Dopamine-sensitive adenylyl cyclases in neuronal development: physiopathological and pharmacological implications

Barbara Pavan[1], Guglielmo Paganetto[1] and Alessandro Dalpiaz[2]

[1] Department of Biology and Evolution, General Physiology Section, University of Ferrara, via L. Borsari 46, 44100 Ferrara, Italy
[2] Department of Pharmaceutical Sciences, University of Ferrara, via Fossato di Mortara 19, 44100 Ferrara, Italy

Pharmacological studies of molecular mechanisms leading to the differentiation of neurons with retained dopaminergic fate and function suggest that such differentiation could be a form of treatment of neurodegenerative disorders, such as Parkinson's disease (PD) and schizophrenia. This goal could be achieved by neuronal replacement therapies based upon the manipulation of endogenous precursors *in situ* or by transplantation-based approaches. Signals conveyed by the adenylyl cyclase (AC) pathway appear to be crucial for the suitable differentiation of neurons. Here, we discuss dopamine (DA)-sensitive isoforms of AC as key cues for dopaminergic neuronal patterning and as interesting therapeutic targets for the induction of regenerative processes or to drive correct neuronal development.

Introduction

Attempts to discover more about the *in vivo* signaling molecules and mechanisms responsible for neuronal development are of fundamental importance to the progress of cell replacement therapies targeting the central nervous system (CNS) [1]. Neurogenesis, as the production of new neurons, was originally considered to occur only during the embryonic and early postnatal periods; however, various degrees of neurogenesis have been recently recognized in selected regions of the adult mammalian brain, namely the hippocampus and olfactory bulb. By contrast, other brain regions, including the cerebellum, brainstem, basal ganglia and spinal cord, appear to be non-neurogenic [2]. Although neurogenesis can be stimulated in response to injury in neurogenic as well as in other brain regions, it remains unclear whether any constitutive neurogenesis occurs in these regions under normal conditions. This possibility has received recent support from the finding that a resident population of neural progenitor cells exists in adult brain, obviating the need for mature neurons to become mitotic [2]. The molecular mechanisms leading to the differentiation of neural stem cells (NSCs) into neuronal progenitors or precursors and finally into mature neurons are now gradually being discovered [2]. The events that are responsible for the definition of the number and distribution of neurons that compose the mature tissue in the CNS are detailed in Fig. 1.

NSCs, with their intrinsic capacity to self-renew and differentiate, have sparked great interest as potential tools for aiding recovery in patients with neurodegenerative disorders, such as Parkinson's disease (PD) and schizophrenia. Although most clinical applications of NSCs are likely to rely on the *in vitro* expansion and differentiation strategies before cell transplantation, the knowledge of the mechanisms and methods required to differentiate NSCs into specialized cell types is still a major limitation [3]. As the supply of fetal-derived neuroblastic tissue is limited, attaining a stable and homogeneous population of dopaminergic neurons would provide a useful supply of neural tissue for developmental studies and clinical applications.

Cumulative evidence indicates that neuronal fate is determined by both intrinsic and extrinsic factors in a coordinated way [4]. In this review, we discuss the role of dopamine (DA)-sensitive isoforms of the adenylyl cyclase (AC) enzyme as cues for dopaminergic neurons to retain their mature functional differentiation. We suggest that such isoforms could be valuable pharmacological targets for the treatment of aberrant or damaged neurons.

Corresponding author:. Pavan, B. (pvnbbr@unife.it)

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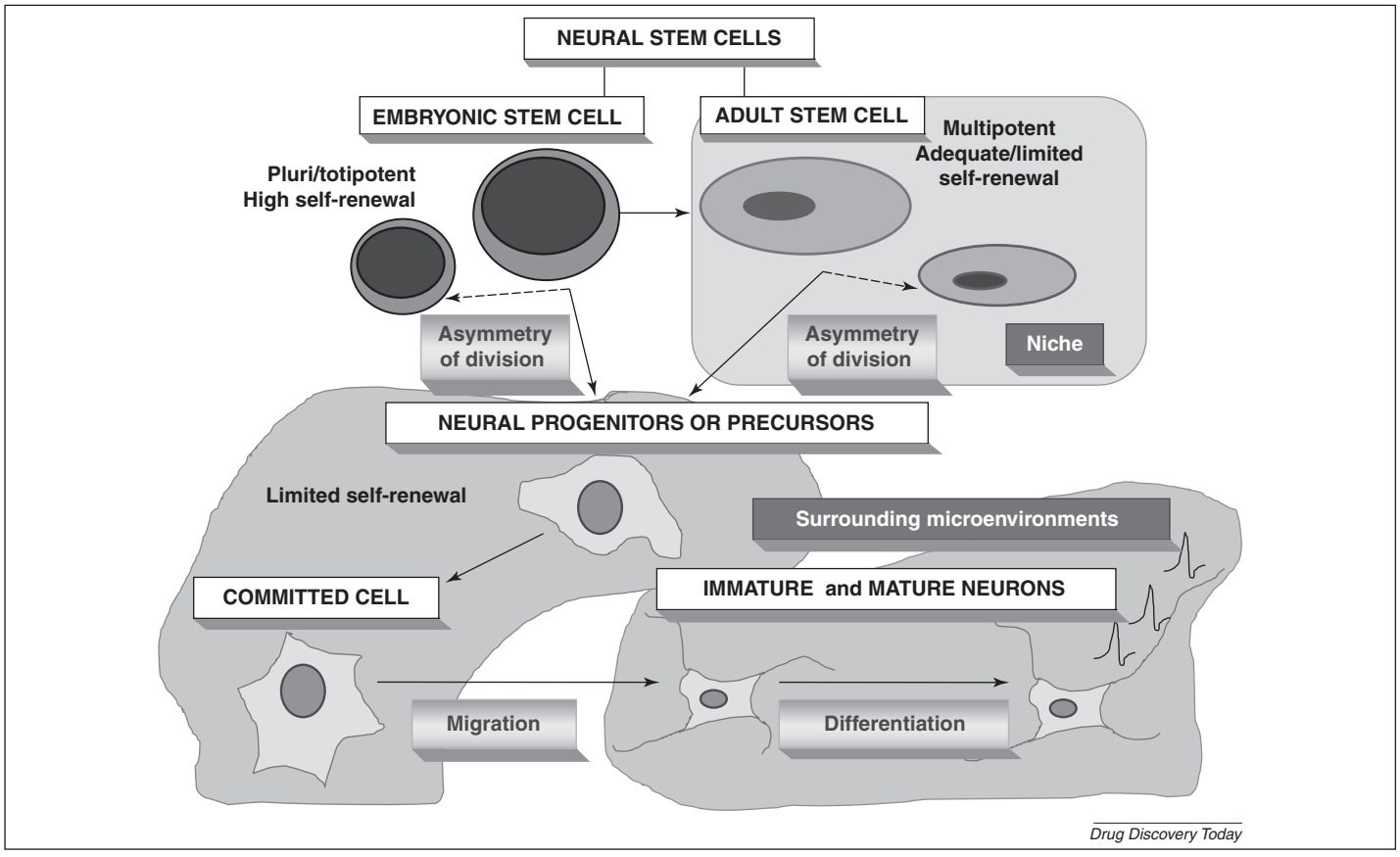

FIGURE 1

Neurogenesis steps. Neural stem cells derive from both embryonic and adult tissues. Embryonic tissues are the source of all adult stem and differentiated cells. A neural stem cell gives rise by asymmetric division either to another daughter stem cell (self-renewal) or to a neural progenitor or precursor with a more restricted proliferation potential. The progenitor is committed to differentiate into a specific fate in response to cell intrinsic and/or extrinsic cues arising from the stem cell microenvironment or niche. As a process that is necessary for neural differentiation, the departure (migration) of progenitors and functional immature neurons from the stem cell niche toward the surrounding microenvironments is also orchestrated by different internal and external signals.

AC signaling in neural differentiation

Neural differentiation *in vivo* as well as *in vitro* is known to require elevation of the intracellular cAMP concentration [5–7], which is controlled by the activity of nine transmembrane AC isoforms (AC1–9) and one 'soluble' AC (sAC). These AC molecules have distinct basal activity, tissue expression patterns and regulatory properties during postnatal development [8,9]. AC–cAMP signaling has been identified as a key intracellular pathway activated by environmental factors that lead to the induction of genes controlling differentiation in lineage-committed progenitor cells [10]. It is also important in the generation of pure populations of dopaminergic neurons from human NSCs in developing methodologies [11,12]. Therefore, the different AC isoforms are of particular interest to researchers because of their regulatory impact on cAMP as an ultimate determinant of neural cell fate [7,13]. AC is also known to be the rate-limiting component distal to G-protein coupled receptors and to be involved in integrating multiple signaling pathways into a single second messenger [8]. Alterations in post-receptor activation of the AC complex during proliferation and differentiation in a myoblast model have already been observed [14]. Interestingly, changes in the concentration of intracellular cAMP have also been identified as key events in neuronal and glial cell migration modulated by neurotrophins, such as nerve growth factor (NGF), neuropeptides and ethanol [15,16].

The dopaminergic system

The neurotransmitter DA is linked to physiological functions such as motor control, cognition and reward, as well as to several syndromes, including PD and schizophrenia [17]. Dopaminergic neurons (Fig. 2) are characterized by the expression of tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC), the rate-limiting and key enzymes in the biosynthesis of DA, the DA-uptake transporter (DAT) and the vesicular monoamine transporter 2 (VMAT2) [18]. These three DA systems have been studied most extensively in the brain [19]. The cell bodies of the nigrostriatal pathway reside in the substantia nigra pars compacta (SNpc) and project to the dorsal striatum (caudate putamen), the center of sensorimotor integration within the basal ganglia. The mesolimbic pathway originates in the ventral tegmental area (VTA) and terminates mainly in the nucleus accumbens (NA); one function of this system is the mediation of natural and drug-induced reward. The mesocortical DA pathway, which also originates in the VTA but terminates in the prefrontal cortex (PFC), regulates complex cognitive processes, such as selective attention and working memory [19]. These systems have no direct role in the

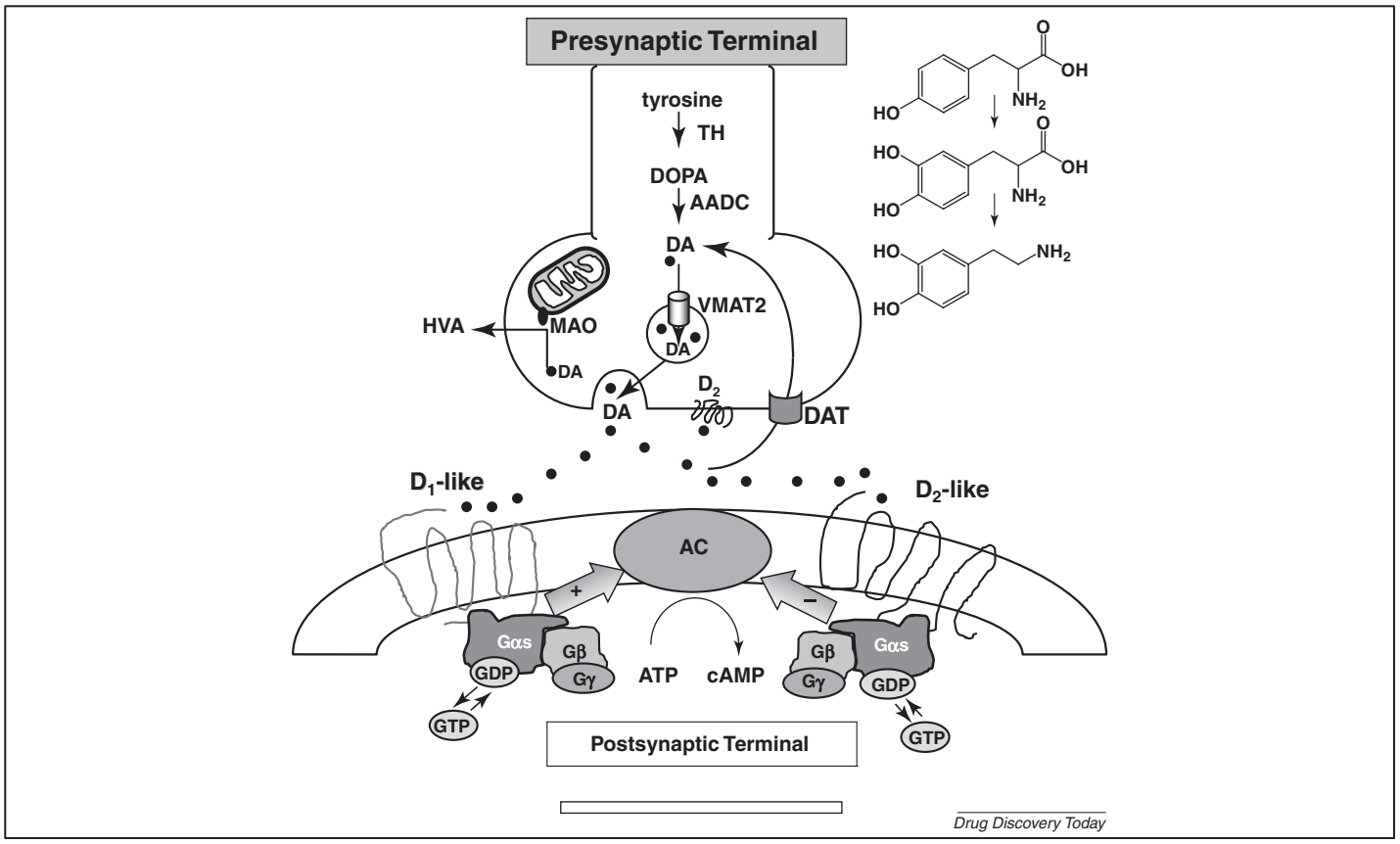

FIGURE 2

Dopaminergic pre- and postsynaptic terminals and antagonistic coupling of D_1-like (D_1/D_5) and D_2-like ($D_2/D_3/D_4$) DA receptors to DA-sensitive ACs. Chemical structures correspond with tyrosine, L-DOPA and DA, respectively. *Abbreviations*: HVA, homovanillic acid; $G_{\alpha i}$, protein G inhibitory α subunit; $G_{\alpha s}$, protein G stimulatory α subunit; G_β and G_γ, protein G β and γ regulatory subunits; MAO, monoaminoxidase.

regulation of prolactin secretion from the anterior pituitary, which is instead affected by the tuberoinfundibular dopaminergic system [20]. All five DA receptor isoforms cloned in mammals have been linked to the AC enzyme. After activation, D_1-type DA receptors (D_1 and D_5) stimulate AC, whereas D_2-type DA receptors (D_2, D_3 and D_4) inhibit AC (Fig. 2).

AC isoforms involved in dopaminergic development

There is little information currently available regarding the transcriptional control of specific AC isotypes in dopaminergic patterning. The DA-sensitive AC5 and AC6 isoforms belong to the same subfamily and both are inhibited by physiological concentrations (0.1–1.0 μM) of Ca^{2+} [21]; they also share high degree of identity [22], but are differentially regulated by the D_2 and D_3 DA receptors [23]. The activity of both AC5 and AC6 is inhibited by the D_2 receptor, whereas only the activity of AC5 is inhibited by D_3 receptor [24]. AC5 is highly enriched in brain dopaminergic regions and largely localized in the mammalian striatum as well as in other DA-innervated structures (i.e. the NA and olfactory tubercle), where the cAMP pathway regulates diverse behavioral functions [25,26]. AC5 often is referred to as 'striatal AC' and its importance in striatal functions involving dopaminergic pathways has been further emphasized by the finding of cells expressing AC5 colocalized with D_1 and D_2 receptors [27]. Among various intriguing studies over the past 90 years, Matsuoka *et al.* [28] demonstrated that mRNA for AC5 and the Ca^{2+}/calmodulin-activated AC1 [8,9] showed opposite expression patterns during the development of rat striatum, where AC1 was the major isoform expressed during the early phases of development, whereas AC5 expression increased dramatically during postnatal stages. Both expression patterns mirrored the developmental changes in Ca^{2+}/calmodulin-sensitive AC activity (from stimulation to inhibition of cAMP production). The developmental expression of AC5 mRNA was associated with the maturation of striatal neurons [28]. Interestingly, ontogenic studies in heart have reported very low levels of AC5 mRNA expression at early stages of cardiac development, which markedly increased as development progressed [29]. This expression pattern requires changes in the regulation of cAMP formation throughout the neurogenesis, perhaps incorporating distinct AC isoforms that are recruited according to the type and timing of several extracellular signals. Therefore, a shift in calcium sensitivity of AC could be regarded as a hallmark of striatal dopaminergic neurons maturation. Indeed, spontaneous Ca^{2+} transients, observed in developing neurons, were reported to be sufficient and necessary to drive normal differentiation and to regulate neurite extension [13], and the possibility that ACs could be voltage sensitive in the CNS has been proposed [30]. However, as for all the AC subtypes, the tissue distribution and developmental expression of AC5 and AC6 have only been determined at the mRNA level [29,31], as antibodies

with the requisite sensitivity for detection at the protein level are still lacking [32]. Interestingly, the successful generation of an AC5 isoform-specific mouse monoclonal antibody has recently been reported [33]. This novel AC5 antibody could lead to a better understanding of the tissue distribution and ontogenic regulation of AC5 expression, as well as providing new insight into the pathogenesis of AC5-related diseases.

The other Ca^{2+}/calmodulin-activated type 3 isoform of AC (AC3) is known to be a crucial element in the odorant-induced transduction cascade and a pivotal player in axonal guidance during migration of dopaminergic neurons from the olfactory bulb toward the brain [34]. In this link, olfactory tissues are also regarded as an open 'window to the developing brain', enabling, for example, early diagnosis of schizophrenia [35]. These tissues are also a valuable route for drug delivery bypassing the inherent barriers associated with the CNS. Therefore, AC3 could be an elective target for promoting olfactory neurogenesis as a therapeutic resource for the CNS.

The proposed pattern of localization of DA- and calcium-sensitive AC isoforms in the CNS is illustrated in Fig. 3. Only the calcium-sensitive AC1, AC3 and AC5/AC6, among the DA-sensitive ACs, are considered because of their direct modulation by calcium, which makes them particularly interesting for neural developmental mechanisms. Indeed, together with cAMP, calcium is one of the major signals involved in cell growth, migration, differentiation and synaptogenesis [36,37].

Dopaminergic system failures

PD and schizophrenia are neuronal diseases characterized by altered dopaminergic signals in the CNS. Both the disorders appear to share the D_1–D_2/AC5 pathway, as described below.

Parkinson's disease

PD is one of the most common late-life neurodegenerative diseases, affecting approximately 2% of the population above 60 years of age. It is characterized by the unilateral onset of resting tremor in combination with varying degrees of rigidity and bradykinesia [38,39]. The etiology of PD is still not completely understood. It is likely to result from a combination of several factors, among which the first is an age-related attrition and death of the dopaminergic neuronal projections from the SNpc to the striatum [39]. Furthermore, PD might arise as a consequence of the ongoing aging process coupled with environmental neurotoxins exposure that accelerates the process of nigral cell death [40]. Another possibility is that some people might have a predetermined genetic susceptibility to these environmental insults [41], suggesting an important role for genetic factors in the onset of PD.

Levodopa (L-DOPA; 3,4-diidrossi-l-fenilalanina), which is converted to DA in the brain, remains the gold standard for treating PD. However, long-term complications of this therapy include dyskinesia [42] and cognitive difficulties altering the working memory [19]. The positively AC-coupled D_1 receptor is known to be involved in the anti-parkinsonian function of L-DOPA as well as in inducing dyskinesia [43]. Studies in animal models of PD have suggested an incoming supersensitivity of the D_1 receptor after DA depletion in the striatum and frontal cortex, which is likely to be further enhanced by L-DOPA treatment [44]. Tong *et al.* [45] studied the status of D_1-stimulated AC activity in the striatum and cerebral cortex of patients with PD. Interestingly, the observed significant increase of DA-stimulated AC activity in these patients is likely to be the result of enhanced coupling between the D_1 receptor and stimulatory G-protein. Therefore, the supersensitivity of the D_1 receptor as a compensatory attempt to ameliorate the

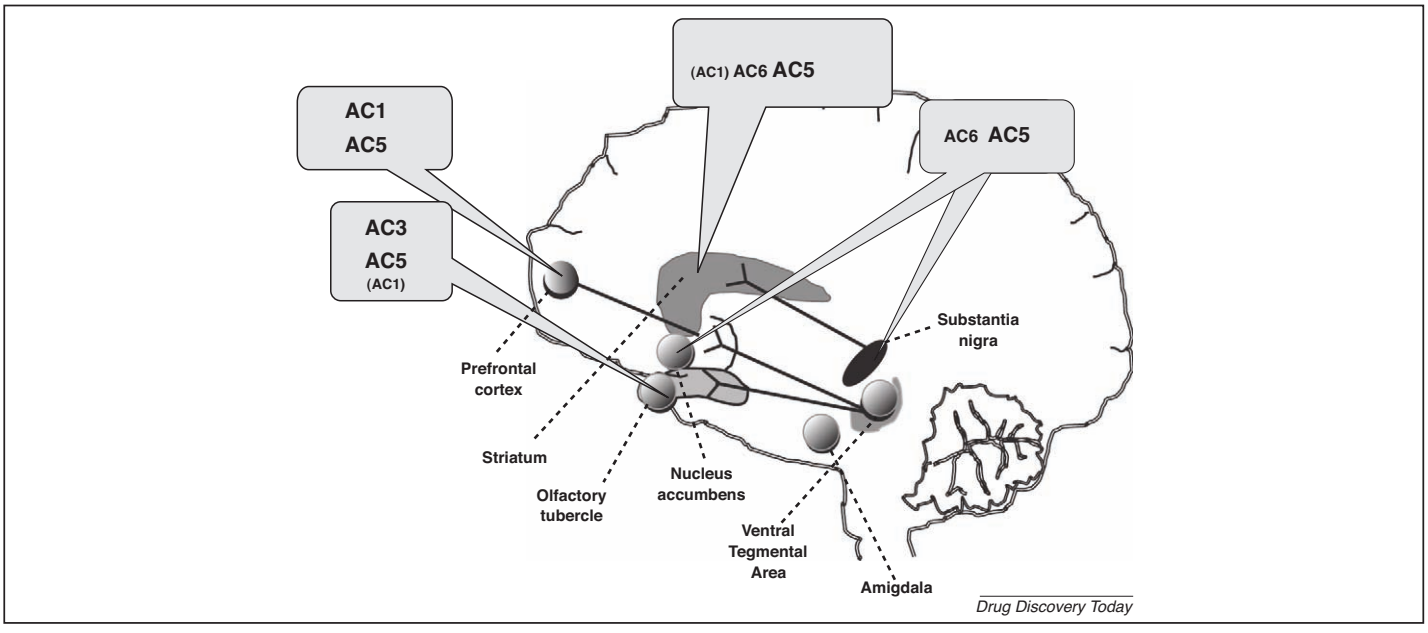

FIGURE 3

Proposed pattern of localization for DA- and calcium-sensitive AC isoforms in the different areas of the three main dopaminergic inputs in the brain. The nigrostriatal pathway resides in the substantia nigra pars compacta and projects to the dorsal striatum (caudate putamen). The mesolimbic pathway originates in the ventral tegmental area and targets the nucleus accumbens and prefrontal cortex. The mesocortical pathway also originates in the ventral tegmental area, but terminates in the prefrontal cortex.

parkinsonism was also suggested to contribute to the development of L-DOPA-induced dyskinesia [46].

Presynaptic dopaminergic deficiencies might also involve the so-called 'Parkinson plus' syndromes, including multiple system atrophy, progressive supranuclear palsy, corticobasal degeneration, dementia with Lewy bodies, Parkinson's disease dementia and frontotemporal dementia. All these disorders are characterized by a reduction in the synthesis and storage of DA, and alterations in its binding to DAT and to the D_1 and D_2 receptors. Among these disorders, Huntington's disease is characterized by both pre- and postsynaptic dysfunctions [47]. AC5 is known to be the major AC subtype in transducing D_1 and D_2 receptor signaling in the adult striatum and its loss or inhibition is a crucial regulatory property for cAMP-dependent motor control, especially in balancing and maintaining both coordination and locomotion [48]. In the absence of AC5, the AC6 and AC1 isoforms are still present, but are unable to compensate fully for the function of AC5 [48]. Accordingly, AC5-null mice were found to exhibit parkinsonian-like motor dysfunctions that were only partially compensated by selective D_1 or D_2 dopaminergic stimulation [48]. A pharmacological modulation of the activity and/or expression of AC5 could be an alternative way of controlling the detrimental effects of long-term L-DOPA treatment. It is interesting to observe that the effects of L-DOPA against Parkinson's disease can be enhanced by the co-administration of adenosine (G_s-coupled) A_{2A} receptors antagonists. Such a strategy allows a reduction in L-DOPA dosage with the consequent reduced intensity of unwanted effects that can result from long-term treatment [49]. This phenomenon has been attributed to heteromeric receptor complexes between the A_{2A} and D_2 receptors, where the activation of the A_{2A} receptor induces a reduction of the D_2 receptor affinity for DA [50]. As a consequence, A_{2A} antagonists enhance the *in vivo* DA effects against PD by increasing the affinity of the D_2 receptors. In such a manner, DA and A_{2A} antagonists converge to inhibit AC5 activity, inducing the appropriate conditions for motor control of patients with PD. The same result can be obtained by using a direct inhibitor of the activity of AC5, which could therefore be a key enzyme to target for new clinical implications against PD.

Schizophrenia as a prototype of dopaminergic overuse

Schizophrenia is a complex brain disorder that induces cognitive impairment associated with positive and negative psychotic symptoms. This disease affects one in 100 individuals, who usually show a clinical schizophrenic onset during late adolescence or early adulthood [51,52]. As for PD, the etiology of this disease is not fully understood [52], although several pieces of evidence exist that suggest that perinatal brain disturbances underlie the initial risks for the disease [53,54]. In particular, these disturbances can arise from genetic and environmental factors that are able to affect the normal postnatal program of brain maturation, leading to manifestation of the disease during late adolescence or early adulthood [55,56]. After disease manifestation, neurodevelopment continues to deviate further [57,58]. This suggests that useful therapeutic approaches would still be possible after the onset of the overt symptoms. Among the susceptible genes that might be responsible for the pathophysiology of schizophrenia, those encoding neuregulin-1 (*NRG1*) and Disrupted-in-Schizophrenia (*DISC1*) appear to have important roles during neurodevelopment [56,59]. A marked decrease in TH expression and extracellular levels of DA was detected in the PFC of *DISC1* knock-down mice after puberty [60]. These data reflect a disturbed maturation of dopaminergic neurons [61]. Interestingly, it has been demonstrated that the adult schizophrenic brain is characterized by underexpression of D_1 receptors in PFC and overexpression of D_2 receptors in striatum, compared with healthy subjects [62]. This D_1–D_2 imbalance leads to alterations in dopaminergic transmission that could be related to the etiology of dopaminergic-based neurodevelopmental disorders [63]. Accordingly, excessive stimulation of striatal DA D_2 receptors and deficient stimulation of prefrontal D_1 receptors have been recognized as conditions associated with schizophrenia [64]. Pharmacological activation of the D_1 receptors has therefore been suggested as a potential adjunct in the treatment of schizophrenia [65]. Given that D_1 receptors stimulate AC activity via G_s proteins, selective activators of the DA-sensitive isoforms of AC expressed in the PFC, such as AC5, could be investigated as potential new drugs for the treatment of schizophrenic diseases.

The physiological consequences of D_2 receptor overexpression have been studied by generating mice with reversible increased levels of D_2 receptors restricted to the striatum. This tissue showed a reduction of DA-induced AC activity in the case of D_2-transgenic mice [66], a phenomenon consistent with an excess of D_2 receptors that, in striatum, are coupled with G_i proteins inhibiting AC activity [67]. The selective D_2 receptor overexpression also resulted in increased levels and decreased turnover of DA in striatum. Moreover, D_2-transgenic mice exhibited cognitive deficits even after the transgene was switched off, suggesting that cognitive diseases could be attributed to the excessive expression of D_2 receptors during neuronal development, rather than to their continued overexpression. Interestingly, striatal D_2 receptors alterations have also been associated with other mental and affective disorders, such as bipolar affective illness, some inherited neuropsychiatric tics (Tourette syndrome) and anxiety disorders [68]. Experiments carried out on AC5 knockout mice revealed that the inhibitory effect of D_2 activation on directly stimulated AC activity was completely abolished in striatum, where AC5 is preferentially expressed under normal conditions. Moreover, the administration of D_2 antagonists to mice lacking AC5 did not produce typical neuroleptic effects [69]. All these data suggest that AC5 is the physiological relevant effector for the D_2 receptors in striatum. In addition, AC5 deficiency in mice induced a consistent anxiolytic effect that was either increased or reduced by D_1 agonists or antagonists, respectively [70], probably as a result of the compounds acting on effectors that were different from AC5.

Taken together, these studies indicate that an important role in the mechanism of anxiety can be attributed to the AC5 isoform. As a consequence, selective AC5 targeting is likely to be of great importance in the discovery novel drugs against aberrant neuronal development involved in anxiety and schizophrenia. Accordingly, it is generally recognized that post-receptor mechanisms can be considered as promising targets for the development of novel drugs characterized by long-term clinical efficacy against schizophrenia [71].

Therapeutic implications

Neuronal replacement therapies can be performed by cell transplantation-based approaches together with manipulation of endogenous or grafted precursors *in situ*. Hence, a rapid and efficient approach aimed at discriminating neuronal differentiation in cell culture can support pharmacological studies directed at differentiating cells and screening for new pro-neurogenic factors [72].

In vitro *cell lines models*

Cell lines provide a powerful model for investigating the role and degree of regulation of AC isoforms during neuronal development. Indeed, they can often undergo a de-differentiation process leading to either the acquisition of an intermediate progenitor cell phenotype or to a direct neuronal *trans*-differentiation process. Embryonic carcinoma P19 cells were the first recognized cell line expressing basal Ca^{2+}/calmodulin-stimulated AC activity, where

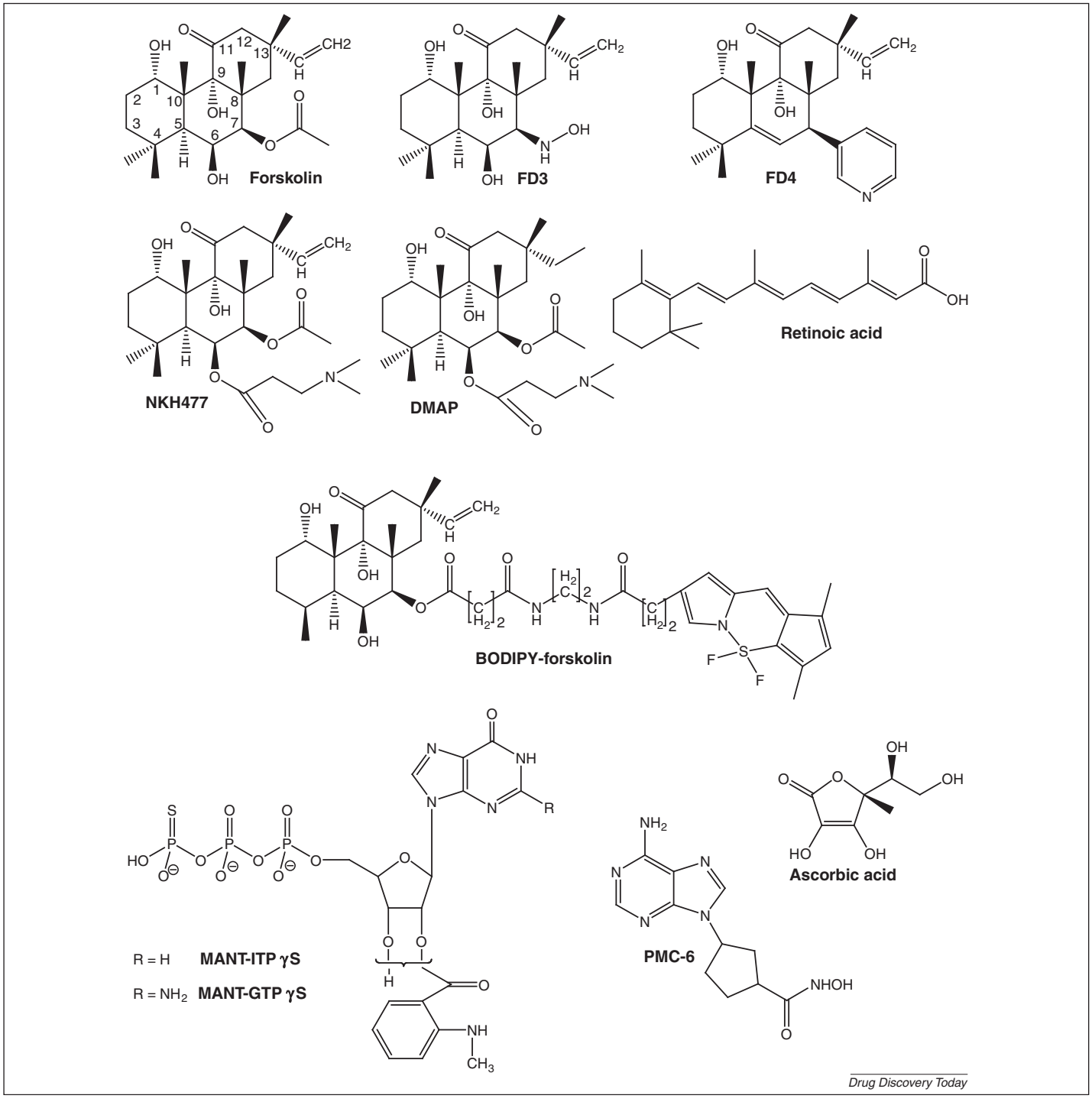

FIGURE 4

Chemical structures of AC activators [forskolin; FD3; FD4; NKH477; DMAP; and BODIPY-FSK], AC inhibitors [MANT-ITPγS and MANT-GTPγS; PMC-6; and ascorbic acid] and RA, acting in concert with forskolin to promote neuronal differentiation.

inducted differentiation was accompanied by specific upregulation of AC2, AC5 and AC8 mRNA and downregulation of AC3 mRNA [13]. Moreover, differentiation in the catecholaminergic (CAD) mouse neural cell line, which expresses the TH enzyme and accumulates L-DOPA, was accompanied by a significant increase of AC6 and a dramatic loss of AC9 mRNA expression [7], showing, once again, a change in calcium-sensitive AC expression during neural development. Furthermore, a simple and reliable method of generating DA neurons has been recently provided by the mouse neural crest-derived cell line Neuro2A, which was stimulated to enhance significantly both TH and DA levels in the presence of a non-hydrolysable analog of cAMP [73]. Experimental evidence from rat pheochromocytoma 12 (PC12) cells, a classic *in vitro* model for neuronal development, is being used to help elucidate how neurotrophins, including NGF, facilitate AC–cAMP-mediated commitment of neuronal stem cells to a dopaminergic phenotype [74]. Interestingly, adult mammalian retinal pigment epithelium (RPE) cells were also shown to express neural progenitor properties as a potential source of neural regeneration under appropriate conditions for cell replacement [75].

Niche or stem cell microenvironment: drug-like differentiating agents

Several small molecules are known to promote NSC differentiation (reviewed in [76,77]) and some might target the AC pathway, such as ascorbic acid (AA; vitamin C; Fig. 4), retinoic acid (RA; vitamin A; Fig. 4) and trophic factors. Discovery of AA as a competitive inhibitor of AC activity has opened new fascinating research areas investigating its potential novel therapeutic properties [78]. In developing rat striatum, RA, through its nuclear receptors, is known to establish the infrastructure of DA neurotransmission by upregulating the expression of striatum-enriched DA signaling molecules, including the D1 receptor, G_{olf}, AC5 and DA- and cAMP-regulated phosphoprotein (DARPP-32) [79]. This suggests that their promoters contain RA response elements (RAREs) or responsive elements for the RA-activated transcription factors. This feature could be used to investigate RA or its more selective derivatives as feasible candidates in PD therapy. The best-known direct activator of AC, the di-terpene forskolin (FSK; Fig. 4), has been reported to act in concert with RA to promote neuronal differentiation of adult NSCs [77]. This information led to the revival of the idea that an endogenous FSK-like small molecule activator of AC might exist [80]. The activity of some AC isoforms is also known to be regulated by receptors of trophic factors, such as the epidermal growth factor (EGF), which specifically activates AC5 only via tyrosine phosphorylation of the stimulatory protein G [9], and NGF, which increases the responsiveness of PC12 cells to the cAMP-elevating property of FSK [74]. These results expand management of cAMP production during neural differentiation to several small drug-like molecules and neurotrophins. Therefore, a microenvironment containing these factors with selective modulators toward DA-sensitive ACs, such as AC3 and AC5/AC6, and also toward the Ca^{2+}/calmodulin-activated AC1, as crucial link between neuronal activity and intracellular cAMP, could be hypothesized. Detailed structure–activity relationships for these selective modulators has been recently reviewed [8,81]. We highlight some of them here for their potential use in streamlining the maturation and retained functionality of the dopaminergic network. Currently, the best-known AC5-selective stimulators are the FSK-derivative 6-[3-(dimethylamino)propionyl]FSK (NKH477; Fig. 4), in use clinically against acute heart failure [8], and 6-[3-(dimethylamino)propionyl]-14-15-dihydroFSK (DMAP; Fig. 4). These compounds show AC5 selectivity with respect to AC2 and AC3 [8,82–85]. Together with their ability to discriminate between the two DA-sensitive AC3 and AC5, the molecular framework of these derivatives could be further engineered for crossing the blood–brain barrier (BBB). The most potent AC1 stimulator BODIPY-FSK (Fig. 4), which is selective with respect to AC5 but not AC2 [8,86], as well as a new lead candidate AC1 inhibitor, NB001 [87], could be valuable tools for probing developing striatal neurons in culture [28]. Two other FSK-derivatives, 7-deacetyl-7-hydroxaminoFSK and 5,6-dehydroxy-7-deacetyl-7-nicotinoylFSK (FD3 and FD4, respectively, Fig. 4), are selective activators for AC3 with respect to AC2 and AC5 [82], and could be tested in dopaminergic neuronal migration.

Among the P-site inhibitors with metal chelating properties (PMC), the derivative 1R,4R-3-(6-aminopurin-9-yl)-cyclopentane-carboxylic acid hydroxamide (PMC-6, Fig. 4) has been identified as a potent AC5 inhibitor that is selective with respect to AC2 and AC3 [88–90]. Moreover, the 2′(3′)-*O*-(*N*-methylanthraniloyl) (MANT) nucleotides, such as MANT-guanosine 5′-[γ-thio]triphosphate (MANT-GTPγS) and MANT-inosine 5′-[γ-thio]triphosphate (MANT-ITPγS, Fig. 4) have also been found to be potent AC inhibitors [91], showing weak AC5 selectivity with respect to AC2, but not with respect to AC1 and AC6 [92].

However, the selectivity of the compounds reported here has not yet been tested for all AC isoforms [8]; therefore, it is currently not possible to make definitive statements about isoform selectivity. A drawback of MANT-nucleotides is their inability to penetrate the BBB, so they do not appear to be suitable drug candidates for neurological diseases. By contrast, appropriate devices could be prepared to manage the uptake of MANT-nucleotides into the CNS, taking into account the fact that polymeric micro- and nanoparticles can be useful for the brain targeting of drugs unable to cross the BBB [93–95]. Similarly, as for NKH477 and DMAP, PMC-6 and MANT-nucleotides could be adapted to cross the BBB and be used potentially to counteract the D_1 supersensitivity effect in the L-DOPA treatment of PD.

Conclusion and future perspectives

As they differentiate and migrate, neurons encounter different cues that are spatially and temporally regulated. The exact sequence of encountered cues can be crucial for the retained functionality of neurons. Neuronal replacement therapies of lost neurons require new neurons to integrate appropriately into the host brain. Alternatively, therapies based upon the manipulation of endogenous precursors *in situ* might have the most obvious advantage over transplantation-based approaches in that they work without an external source of cells. However, there are also potential limitations. First, such an approach might be limited to particular regions of the brain, because multipotent neural precursors are more densely distributed in particular subregions of the adult brain. As a consequence, it is possible that there simply are not sufficient numbers of precursor cells to bring about functional recovery. In addition, the potential differentiation fates of endogenous precursors might be too limited to allow their integration into varied portions of the brain. However, the more relevant difficulty is that it

could be hard to provide the precise combination and sequence of molecular signals necessary to induce endogenous precursors to proliferate efficiently and differentiate precisely into appropriate types of neuron deep in the brain. Therefore, knowledge of the sequence of biochemical events is essential not only for the therapeutic use of exogenous NSCs, but also for human adult neurogenesis, with the aim of stimulating endogenous neuronal repair during brain failure, including PD and schizophrenia.

A rapid and efficient approach oriented to discriminate neuronal differentiation in cell culture can support pharmacological studies directed at differentiating cells and screening for new proneurogenic factors. This field is only now beginning to understand the complex interplay between neural precursor potential and signals in the local microenvironment; much remains to be learned about precursor heterogeneity and how to take advantage of what might be partial cell-type restriction, permissive and instructive developmental signals, and modulation of specific aspects of neuronal differentiation and survival. These goals could be approached by modulating promising post-receptor targets, therefore bypassing unwanted phenomena such as the under- and overexpression or supersensitivity of receptors. The potential of AC as object of drug therapy has been already discussed elsewhere [8,81], highlighting the different AC isoforms as specific and integrative detectors for environmental signals. Therefore, ACs could be a site of convergence for extrinsic and intrinsic factor signaling during neurogenesis. Recruitment of DA/Ca^{2+}-sensitive AC1, AC3 and AC5/6 in a subtype-specific manner could provide both instructive and permissive cues for the production of dopaminergic-specified neurons. In other words, it would be interesting to know whether the change in Ca^{2+}-regulated ACs is either a consequence or the driving cue of dopaminergic differentiation. Moreover, the role of individual ACs in neural differentiation should be clarified by evaluating the potential discrimination of cAMP signals among different lineages of neurons.

An answer to these questions requires not only potent ligands whose selectivity has to be systematically examined across all the ACs, but also antibodies with the requisite sensitivity for each isoform [8,81]. As promising investigative tool, a selective AC5 antibody has recently been identified [33]. However, evidence that only the catalytic activity of an isoform is usually predominant in a specific tissue, such as the striatal AC5 or the olfactory AC3, in combination with the recent approach using specific AC knockout mice [8], encourages AC-selective drug targeting. Olfactory AC3-discerning ligands, framed potentially on the structure of the two 7-deacetyl-7-hydroxaminoFSK and 5,6-dehydroxy-7-deacetyl-7-nicotinoylFSK derivatives, could be used to enhance neuronal migration performance, resulting in an addition route for drug delivery that obviates the inherent barriers associated with the CNS. AC1 and AC5 targeting might enable researchers to discern the relevance of the shift in calcium- and voltage-sensitive AC expression and activity during development of striatal neurons. As the supply of fetal-derived neuroblastic tissue is limited, *in vitro* studies on cell lines, expressing constitutive and/or induced DA/Ca^{2+}-sensitive ACs during their differentiation, can offer an easy-to-handle tool for performing new structure–differentiation relationships in AC drug targeting. In this context, functional neurons have been recently obtained directly from fibroblasts, without inducing the first cell undifferentiated pluripotent state [96]. This new approach mirrors increasing evidence on the epigenetic stability of differentiation, where distinct states of pluripotency can interconvert through the modulation of both cell intrinsic and exogenous factors. Interestingly, FSK has been reported to be one of the crucial exogenous factors involved in the stabilization of naïve pluripotent state in human fibroblasts *in vitro* [97]. These studies will help to generate specific neurons in a patient without the risk of carcinogenesis, which is currently associated with stem cell replacement therapy.

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