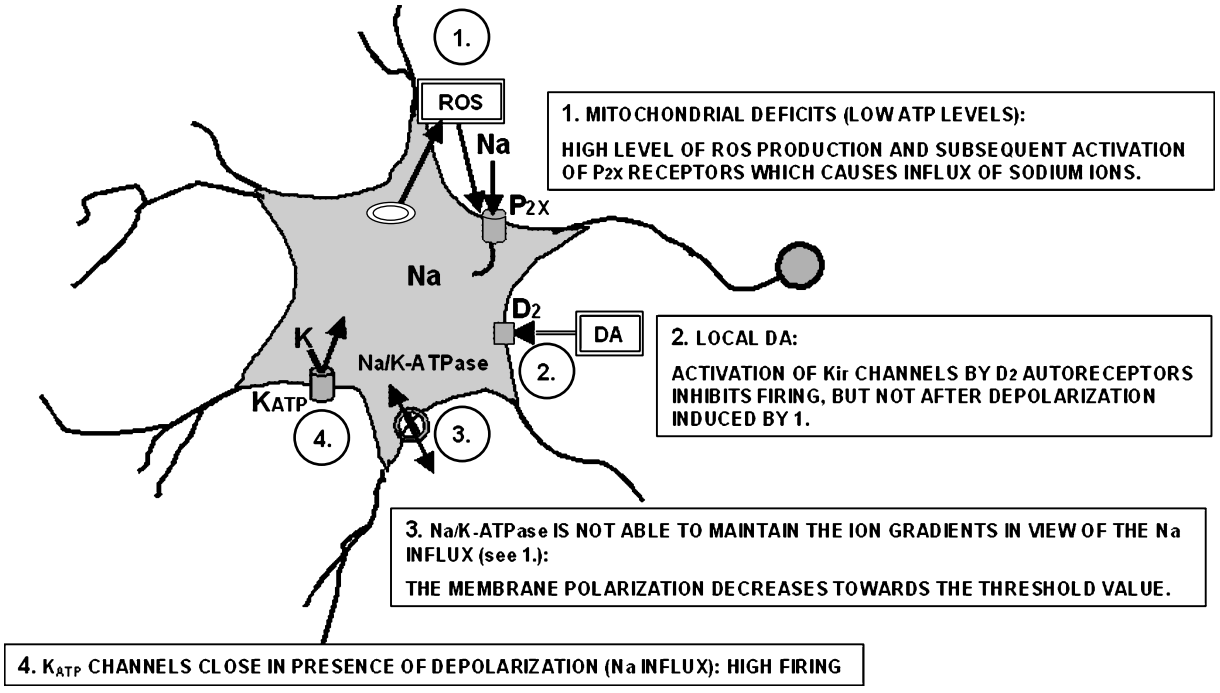

Fig. 3. Hypothesis on the possible role of P2x receptors in nigral DA nerve cells as to their degeneration in PD. Due to mitochondrial dysfunction in PD, ROS may be formed thereby activating the P2x receptors together with ATP released from degenerating nerve cells and glial cells with influx of especially Na ions, leading to depolarization, since the Na/K ATPase cannot maintain the ion gradients. The KATP channels close as the membrane depolarizes and cannot act as a brake nor can the D2 autoreceptors that activate inwardly rectifying K^+ channels operating close to the equilibrium membrane potential. The resulting high firing rate will cause a progressive ATP depletion of the DA neuron leading to cell death. Similar events may take place in other vulnerable neurons in PD and P2x receptors have been demonstrated in the dorsal motor nucleus of the vagus (Burnstock and Knight, 2004) where the pathology begins (Braak et al., 2004)

seconds of P2x7 activation, permeability to larger organic cations increases. This may either be related to a dilation of the pore of the P2x7 channel or to the activation of a distinct channel protein (North, 2002). After prolonged agonist application, the P2x7 activation leads to membrane blebbing, seen as large hemispherical protrusions, and finally to cell death (North, 2002). The pore formation with influx of extracellular chloride ions seems to play a major role for the apoptotic cell death induced (Tsukimoto et al., 2005). Antagonists of P2x7 receptors are presently being developed against inflammatory processes (Baraldi et al., 2004).

Based on the present hypothesis it will be of substantial interest to study the distribution pattern of P2x and P2y receptors in the nigrostriatal DA pathway and its subsystems, as well as in the adjacent astroglia and microglia, and how the receptor distribution pattern may change in models of PD, and in PD with focus on P2x7 receptors. This approach may reveal new aspects of the mechanisms of neurodegeneration in the nigrostriatal DA neurons in PD, and initiate novel strategies for neuroprotective treatments of PD based on, for example, the development of P2x receptor antagonists.

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