Chapter 12
The Role of Phosphodiesterase-2 in Psychiatric and Neurodegenerative Disorders

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Abstract Cyclic nucleotide PDEs are a super-family of enzymes responsible for regulating intracellular levels of the second messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Through their catalysis, PDEs are able to exert tight regulation over these important intracellular signaling cascades. Previously, PDEs have been implicated in learning and memory, as well as in mood disorders, such as anxiety and depression. PDE2 is of special interest due to its high level of expression in the forebrain, specifically in the isocortex, entorhinal cortex, striatum, hippocampus, amygdala, and medial habenula. Many of these brain regions are considered participants of the limbic system, which is known as the emotional regulatory center of the brain, and is important for modulating emotion and long-term memory. Therefore, PDE2s coincidental expression in these areas suggests an important role for PDE2 in these behaviors, and researchers

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are continuing to uncover the complex connections. It was shown that PDE2 inhibitors have pro-cognitive effects in tests of memory, including the object recognition test. PDE2 inhibitors are also protective against cognitive deficits in various models of cognitive impairment. Additionally, PDE2 inhibitors are protective against many different forms of stress-induced anxiety-like and depression-like behaviors. Currently, there is a great need for novel therapeutics for the treatment of mood and cognitive disorders, especially anxiety and depression, and other neurodegenerative diseases, such as Alzheimer's disease, and PDE2 is emerging as a viable target for future drug development for many of these diseases.

Keywords PDE2 • Memory and cognition • Stress • HPA axis • Depression • Anxiety • Pain

12.1 History of PDE2

The 3′5-cyclic-guanosine monophosphate (cGMP)-stimulated cyclic nucleotide phosphodiesterase (PDE) that hydrolyzes cAMP was first identified in a rat liver by Beavo, Hardman and Sutherland in 1971 (Beavo et al. 1971). They noted that, while cGMP tended to inhibit PDE activity in bovine heart, it was able to stimulate PDE in rat liver, indicating a likely novel subtype. Similar activity from this novel enzyme was subsequently found in numerous other tissues, including bovine adrenal and heart tissue (Martins et al. 1982), and was eventually given the name PDE2. PDE2 was later recognized as a dual specificity enzyme, capable of hydrolyzing both cGMP and cAMP. This ability to modulate activity in response to cGMP allows the enzyme to mediate the 'cross-talk' between the cGMP and cAMP signaling cascades.

PDE2 was cloned and sequenced in 1986 (Chen et al. 1986; Sass et al. 1986). Three different splice variants of PDE2 are encoded by a single gene (PDE2A) and differ only in their N-terminal, which determines intracellular localization to the membrane (PDE2A2 or PDE2A3) or soluble fraction (PDE2A1). PDE2A2 was originally cloned from rat brain cDNA (Repaske et al. 1992; Yang et al. 1994), while PDE2A1 and 2A3 were cloned from bovine heart cDNA (Sonnenburg et al. 1991; Tanaka et al. 1991). An identical PDE2A3 variant from bovine was also cloned from human brain tissue (Rosman et al. 1997). Researchers have yet to find any 2 of the splice variants co-expressed in one of these systems. A similar cAMP-stimulated PDE2, is found in the parasite *Trypanosoma brucei,* but has yet to show significant cGMP activity (Laxman et al. 2005; Rascón et al. 2002).

Though PDE2 activity has been demonstrated in a variety of tissues over the last three decades, the lack of a brain penetrant PDE2-selective inhibitor has limited progress in fully understanding the role of PDE2 in biological systems and signaling. Nevertheless, behavior work that has been carried out over the last 20 years has shown PDE2 as a promising target for drug development for the potential treatment of a variety of cognitive disorders (Table 12.1). Though no PDE2 inhibitors have

Table 12.1 Overview of effects of PDE2 inhibitors in the CNS

Task		Model (species)	PDE2 inhibitors used	Results	Reference
Memory and cognition	Social and object recognition; T-maze	Unimpaired (rats and mice); MK-801 induced memory impairment (mice)	Bay 60-7550	Memory consolidation improved in unimpaired animals; MK-801 induced memory deficits were reversed	Boess et al. (2004)
	Object recognition	Unimpaired (rat)	Bay 60-7550	Memory consolidation improved	Rutten et al. (2007a, b)
	Object recognition	Age related memory impairment (rat)	Bay 60-7550	Memory acquisition and/or consolidation improved	Domek-Łopacińska and Strosznajder (2008)
	Object recognition	Acute tryptophan depletion induced memory deficits (rats)	Bay 60-7550	Memory acquisition impairment was reversed	van Donkelaar et al. (2008)
	Object location	Unimpaired (rats)	Bay 60-7550	Memory consolidation improved	Rutten et al. (2009)
	Object location; Y-maze	AD model (APPswe/PS1dE9 mice)	Bay 60-7550	Chronic treatment reversed memory deficits in AD mice	Sierksma et al. (2013)
	Object recognition	Scopolamine or MK-801 induced memory impairment (rats)	Bay 60-7550	Memory acquisition impairment was reversed	Reneerkens et al. (2013)
	Object recognition	Unimpaired (rats)	Bay 60-7550	Memory consolidation improved	Bollen et al. (2014)
	Object recognition	Unimpaired (mice)	Bay 60-7550, ND7001	Memory acquisition, consolidation and recall improved	Lueptow et al. (2016)
	Morris water maze; object recognition; object location	Chronic stress induced memory impairment (mice)	Bay 60-7550	Chronic treatment reversed memory deficits induced by chronic stress	Xu et al. (2015)

(continued)

Table 12.1 (continued)

Task		Model (species)	PDE2 inhibitors used	Results	Reference
Schizophrenia-related cognition	Object recognition; ED/ID; P20-N40 auditory gating	PCP induced memory deficits (rats); DBA/2 mouse model with symptoms related to schizophrenia	Lu AF64280	PCP-induced or genetic executive functional deficits were reversed	Redrobe et al. (2014)
	ED/ID	PCP induced memory deficits (rats)	Bay 60-7550	Executive functional deficits were reversed	Rodefer et al. (2012)
Anxiety disorder	Elevated plus maze; open field; hold-board	BSO (oxidative stress) induced behavioral deficits (mice)	Bay 60-7550	Anxiolytic-like effects	Masood et al. (2008)
	Elevated plus maze; (?)	Unimpaired or acute restraint stress induced behavioral deficits (mice)	Bay 60-7550; ND7001	Anxiolytic-like effects	Masood et al. (2009)
	Elevated plus maze; marble burying	Chronic stress induced behavioral deficits (mice)	Bay 60-7550	Anxiolytic-like effects	Ding et al. (2014)
Depressive disorder	Tail suspension; novelty suppressed feeding	Chronic stress induced behavioral deficits (mice)	Bay 60-7550	Antidepressant-like effects	Xu et al. (2013)
	Tail suspension; forced swim	Chronic stress induced behavioral deficits (mice)	Bay 60-7550	Antidepressant-like effects	Ding et al. (2014)
Inflammatory pain	Measurement of body weight shift	Medical meniscal transection (MMT) model of osteoarthritis pain (mice)	Compound 22	Alleviation of inflammatory pain	Plummer et al. (2013b)

completely made it through clinical trials so far, interest in PDE2 as a drug target has been growing in recent years. Numerous novel PDE2 inhibitors are currently in development.

12.2 Biology of PDE2

PDE2 is a homodimer, with each monomer being approximately 105 kDa (Gesellchen and Zaccolo 2011; Martinez 2006). X-ray structural studies have revealed that the N-terminal consists of two GAF (cGMP-binding PDE, Anabaena adenylyl cyclases, Eschericihia coli FhlAs) domains, GAF-A and GAF-B. PDE2 is activated upon binding of cGMP to the GAF-B domain, which induces positive cooperativity for the hydrolysis of both cAMP and cGMP. cAMP has a 20-fold lower affinity for the GAF-B binding site, and is not shown to induce hydrolysis. Endogenous substrate cAMP/cGMP binds to the catalytic pocket that consists of a purine binding site, where the hydrolysis reaction takes place. Notably, there is invariant conserved residue, Gln859 in PDE2A, forming hydrogen bonds with the purine ring of the substrate at the apex of the purine-binding site. The free rotation of Gln859 in PDE2 makes it possible to form hydrogen bonds with the exocyclic amino group of cAMP and the exocyclic carbonyl oxygen of cGMP, which determined the selectivity for both cAMP and cGMP (C.-R. Yang et al. 2012). However, the rate of cAMP hydrolysis is increased nearly 30-fold in the presence of low, micromolar concentrations of cGMP, and once activated, PDE2 is able to hydrolyze both cAMP and cGMP with a similar Km value (30 μM and 10 μM, respectively) (Gesellchen and Zaccolo 2011; Martinez 2006).

Expression of PDE2 in the central nervous system (CNS) is fairly restricted to forebrain regions, and very limited in mid or hind brain regions. *In situ* hybridization of PDE2A in the rat brain showed high levels of PDE2 in the gray matter forebrain, the pyramidal cell layer, and to a lesser extent the granule cell layer, of the hippocampus, and amygdala, but not in the substantia nigra or other mesencephalic or diencephalic brain regions (Stephenson et al. 2012) (Fig. 12.1). Immunohistochemistry shows similar expression patterns (Stephenson et al. 2012; Diane T Stephenson et al. 2009). Furthermore, human, primate, dog and mouse cortex share a similar protein expression pattern in cortex with rat (Diane T Stephenson et al. 2009; Sadhu et al. 1999). In human, PDE2 mRNA expression was observed with comparable levels in healthy adults, patients with Alzheimer's disease and age matched controls (Lakics et al. 2010; Reyes-Irisarri et al. 2007). For most of the brain regions that have PDE2A immunoreactivity, PDE2A expression is mainly found in the neuropil rather than cell bodies, with a notable exception of the medial habenula (Stephenson et al. 2009). Therefore, PDE2 has a complex biochemical regulation over a variety of different physiological processes and functions in the brain. However, to date PDE2 inhibitors have only served primarily as research tools, rather than being used in clinical studies. Again, a unique property of PDE2 is its ability to mediate negative "cross talk" between the cGMP and cAMP pathways, because of the cooperative kinetics in hydrolysis of cAMP and cGMP by this enzyme.

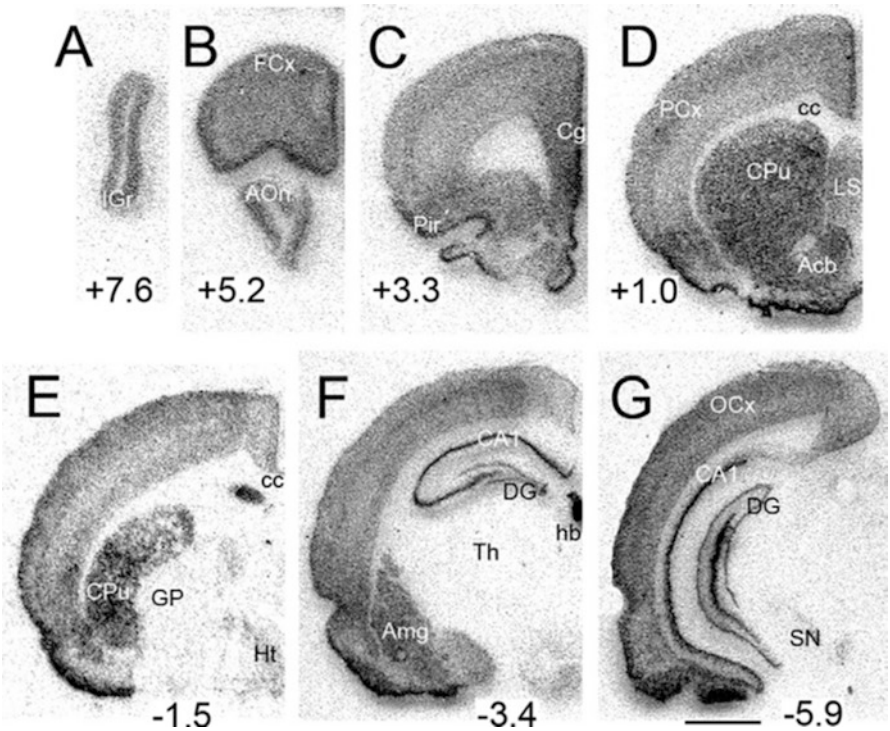

Fig. 12.1 Distribution of PDE2A mRNA in coronal sections of rat brain, determined by in situ hybridization. Hybridization signal is black. Numbers at bottom of panels are distance from bregma, according to the atlas of Paxinos and Watson (1997). Message is high in cortical regions, striatum, and the habenula. *A* cerebral aqueduct, *ac* anterior commissure, *Acb* n. accumbens, *ACo* anterior cortical amygdaloid n, *Amg* amygdaloid complex, *AOn* anterior olfactory n., *BLA* basolateral amygdaloid n, *CB* cerebellum, *CC* central canal, *cc* corpus callosum, *Cg* cingulate cortex, *Ce* central amygdaloid n, *CP* caudate-putamen, *CPu* caudate n. and putamen, *dc* dorsal columns, *dcs* dorsal corticospinal tract, *DG* dentate gyrus, *DR* dorsal raphe, *FCx* frontal cortex, *GC* granule cell layer, *GP* globus pallidus, *GPe*, external part of globus pallidus, *hb* habenula, *Ht* hypothalamus, *IC* inferior colliculus, *IGr* internal granule cell layer of olfactory bulb, *In* interpeduncular nucleus, *LL* lateral lemniscus, *LS* lateral septal n., *M* molecular layer, *MR* median raphe, *MVe* medial vestibular nucleus, *OCx* occipital cortex, *Opt* optic tract, *PC* Purkinje cell layer, *PCx* parietal cortex, *Pir* piriform cortex, *Pl* plexiform layer of olfactory bulb, *Py* pyramidal cell layer, *RF* rhinal fissure, *SG* substantia gelatinosa, *SL* stratum lucidum, *SN* substantia nigra, *SNc* substantia nigra pars compacta, *SNr* substantia nigra pars reticulata, *SO* stratum oriens, *SR* stratum radiatum, *Th* thalamus, *3v* 3rd ventricle, *VH* ventral horn. Scale bar in G = 2.5 mm. (Reprinted by permission from Macmillan Publishers Ltd.: Neuroscience, Stephenson et al. (2012) copyright 2012)

In vivo analysis of the role of PDE2 in rodent behavior was only first started in 2002, when Suvarna and O'Donnell began investigating the role of PDE2 in cAMP/cGMP hydrolysis in the central nervous system using rat primary cerebral cortical and hippocampal cultures, a mostly unexplored area at the time in regards to PDE2 function. Using this neuronal preparation, they were able to demonstrate the differential role of PDE2 and PDE4 in cGMP and cAMP hydrolysis, respectively (Suvarna and O'Donnell 2002). While PDE2 is a dual-hydrolysis enzyme,

capable of degrading both cAMP and cGMP, they showed that NMDA-mediated increases in cAMP were enhanced by the PDE4 inhibitor rolipram, while NMDA-mediated increases in cGMP were blocked by PDE2 inhibitor EHNA. Subsequent research has continued to focus on the role of PDE2 in CNS and behavior, and is reviewed below.

12.3 Current PDE2 Inhibitors

Though PDE2 was identified almost 50 years ago, it wasn't until the early 2000s and the development of a selective inhibitor that researchers were confidently able to begin to analyze the role of PDE2 in mammalian systems. Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), a potent adenosine deaminase inhibitor, was the first compound discovered to have PDE2 isozyme selective properties, with an IC_{50} of ~0.8 uM for human PDE2A (Méry et al. 1995; Podzuweit et al. 1995). Unfortunately, because EHNA is also a potent adenosine deaminase inhibitor, studies require additional controls and careful interpretation of any findings. A major advancement came with the synthesis of Bay 60-7550 by Bayer in 2002 (Niewohner et al. 2003). Bay 60-7550 has an IC_{50} value of 2.0 ± 0.7 nM when used on purified PDE2 from bovine heart, and an IC_{50} of 4.7 ± 1.0 nM when used on human recombinant PDE2 (Boess et al. 2004). Additionally, Bay 60-7550 has 50-fold selectivity for PDE2 over PDE1, and 100-fold or more selectivity over other PDEs (Boess et al. 2004; Buijnsters et al. 2014; Gomez and Breitenbucher 2013; Heine et al. 2013; Martinez 2006; Masood et al. 2009). And despite having solubility and pharmacokinetic issues, Bay 60-7550 has opened the door for uncovering the role of PDE2 in mood and cognitive disorder that will be reviewed here.

One published study measured the amount of Bay 60-7550 in the rat brain following oral administration. Brain and plasma exposure of 1 mg/kg Bay 60-7550 was only detectable 30 min after drug administration (about 0.045 ng/g). Recent data from our lab suggests that brain exposure to Bay 60-7550 might be higher than that using a modified LC/MS method (data not published). However, even if minimal amounts of Bay 60-7550 are able to cross the blood brain barrier, it can still be taken into consideration that a low brain concentration of the compound would be sufficient to produce the cognitive enhancement. This is probably due to the rich expression of PDE2 in brain regions responsible for the object recognition memory, and the fact that Bay 60-7550 could affect both cyclic nucleotide (cAMP and cGMP) signal cascades, thereby causing biological responses because of signal amplification in the cascades. To support these hypotheses, a second, potent PDE2 inhibitor would be required in similar studies. Moreover, it is also possible that an active metabolite instead of Bay 60-7550 itself is responsible for the memory enhancing effects.

Though Bay 60-7550 is the primary PDE2 inhibitor used in the literature, ND7001 was synthesized around the same time (Abarghaz et al. 2005). It has an IC_{50} of about 50 nM for human PDE2, with high selectivity over other PDEs (Gomez

and Breitenbucher 2013). Both Bay 60-7550 and ND7001 increase levels of cGMP in primary neuronal cultures; however, unlike Bay 60-7550, ND7001 is unable to significantly increase levels of cAMP (Masood et al. 2009). In France in 2005, Neuro3d (now Evotec AG) initiated Phase I clinical trials with ND7001 for the treatment of anxiety and depression. The results of these trials are unknown, and no further development has since been reported. As of 2010, the clinical trial appears to have been discontinued. Wyeth also published series of PDE2/10 dual inhibitors around that time, which exhibited slightly higher potency for PDE2, improved memory and cognition, and reduce anxiety behaviors after oral dosage to rodents (Gomez and Breitenbucher 2013). Based on modification of these structures, Boehringer Ingelheim International Gmbh published series of patents application disclosing two compounds with similar SAR, high selectivity for PDE2a over PDE10 (either 15- or 250-fold), and reported pro-cognitive, antidepressant, anxiolytic, and anti-convulsant effects in *in vivo* models in rodents. Janssen and Lundbeck also published a number of patent applications related to triazolopyrazine analogs, which could possess about 25-fold selectivity on PDE2, even though behavioral-related data were reported. Further down the road, Pfizer published a novel series of pyrazolopyrimidine PDE2 inhibitors, among which PF-05180999 (IC_{50} = 0.001 μM, 2000-fold selective over PDE10) is now available from Sigma (CAS Number 1394033-54-5), and exhibited free brain/plasma ratio of 0.5 in rat (Gomez and Breitenbucher 2013). Pharmacological evaluation of the compound showed that it attenuated the ketamine-induced disruption of working memory, as well as scopolamine-induced spatial learning and memory. Between 2011 and 2012, the compound was reported to be well tolerated at doses between 0.1 and 60 mg in a phase I clinical trial aimed at determining the safety, tolerability, and pharmacokinetics after 14 days of treatment in healthy subjects. However, a similar phase I study planning to use twice daily doses over 14 days was withdrawn prior to enrollment due to safety concerns. For the purpose of evaluating the potential benefit from PDE2 inhibition in the central nervous system, most of the studies reviewed here is based on use of Bay 60-7550.

12.4 PDE2 in Cognitive Function

12.4.1 Basic Concepts of Memory and Cognition

There are four main stages involved in learning and memory: acquisition, consolidation, storage and retrieval (García-Osta et al. 2012). Memory can further be divided into short-term memory (STM) and long-term memory (LTM). STM, also known as working memory, is able to maintain several items simultaneously for brief periods of time. Working memory provides temporary storage and manipulation of information for the brain and plays an important role in on-demand human cognition. Formation of LTM, on the other hand, may be based on the successful

processing of working memory. LTM can be divided into three categories based on the type of information: explicit memory (declarative memory), implicit memory (procedural memory), and autobiographical memory. The role of PDE2 in these memory processes will be discussed in this section.

12.4.2 Evidence for Involvement of PDE2 in Cognitive Function

The expression of PDE2 in the CNS is primarily in limbic and cortical brain regions, including the hippocampus, all of which have been implicated in learning and memory (Gesellchen and Zaccolo 2011; Martinez 2006; Stephenson et al. 2009; Van Staveren et al. 2003). In fact, research has shown PDE2 inhibition is linked to enhance learning and memory in numerous cognitive tasks in rodents (Boess et al. 2004; Bollen et al. 2014; de Vente et al. 2006; Domek-Łopacińska and Strosznajder 2008; Lueptow et al. 2016; Redrobe et al. 2014; Reneerkens et al. 2013; Rodefer et al. 2012; Rutten et al. 2007a, b, 2009; Sierksma et al. 2013; van Donkelaar et al. 2008; Xu et al. 2015). Additionally, the underlying signaling involved in mediated memory enhancement following PDE2 inhibition appears to be primarily the nitric oxide synthase (NOS)/cGMP/protein kinase G (PKG) pathway, over the cAMP/protein kinase A (PKA) pathway (Bollen et al. 2014; Lueptow et al. 2016; Rutten et al. 2007a, b). Though, as will be discussed below, there is likely more to the story, including differential, time sensitive elevation of cAMP vs cGMP (Bollen et al. 2014; Rutten et al. 2007a, b).

In 2004, Boess and colleagues first reported the significant memory enhancing effects of Bay 60-7550 in the object recognition test (ORT) and social recognition test (SRT) (Boess et al. 2004). In the ORT, mice and rats were exposed to 2 identical objects. Immediately after training, they were injected with vehicle or Bay 60-7550. Twenty-four hours later the rodents were returned to apparatus and exposed to one of the familiar objects and one novel object. Mice treated with Bay 60-7550 showed significantly enhanced memory (Fig. 12.2). Similarly, an adult rat and juvenile rat were allowed to interact in the SRT. Immediately after the interaction, they were injected with vehicle or Bay 60-7550. Twenty-four hours later, they were reintroduced, and those treated with Bay 60-7550 showed significant decreases in time exploring the juvenile rats, indicated enhanced memory (Fig. 12.2).

In addition to behavior changes observed following Bay 60-7550 administration, these researchers have also demonstrated some of the *in vitro* mechanisms of Bay 60-7550 and PDE2. Incubation of Bay 60-7550 in primary cerebral cortical neurons from both mice and rats resulted in a dose-dependent increase in both cAMP and cGMP, though the changes of cGMP levels were much more dramatic than those of cAMP. This is perhaps due to the presence of several other cAMP metabolizing PDEs in the incubation systems. The results were consistent with the observation that the PDE2/adenosine deaminase inhibitor EHNA enhanced the NMDA receptor-

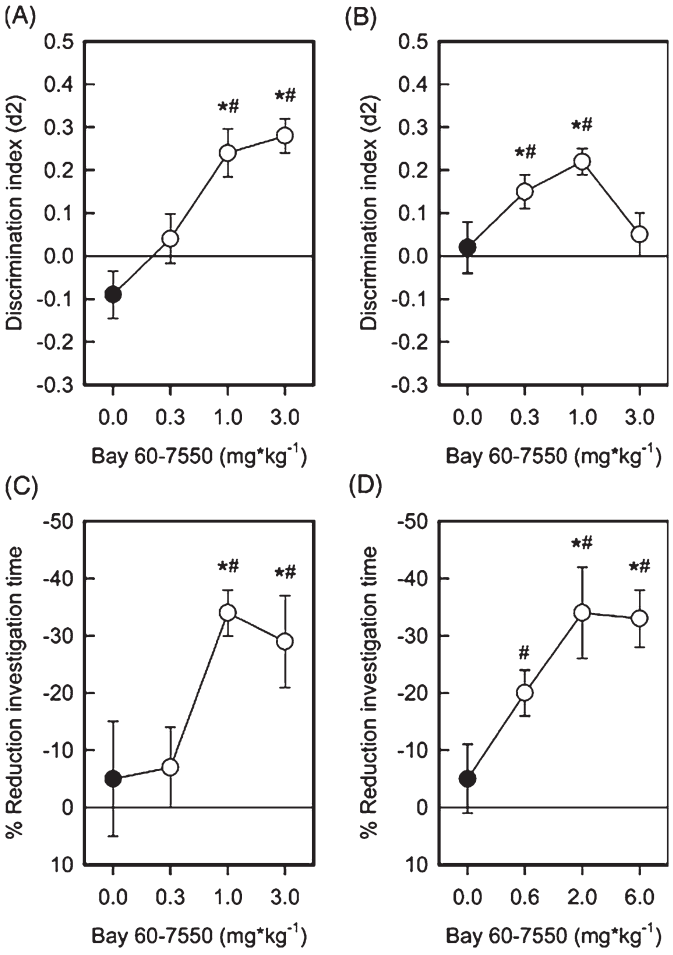

Fig. 12.2 Effects of Bay 60-7550 on object recognition and social memory. Bay 60-7550 improved object recognition performance in rats (A), and mice (B), measured as an increase in the discrimination index d2 [d2 = exploration time new object/exploration time familiar object)/(total exploration time)]. Independent groups of rats were tested (12 rats were used for each treatment group). All mice (n = 24) were tested in all treatment conditions using a within sub- jects design with three or more days between treatments. Bay 60-7550 improved social memory in rats (C and D) measured as a reduction of the time a juvenile is investigated by an adult rat during the second encounter. Independent groups of rats were tested (10 rats were used for each treatment group).? $p < 0:05$ different from vehicle control (Fischer's LSD post hoc comparison). #$p < 0:05$ different from zero (one-sample t-statistics). (Reprinted by permission from Macmillan Publishers Ltd.: Neuropharmacology, (Boess et al. 2004) copyright 2004)

induced elevation of cGMP (but not cAMP) levels in rat cortical and hippocampal neurons in primary culture (Suvarna and O'Donnell 2002).

Bay 60-7550 also significantly increased long-term potentiation (LTP) in the CA1 region but did not affect basal synaptic transmission, suggesting a use-dependent enhancement of synaptic function. Based on these findings, the effect on

synaptic efficacy may be due to a PKG-mediated increase in neurotransmitter release. It is also possible that postsynaptic changes due to PKG-mediated phosphorylation of ion channels or other elements of signal transduction pathways, may also contribute to the changes in the strength of the postsynaptic response during LTP. Nevertheless, there is a clear role of NO/cGMP signal transduction in synaptic plasticity, and PDE2 is an important element of this pathway. Moreover, since both cAMP and cGMP are known to induce vasodilation, which could contribute to improved cognitive functions, Rutten et al. (2009) examined this possibility by measuring the effects of PDE inhibitors on local cerebral blood flow and glucose utilization in rats. It was shown that the cGMP hydrolyzing PDE5 decreased the ratio between blood flow and glucose utilization, indicative of general oligaemia, whereas the cAMP hydrolyzing PDE4 increased this ratio, indicative of general hyperemia. Both oligaemic and hyperemic conditions do not explain memory enhancement, suggesting that the memory enhancement induced by PDE inhibitors are not likely due to cerebrovascular effects.

12.4.3 Time-Sensitive Improvement of Memory Induced by PDE2 Inhibition

Decades of memory research have suggested that memory enhancement as a result of increases in either cyclic nucleotide (cAMP or cGMP) is time-dependent. cGMP and cAMP have been proposed to participate in the early and late stages of LTP, respectively. Accordingly, cGMP-regulated processes in the hippocampus play an important role in the early stages of memory consolidation and cAMP signaling pathways are involved in the later consolidation mechanism.. It was shown that a post-training, intra-hippocampal infusion of 8 Br-cGMP (1.25 micrograms per side), a membrane-permeable analogue of cGMP, enhanced retention test performance when given immediately (0 min) after training, but not when given 180 min after training. Conversely, intra-hippocampal infusion of the same dose of 8 Br-cAMP facilitated memory consolidation when given 180 min, but not 0 min, after training. Moreover, rats submitted to an inhibitory avoidance task showed a significant increase in the amount of cGMP in the hippocampus at 0 and 30 min after training, and in the amount of cAMP at 30 and 180 min after training. By injecting the protein synthesis inhibitor anisomycin or the PKA inhibitor Rp-cAMPs at various time windows after training, it was discovered that contextual memory has either one or two brief consolidation periods requiring both synthesis of new proteins, and activation of PKA. Of these, weak training results in two time periods of sensitivity to inhibitors of protein synthesis and PKA, whereas stronger training exhibits only one. Inhibition of PDE2 may lead to increases in either cAMP, cGMP, or both. Therefore, use of a brain penetrant PDE2 inhibitor may not only affect different phases of memory formation and recall but also provide a better understanding of the underlying pathways that are involved in PDE2 inhibitor-induced changes in memory. Unfortunately, different research groups tend to use individual protocols

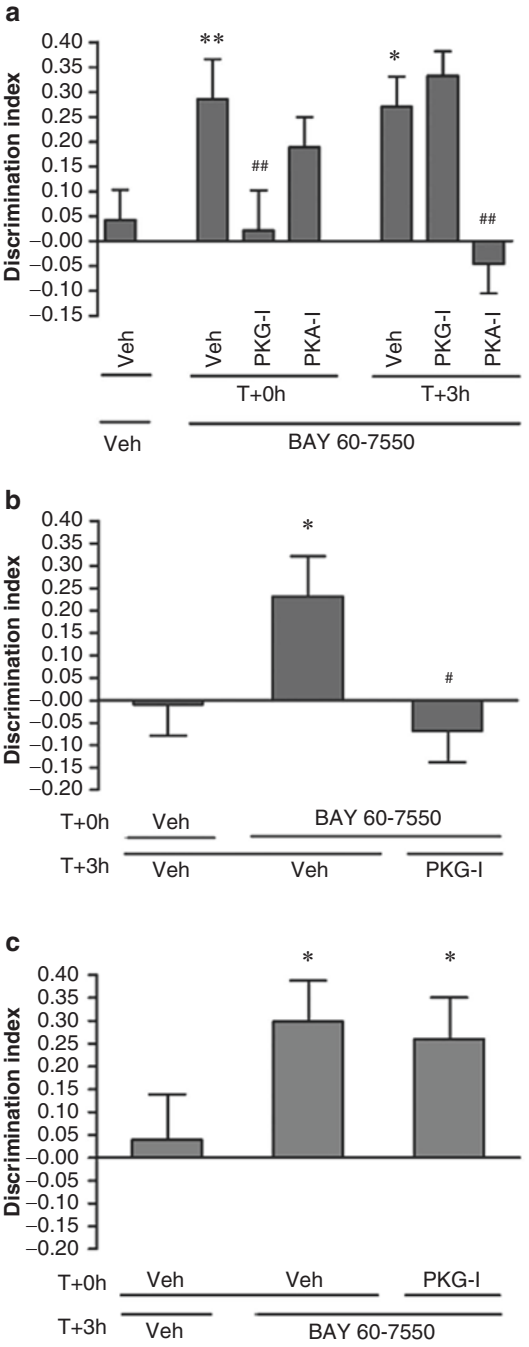

Fig. 12.3 Role of cAMP vs cGMP in PDE2 inhibition. (**a**) Bay 60-7550 3 mg/kg, p.o., n = 26/19/18/19/18/16/19 on discrimination performance (discrimination index; means ± SEM) in a 24-h-delay object recognition task. PDE inhibition was administered immediately after

with different training and testing parameters, as well as different apparatuses, so observed time-dependent changes may be due to these protocol differences, rather than replicable changes in cAMP and cGMP (Bourtchouladze et al. 1998).

Studies by Rutten et al. have shown that Bay 60-7550 enhanced memory in the ORT to be time-sensitive as well. When administered immediately after training or 3 h. after training, Bay 60-7550 significantly enhanced memory in the ORT in rats (Fig. 12.3a). However, when given 1 or 6 h. after training, Bay 60-7550 did not affect memory in the ORT, suggesting PDE2 plays a role in specific early and later consolidation processes (Rutten et al. 2007a, b). In this same test, treatment with vardenafil, which is a PDE5 (a cGMP selective PDE) inhibitor immediately after training, but not 1 or 3 h after training resulted in enhanced memory, suggesting the efficacy of Bay 60-7550 during early consolidation is due to cGMP Alternatively, when rolipram, which is a PDE4 (cAMP selective PDE) inhibitor was given 3 h after training, there was a significant enhancement of memory, but not immediately after training or 1 h. after training, which suggests he memory enhancing effects of Bay 60-7550 when administered 3 h after training is due to enhacement of cAMP. Since Bay 60-7550 has a rather short elimination half-life of approximately 45 min after p.o. injection, it suggests that time windows for influencing early consolidation processes that are possibly via cGMP, and late consolidation processes that are possibly through cAMP, are specific and narrow. The results from the experiment with Bay 60-7550 support the notion of a specific and time-critical role of cGMP and cAMP in memory consolidation, which is also supported by data from the PDE4 inhibitor rolipram and the PDE5 inhibitor vardenafil (Rutten et al. 2007a, b).

Time-course analysis in mice resulted in a slightly different effect. Lueptow et al. showed that Bay 60-7550 administration 30 min prior to training, immediately after training, or 30 min prior to recall resulting in significantly enhanced object recognition memory, with no effect when administered 1 or 3 h. after training (Lueptow et al. 2016). These differences may be due to methodological disparities between the research groups, including between-subjects vs within-subjects design, different training schedules, as well as different apparatuses. Despite the species difference, housing protocols also vary. In studies with rats, subjects were singly housed, which

Fig. 12.3 (continued) (T + 0 h) or 3 h (T + 3 h) after the first trial. When PDE inhibition combined with saline intra-hippocampal injections (veh) yielded significant improvement of discrimination, we subsequently combined PDE treatment with inhibitors of PKG (PKG-I; RP-8-Br-cGMPS 1 mg/side) or PKA (PKA-I; RP-8-Br-cAMPS 1 mg/side). (**b**) Bay 60-7550 3 mg/kg, n = 28/12/13) in combination with PKA inhibition (PKA-I; RP-8-Br-cAMPS; i.h.; 1 mg/side) 3 h (T + 3 h) after the first trial on discrimination performance (discrimination index; means ± SEM) in a 24-h-delay object recognition task. (**c**) PDE2 inhibition—Bay 60-7550 3 mg/kg, n¼9/10/11) administered 3 h after learning in combination with PKG inhibition (PKG-I; RP-8-Br-cGMPS; i.h.; 1 mg/side) immediately after the first trial on discrimination performance (discrimination index; meansþSEM) in a 24-h-delay object recognition task. Asterisks indicate significant differences (*p < 0.05, **p < 0.01) of PDE inhibition treatment from vehicle condition. Hashes indicate a significant reversal of PDE-induced memory improvement ((#)p < 0.056, #p < 0.05, ##p < 0.01). (Reprinted by permission from Macmillan Publishers Ltd.: Neuropsychopharmacology, (Bollen et al. 2014) copyright 2014)

may cause a type of stress that is related to social isolation. Social isolation in rats has been shown to result in a hyperactive state with increased novelty seeking and increases in exploration time during the ORT, which complicates the interpretation of both behavioral and biochemical outcomes following PDE2 inhibition in these rats. Numerous behavioral, neurochemical, and neurophysiological changes, as well as differential responses to psychotropic drugs are indeed associated with social isolation, which in turn can be used as a model for various psychiatric disorders, including schizophrenia (Bianchi et al. 2006; Douglas et al. 2003; Thorsell et al. 2006). In another sense, social isolation also leads to increased levels of anxiety-like behavior and results in altered hippocampal organization and hypothalamic–pituitary–adrenal axis function (Bianchi et al. 2006; Weiss et al. 2004). These changes are important to note because PDE2 inhibition is protective against stress-induced anxiety- and depressive-like behavior, as well as stress-induced deficits in learning and memory, further complicating the interpretation of results in socially isolated rats (Ding et al. 2014; Masood et al. 2009; Masood et al. 2008; Xu et al. 2013, 2015). Also, different route of drug administrations may also contribute to the discrepancies from rat and mouse studies. When Bay 60-7550 was given through oral gavage like most of the rat studies did, an additional gut absorption phase on top of the distribution to the brain needs to be taken into consideration.

Probing a little deeper, Bollen and colleagues have shown that PKG, but not PKA inhibition prior to Bay 60-7550 administration, which was given immediately after training, blocks memory enhancement in the ORT (Bollen et al. 2014). Additionally, PKA but not PKG inhibition prior to Bay 60-7550 administration, which was given 3 h. after training, blocks memory enhancement in the ORT (Fig. 12.3b). These findings again suggest that cGMP enhancement is critical for early consolidation mechanisms, while cAMP enhancement is critical for later consolidation mechanisms (Bollen et al. 2014). Additionally, administration of Bay 60-7550 immediately after training plus a PKA, but not PKG, inhibitor 3 h. after training, blocks memory enhancement in the ORT, suggesting that early cGMP activity requires later cAMP activity (Bollen et al. 2014) (Fig. 12.3c). This highlights the possibility that differences in the window for Bay 60-7550 memory enhancement between mice and rats mentioned previously may be due to a shift in the critical window for cGMP and/or cAMP enhancement. This time-course specific enhancement of cAMP vs cGMP demonstrates the time-dependent importance of both cyclic nucleotides in memory consolidation processes. Additionally, the differential regulation of cAMP/cGMP emphasizes the importance in furthering our understanding of the role of PDE2 at these different time points.

12.5 PDE2 in Cognitive Dysfunction

Memory impairments and cognitive decline are a significant health problem around the world, especially with aged population and individuals with neurodegenerative conditions such as Alzheimer's, depression, and schizophrenia. Extensive research

has been conducted to determine the fundamental biological mechanisms underlying not only learning and memory, but also cognitive dysfunction. Nevertheless, there is still a significant medical need for new drugs which delay or reverse these cognitive deficits. To date, most commercially available drugs are not able to delay or reverse cognitive deficits, but only relieve some symptoms during the mild to moderate stages of disease progression. These drugs are targeted to one of the major neurotransmitter systems, include the acetylcholine, serotonin, histamine, glutamate, and dopamine systems. While these classes of drugs are able to ameliorate some of the cognitive deficiencies temporarily, because they target large transmitter systems, they do come with some adverse side effects An alternate approach is to target downstream signaling cascades, which trigger the long term transcriptional and structural response to neuronal activity that underlies the formation of long term memories. PDE2 serves as an important potential biological target for therapeutic intervention in a variety of disorders, due to its important role in terminating transcriptional cascades triggered by cAMP and cGMP.

As mentioned earlier, research using ORT and similar memory tests has shown PDE2 inhibition enhances learning and memory in both mice and rats. In terms of translational possibilities, it is also important to analyze PDE2 in more pathological systems. To that end, the PDE2 inhibitor Bay 60-7550 has shown to be protective against a number of different memory-disrupting conditions. One approach is to disrupt ORT memory with pharmacologic agents, among which the NMDA receptor antagonist MK-801, as well as the anticholinergic agent scopolamine are two most commonly used ones. Scopolamine is a widely used anti-cholinergic agent to create a memory deficit model. It is often used to study the effects of drug administration before the learning trial (Klinkenberg and Blokland 2010). This has shown to impair memory in several behavioral tests including ORT. It was demonstrated that the cAMP-selective PDE4 inhibitor rolipram reversed a scopolamine-induced memory deficit in the ORT and other behavioral tests in rodents (Egawa et al. 1997; Rutten et al. 2006; Zhang et al. 2000). MK-801 is another commonly used agent to induce memory deficit in preclinical cognition research. It is an N-methyl-D-aspartic acid (NMDA) antagonist which impairs memory function and attention processes and is therefore assumed to be more affiliated to cognitive deficits related to schizophrenia (van der Staay et al. 2008). Treatment with Bay 60-7550 30 min before training reversed the memory impairment induced by both scopolamine and MK-801 (Reneerkens et al. 2013). With a 24 h. inter-trial interval, the discrimination index of control animals has been reported close to 0, therefore a 1 h. interval between T1 and T2 was used in this study in order to create a differentiated baseline between the normal animals and the scopolamine treated animals. Similarly, T-maze continuous alternation task is a measurement of predominantly spatial working memory. Boess et al. (Boess et al. 2004) demonstrated that administration of 3 mg/kg Bay 60-7550 (p.o.) 30 min before the test session reversed a MK-801-induced working memory deficit in the T-maze in mice, while vehicle-treated mice had an alternation rate higher than the chance level, mice injected with MK-801 (0.04 mg/kg) 30 min before the test had a lower continuous alternation rate.

Another method for disrupting memory involves depletion of tryptophan (ATD, acute tryptophan depletion), which results in depletion of serotonin in the brain and ultimately temporary deficits in cognition. ATD is frequently used to induce short-term deficit in brain 5-HT synthesis through manipulation of the essential amino acid tryptophan (TRP), the dietary 5-HT precursor. 5-HT along with its receptors have been found to be critically important for learning and memory. Through depletion of TRP from ingestion, less peripheral TRP is available to enter the brain for synthesis into 5-HT, thereby creating a so-called "serotonergic deficit model" and has been widely used to interfere with short-term memory in the rat object recognition task (Jans et al. 2007, 2010; Jans and Blokland 2008; Lieben et al. 2004; Olivier et al. 2009; Rutten et al. 2007a, b; van Donkelaar et al. 2008). It has been shown that only moderate peripheral TRP depletion or a single administration of a TRP-free nutritional mixture is required to generate the memory deficit model. Following serotonin depletion, 3 mg/kg Bay 60-7550 restored memory in the ORT with a 2 h. inter-trial interval (Eva L van Donkelaar et al. 2008). However, these behavioral changes induced by inhibition of PDE2 were not accompanied by altered levels of 5-HT or its precursor 5-HIAA (van Donkelaar et al. 2013), suggesting that mechanisms other than direct regulation of 5-HT neurotransmitter is involved in the process.

Memory loss can also result from unwanted daily stress. In learning and memory studies using rodents, two commonly used types of stress include the chronic unpredictable stress (CUS) and oxidative stress. CUS involves daily, randomly administered mild stressors, including wet cage bedding, short-term food/water deprivation, overnight lights on, etc., that, over time, result in increases in anxiety and depression, as well as decreases in cognitive abilities. In response to these repeated stressors, the brain undergoes a complex array of cellular and molecular changes that lead to maladaptive remodeling, which are correlated with the observed changes in behavior. Therefore, while much of stress research has focused on interventions for anxiety and depression, attenuating or reversing the deleterious effects of stress on cognitive processes is also important. PDE2 inhibition appears to be a promising avenue for such an intervention. Xu et al. found that treatment with Bay 60-7550 during the administration of CUS prevents cognitive impairment in ORT, object location test (OLT), and Morris Water Maze (MWM). Furthermore, the protective effects of PDE2 inhibition were shown to be mediated via the NO/cGMP/PKG pathway (Xu et al. 2015).

Oxidative stress, on the other hand, is often characterized by increased reactive oxygen species (ROS) generation and decreased antioxidant capacity, i.e. an unbalanced oxidant-antioxidant status in brain. Specifically, the brain consumes large amounts of oxygen, and when damaged, cellular respiration and enzymatic activity increase, resulting in increased ROS, which interact with fatty acids and proteins. The brain has an abundance of polyunsaturated fatty acids, but relatively less of antioxidant agents compared to other organs (Cheng et al. 2010; Mehta et al. 2012; Shelat et al. 2008). This results in damage to cell membranes, interruption of membrane-bound proteins, and altered cellular permeability, i.e. a pro-oxidative state. If such cellular stress is not effectively removed, ROS may cause oxidative cell injury, protein damage, and lipid peroxidation, and energy failure that finally

causes altered neuronal function and overall brain activity, leading to onset of neurodegenerative disease, such as cognitive deficits in AD, Parkinson's disease, as well as symptoms observed following traumatic brain injury.

The link between PDE2 and oxidative stress has been demonstrated in several in vitro studies (Drobna et al. 2012; Masood et al. 2008). In the mouse hippocampal cell line HT-22, oxidative stress results in an increase in the expression of PDE2. In endothelial cells, PDE2 promotes activation of NADPH oxidase-dependent ROS production and subsequent endothelial proliferation and angiogenesis. In yeast, PDE2 hydrolyzes cAMP, prevents PKA activation, and decreases the cellular stress responses. Recent *in vivo* data from our lab (not published) suggests that the memory enhancing effects of Bay 60-7550 may be through regulation of several subunits of the NADPH oxidase enzyme, which is a critical component responsible for a number of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and multiple sclerosis.

Besides the pharmacologically induced memory deficit models, a more clinically relevant model in terms of pathology is the triple transgenic mouse line that results in Alzheimer's disease (AD) -like state. AD is one of the most prevalent neurodegenerative diseases, characterized by memory loss, personality changes, psychiatric symptoms, and severe cognitive dysfunction. It is the most frequent form of dementia found in the elderly. In the early stages of AD, there are more manageable symptoms such as impaired short-term memory; however, in later stages of the disease, individuals have almost complete loss of their cognitive abilities and are unable to function on their own, putting a great burden on friends and family members (Taylor et al. 2013).

Currently, there are only a few FDA approved drugs to treat AD symptoms, such as cholinesterase inhibitors (ChEIs) (tacrine, donepezil, rivastigmine and galantamine) and N-methyl-D-aspartate (NMDA) receptor modulators (memantine) (Nguyen and Woo 2003). However, while these drugs may temporarily ameliorate short term memory loss during earlier stages of the disease, no drugs are able to slow or reverse the course of AD (Guan et al. 2011). Therefore, researchers are interested in identifying effective drug targets that may either slow the progression of the disease in order to allow the AD patients to remain socially active and productive or to completely halt, and potentially reverse, the disease course.

The neuropathology of AD involves the overproduction and accumulation of the peptide amyloid β (Aβ), as well as neurofibrillary tangle formation leading to synaptic dysfunction. The first and most severely affected brain regions are those involved in learning, memory and other higher cognitive function (Eckert et al. 2013). Abnormal cleavage of Aβ results insufficient clearance and subsequent accumulation. Research shows that Aβ oligomerization decreases the phosphorylation of the transcription factor CREB and impairs LTP. This reduction in CREB phosphorylation and change in LTP is likely dependent on several kinases, such as PKA and PKG, which are downstream of cyclic nucleotide activation (Y. Chen and Cai 2003; Sanderson and Sher 2013). Coincidentally, reduction in the activity of specific PDEs have shown to enhance memory performance in different animal models of AD (Vitolo et al. 2002). PDE inhibitors that elevate concentrations of cAMP and/or

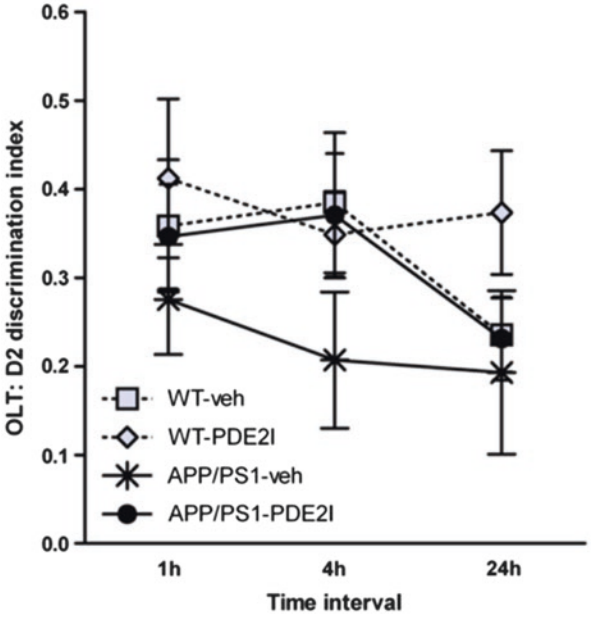

Fig. 12.4 Performance in the object location task (OLT) over 3 intervals (1 h, 4 h, 24 h). Values indicate mean and S.E.M per group. Although all animals were able to signifi- cantly distinguish between the novel and the familiar location at the 1 h and 4 h interval, i.e. $D2 > 0$, $p < 0.05$, it is interesting to note that the APPswe/PS1dE9-PDE2I group has almost the same absolute D2 scores as theWT-veh group in all intervals. At the 24 h interval APPswe/PS1dE9-veh group is not significantly able to distinguish between the novel and the familiar location, while the other groups are. (Reprinted by permission from Macmillan Publishers Ltd.: Neuropharmacology, (Sierksma et al. 2013) copyright 2012)

cGMP result in stimulation of the cAMP/PKA/CREB and cGMP/PKG/CREB pathways, respectively, to enhance synaptic transmission through increasing CREB phosphorylation and BDNF transcription. The effect of PDE2 inhibition in improving memory in the APPswe/PS1dE9 mouse model of AD has been demonstrated by Sierksma et al. (2013). Chronic treatment of the APPswe/PS1dE9 mice with Bay 60-7550 significantly improved their performance in the object location test with 1, 4 or 24 h. inter-trial intervals, and Y-maze test. Behaviors related to depression or anxiety were not affected by such treatment. Interestingly, Bay 60-7550 treatment did no alter Aβ amyloid plaque load, phosphorylation of CREB, BDNF or synaptic density in those animals (Figs. 12.4), suggesting that the memory enhancing effects of PDE2 inhibition in this AD model may not be through canonical signaling transduction.

Other types of memory deficits include those associated with aging or neuropsychiatric disorders. Studies have shown that aged brain contains a lower cGMP concentration and altered nitric oxide synthase activity (Domek-Łopacińska and

Strosznajder 2008; Liu et al. 2003). NO is an important signaling molecule in the brain that is responsible for stimulation of the soluble isoform of guanylyl cyclase (sGC), which synthesizes cGMP. During physiological aging, cognitive performance of the brain decreases, and this is often correlated with decreases of cGMP in the brain. Accordingly, inhibition of PDE2 can not only improve memory function in young adult rats, but also in 12 and 24 month-old rats (Domek-Łopacińska and Strosznajder 2008). All age groups benefited from Bay 60-7550 administration when given during memory acquisition or consolidation. Moreover, nNOS activity in all age groups also increased in response to Bay 60-7550 treatment, and blockade of nNOS activity by its selective inhibitor NAAN prevented the memory enhancing effects of Bay 60-7550. Since phosphorylation of NOS by PKA or PKG diminishes the catalytic activity of NOS rather than reinforcing it (Dinerman et al. 1994), the activity of cyclic nucleotide gated (CNG) calcium channels following elevated cGMP concentrations may be responsible for the increased activity of NOS. Interestingly, only Bay 60-7550 improved the object recognition memory in the 24 month-old rats, suggesting that the activity of PDE2 over other PDEs, specifically PDE5, is the most influential at that time point. Overall, these data suggest that inhibition of PDE2 increases cGMP levels not only by suppression of cyclic nucleotide degradation, but also by further stimulation of cyclic nucleotide synthesis through the NOS/NO/sGC/cGMP pathway.

12.6 PDE2 and Neural Plasticity

Synaptic plasticity is a critically important property of neurons that is thought to underlie learning and memory. Neuroplasticity involves both functional plasticity (regulation of synaptic transmission) and structural plasticity (such as adult neurogenesis) (Lopez et al. 2009; Mohs 2008). In the context of memory and cognition, function neuroplasticity emphasizes the role of LTP, which is a long-lasting increase in the efficacy of synaptic transmission that has been proposed as a cellular substrate underlying mammalian learning and memory (Mehta et al. 2012). Various studies have also shown that LTP and memory formation are dependent on cellular cascades stimulated by an increase of intracellular cAMP/cGMP with the subsequent activation of PKA/PKG (Domek-Łopacińska and Strosznajder 2010). A growing body of evidences indicates that cAMP/PKA/CREB and the cGMP/PKG/CREB pathways are involved in LTP and long-term memory formation (Eckert et al. 2013; Vitolo et al. 2002).

As previously discussed, cAMP is a vital intracellular second messenger involved in signaling pathways that are initiated via neurotransmitters, hormones, and many other molecules. Protein kinase A (PKA) is considered to be the main downstream kinase activated by cAMP. Phosphorylated/activated PKA leads to phosphorylation/activation of CREB and increased production of BDNF. Accumulating data regarding CREB-dependent learning and memory in mice, rats, and drosophila all indicate that CREB activation by phosphorylation at the serine residue 133 is implicated in

plasticity of synapses necessary for the maintenance of LTP and formation of long-term memory (Ermak and Davies 2002; Titus et al. 2013). Moreover, the critical genes known to be involved in neuroplasticity, such as BDNF, are also known to be affected by CREB activation. In both AD and in Huntington's disease models, the PKA inhibitor H-89 can block the activation of CREB as well as memory improvement induced by rolipram (Sanderson and Sher 2013). The dominant-negative CREB mutation as well as CREB isoform deletions can affect memory formation and long-term synaptic changes, as well as alter nerve growth and regeneration in mice (Kishida and Klann 2007). Due to the large involvement of cAMP in such processes, it is reasonable to speculate that synaptic plasticity can be modulated by manipulation of the activity of PDE2 as well (Mseeh et al. 2000). Indeed, numerous studies show that cellular cAMP levels are affected by concentrations of Ca^{2+} indirectly via manipulation of the adenylyl cyclase and PDE activity (Ota et al. 2008; Reneerkens et al. 2013; Rutten et al. 2007a, b).

Similarly, cGMP is an important second messenger generated by two varieties of guanalyl cyclases (GCs). One is cytosolic soluble GC (sGCs) activated by nitric oxide (NO) and the other is membrane-bound particulate GC (pGCs) activated by natriuretic peptides. In the central nervous, the NO-sensitive sGC isoform is the major enzyme responsible for cGMP synthesis. Numerous researchers have shown that the NO/sGC/cGMP/PKG signal transduction pathway plays a significant role in coordinating presynaptic and postsynaptic alterations that underlie long-term synaptic plasticity and memory formation (Maes et al. 1993; Puzzo et al. 2005). NO is a highly reactive radical produced by nitric oxide synthase (NOS), which can be either neuronal (nNOS), endothelial (eNOS) or inducible (iNOS). Several studies reveal that nNOS and eNOS are abundant in brain areas critical for learning and memory such as the hippocampus, relying on the Ca^{2+}/calmodulin enzymes for activation, whereas inducible NO synthase contains tightly bound calmodulin and is permanently activated (Bonkale et al. 1995; Francis et al. 2010; Puzzo et al. 2005). Neuronal NOS is the most relevant enzyme in terms of cGMP activation, as it is strategically positioned near NMDAR to generate NO involved in the cGMP signaling in hippocampus (Tsai and Kass 2009). In vitro research has shown that pharmacological manipulation of NO signaling in the lateral amygdala using either a NOS inhibitor or a membrane-impermeable scavenger of NO impaired memory consolidation in auditory fear conditioning in mice (Fischmeister et al. 2005). Moreover, in AD, cognitive deficits are accompanied by the formation of beta-amyloid (Aβ) plaques, which deactivates the NO/cGMP/PKG pathway and decrease the phosphorylation of CREB during hippocampal synaptic plasticity (Knight and Yan 2012). Accumulating evidence indicates that both the NO donor DEA/NO and cGMP analogs counteract the Aβ-induced impairment in CREB phosphorylation and LTP. Appropriately, deletion of the gene encoding NO synthase 2 (NOS2) results in worsening of the AD phenotype in APP mouse model (Rybin et al. 2000). According to Bonkale et al., the concentration of sGC in AD patients is less than normal (Buxton and Brunton 1983). Additionally, NO-dependent LTP in rat hippocampal and amygdala slices was inhibited by the sGC inhibitor, but enhanced by the sGC activator (Houslay and Milligan 1997). Together, the data suggest that the

NO-mediated modulation of synaptic plasticity is a key step in learning and cognition and that Alzheimer's disease may involve an sGC-cGMP-dependent mechanism. The final concentration of cGMP in cells depends on the balance of not only its synthesis via sGC but also the degradation by PDEs. There is substantial evidence indicating that cGMP-specific PDEs are involved in memory and synaptic plasticity (Mokni et al. 2010). In fact, current research suggests that the primary mechanism of PDE2 inhibitors is mediated through NO-cGMP signaling.

Organotypic slice co-cultures of the nigrostriatal dopaminergic system in combination with biocytin tracing and tyrosine hydroxylase labeling has been used to examine the neural outgrowth induced by PDE2 inhibitors (Heine et al. 2013). The quantification shows a significant increase of tyrosine hydroxylase-positive fiber density in the border region of the co-cultures induced by PDE2 inhibitors BAY60-7550 and ND7001, as well as significantly increased expression of nerve growth factor. This is supported by prominent PDE2A immunoreactivity found near the striatum in the isocortex, hippocampus, and basal ganglia, as well as a high expression in the substantia nigra and ventral tegmental area on cell bodies and terminals. Moreover, the growth-promoting effect of the PDE2 inhibitors was compared with the stimulating capacity of NGF, which is known to promote neuronal survival and elicit neuronal fiber outgrowth. The action of NGF, as well as other neurotrophic factors such as BDNF and GDNF, is mainly through a cAMP-independent signaling pathway involving Ras, PKC, and extracellular signal-regulated protein kinase. It has been shown that both cAMP and cGMP induces neuronal differentiation in PC12 cells (Laasberg et al. 1988; Ng et al. 2009). As a critical enzyme that modulate the concentration of both cAMP and cGMP, PDE2 is surely involved in survival, repair and remodeling of the central nervous system both during development and after injury. Moreover, the cellular and subcellular localization of PDE2 might determine its role in modulating neuronal plasticity. PDE2 staining has been found in the hippocampal CA1 area, especially the stratum oriens, while little expression was observed in somatic regions or proximal dendrites of pyramidal neurons. In CA3, highest staining was observed in the subiculum, the hilus and the area where the mossy fiber terminates. These data suggested that PDE2 might be highly expressed in axons and axon terminals (Stephenson et al. 2012). Moreover, PDE2 staining in CA3 did not overlap with the dendritic marker MAP2 (Stephenson et al. 2012), while in hippocampal cultures PDE2 is partially co-localized with the presynaptic marker synaptophysin (Russwurm et al. 2009), suggesting that PDE2 may modulate LTP at a presynaptic location. However, whether PDE2 inhibitors modulate a component of LTP that is expressed pre-synaptically or postsynaptically has not been directly addressed.

The increase of use-dependent enhancement of synaptic function (LTP) in hippocampal CA1 region induced by Bay 60-7550 is mainly through regulation of cGMP. In the hippocampus, cGMP levels were increased in the perforant pathway, which is next to varicose fibers throughout the hippocampus. The main cGMP increase might take place post-synaptically, followed by a PKG-mediated increase in neurotransmitter release, or phosphorylation of ion channels in the signal trans-

duction pathway, which are characteristic of induced changes in strength of the postsynaptic response during LTP (Lynch 2004). In the study by Boess et al., it was demonstrated that sub-maximal LTP in the CA1 region induced by a weak tetanic stimulation of the Schaffer-collateral pathway was strengthened by 10 or 100 nM of Bay 60-7550 (Boess et al. 2004). Treatment with Bay 60-7550 starting 20 min before the weak tetanic stimulation caused a significantly larger potentiation of the field excitatory postsynaptic potential (fEPSP) slope than that observed in control slices stimulated in a similar fashion. Higher concentrations of Bay 60-7550 induced a longer-lasting strengthening of LTP observable up to 40 min after stimulation. The lowest concentration of Bay 60-7550 tested (1 nM), did not change the potentiation of the fEPSP slope compared to control.

Inhibition of PDE2 by Bay 60-7550 may also reverse the neuronal morphological changes in hippocampal CA1 region induced by chronic stress (Xu et al. 2015). In the stressed animals, the neuronal atrophy was characterized by reduced number of dendritic apical branching points, shortening of the basal and apical dendrites, and the spine density. However, the morphologic changes within the CA1 neurons seen during chronic unpredictable stress were not apparent in mice treated with 3 mg/kg Bay 60-7550. The CA1 neurons are sensitive to stress, even though less well characterized morphologically compared with that of CA3. Restoration of neuronal plasticity, reflected by increased dendritic branches and neuronal sprouting in the CA1, very well correlated with the behavioral changes related to memory and cognition in the same study. Moreover, the neuroprotective effects of Bay 60-7550 were blocked by the NMDA receptor inhibitor MK-801, the calcium calmodulin kinase II (CaMKII) inhibitor myr-AIP, and the PKG inhibitor KT5823, suggesting a role of NMDAR-CaMKII-nNOS-cGMP pathway on dendritic remodeling during stress. Alteration of neuroplasticity affected by stress hormones may also be reflected by change of expression of plasticity-related proteins. The effects of Bay 60-7550 on Egr-1 and BDNF, both related to hippocampal memory, mirror those found with neuronal morphology, further suggesting that function of PDE2 may be involved with both presynaptic and postsynaptic mechanism. Reactive oxygen species have also been shown to impair LTP, learning and memory, and biochemical signal transduction cascades that are believed underlying LTP and memory formation (Du et al. 2013). Tau et al. showed that impairments in hippocampal synaptic plasticity and memory in AD mice can be alleviated by decreasing mitochondrial ROS (Diebold et al. 2009). Additional research has also shown that the age-related LTP impairments in hippocampal area CA1 or the DG are in part due to the increase in ROS levels (Diebold et al. 2009; Ermak and Davies 2002; Mehta et al. 2012; Rutten et al. 2007a, b). It is thought that the destructive properties are mediated through abnormalities in mitochondria and increased NADPH oxidase subunit expression (Du et al. 2013; Ota et al. 2008; Reneerkens et al. 2013; Zhang et al. 2012). While ROS serve an appropriate biological function in regulating synaptic plasticity-related signaling molecules, including NMDA receptors, Ca^{2+} and K^{+} channels, CaMKII, ERK, and CREB, excessive ROS can be extremely destructive (Duszczyk et al. 2012). On the other hand, Aβ-induced inactivation of PKA/CREB signaling can be blunted by SOD/catalase or pharmacological scaven-

gers of ROS, by increasing phosphorylation of PKA at C subunit Thr 197, which functions to eliminate pro-oxidant that could otherwise promote the generation of excessive ROS (Calabrese et al. 2007). Studies from the O'Donnell group demonstrate that the PDE2 inhibitor Bay 60-7550 mediates decreases in oxidative stress, namely ROS expression and NADPH oxidase subunits p47 phox and gp91 phox in cultured neurons (Puzzo and Sapienza 2008). Thus, we conjecture that PDE2 inhibitors increase intracellular cAMP and enhance antioxidant capacity, which would disturb Aβ-induced inactivation of PKA/CREB signaling, and promote the synaptic plasticity.

12.7 PDE2 in Psychiatric Illnesses

Emotional processing takes place in many of the same brain regions responsible for learning and memory. Therefore, it would not be surprising that PDE2 also plays a role in mood disorders and other psychiatric illnesses. While the etiology and pathogenesis of many of these disorders is unknown, current research suggests inhibition of PDE2 may ameliorate some of the symptoms of a variety of mood and psychiatric illness. For example, PDE2 has proven to be protective against stress-induced anxiety and depression in mice (Ding et al. 2014; Masood et al. 2008, 2009; Xu et al. 2013, 2015). The potential role of PDE2 in various psychiatric disorders is discussed below.

12.7.1 PDE2 and Schitzophrenia

Neuropsychological testing of individuals with schizophrenia reveals significant cognitive deficits in attention, working memory, and executive functioning. Administration of phencyclidine (PCP) has been reported to induce positive, negative symptoms as well as cognitive impairments that are characteristic of schizophrenia (Rodefer et al. 2012). Subchronic administration of rats with PCP induces behavioral inhibition based on new or changing information about conditioned stimulus-reward associations, spatial memory, and latent inhibition, and causes neurochemical changes in rats resembling those in patients with schizophrenia. Selective atypical antipsychotics have been shown to attenuate these symptoms. However, since the process involves multiple neurotransmitter systems, there is still lack of efficacy in current treatments. Targeting intracellular second-messenger systems that affect multiple neurotransmitter systems might provide a feasible alternative. Extradimensional-intradimensional (ED/ID) task is a rodent cognitive that is similar to those used in humans and non-human primates for assessing executive function. It is sensitive to the effects of lesions, natural aging, and pharmacological manipulations. Rodefer et al. (Rodefer et al. 2012) demonstrated that sub-chronic treatment with PCP produced a selective impairment on ED shift

(EDS) performance without significant impairment on any other discrimination problem when compared to saline-treated control animals. 3 mg/kg Bay 60-7550 attenuated this cognitive deficit in EDS performance. Similarly, a newer PDE2 inhibitor Lu AF64280 attenuated sub-chronic PCP-induced deficits in novel object exploration in rats, and blocked early postnatal PCP-induced deficits in the ED/ID shift task in rats and attenuated spontaneous auditory gating deficits in DBA/2 mice (Redrobe et al. 2014). As mentioned previously, Bay 60-7550 can improve performance in rodent object recognition tasks, which is mainly dependent on the proper functioning of hippocampus and amygdala. These data suggested that inhibition of PDE2 might also contribute to cognitive enhancement through frontal mechanism.

12.7.2 PDE2 and Depression

Major depressive disorder (MDD) is currently the most common mood disorder in the United States, and is characterized by a pervasive and persistent negative mood, loss of interest in daily activities, and anhedonia. The fundamental mechanism of action of most antidepressants involves the enhancement of levels of monoamines (serotonin, norepinephrine and/or dopamine) at the synaptic cleft. This may result in modulation of the hypothalamus-pituitary-adrenal (HPA) axis and other major neurotransmitter systems, as well as increased neuroplasticity and LTP (Andrew Alt et al. 2006; Hebb and Robertson 2007; O'Donnell and Zhang 2004). While the basic mechanism of action for these drugs is simple, the downstream effects of these drugs may be numerous.

Antidepressants can be broken down into a few categories based on their specific targets. Monoamine oxidase inhibitors (MAOIs), which inhibit the degradation of monoamines, were the first compounds discovered to have antidepressant properties. The next major classes of antidepressants are collectively known as the reuptake inhibitors, which block the reuptake of neurotransmitters such as serotonin, norepinephrine, and/or dopamine. While having different drug targets, the biochemical outcome for both classes of antidepressants is the enhancement of synaptic monoamine levels. However, despite the variety of currently available therapeutics, only about one third of patients respond to their first treatment and up to one third do not respond at all and are considered treatment resistant (Aan het Rot et al. 2009). Other limitations include delayed treatment response, relapse, and intolerable side effects for some patients. Research has indicated that the improvement of depressive symptoms may instead be due to changes in the intracellular messaging systems, such as differential transcription, translation and expression of downstream receptors (Zhang et al. 2002, 2006). As a result, instead of focusing on alterations in neurotransmitter levels, current research is focusing on intracellular signaling cascades, of which the cyclic nucleotide second messenger system has become a primary interest.

More recently PDEs have stepped into the spotlight, due to their role in "turning off" the cyclic nucleotides, mainly cAMP and cGMP. Both cAMP and cGMP regulate respective downstream protein kinases, which activate transcription factors, ultimately controlling the expression of specific genes that have been shown to be involved in the expression of depressive symptoms or symptom relief (Burgin et al. 2010; Zeller et al. 1984; Zhang et al. 2002). Inhibition of PDE4 has shown robust antidepressant-like effects in rodent behavior testing (Benes et al. 2001; Lakics et al. 2010; Shaywitz and Greenberg 1999), especially the prototypic inhibitor rolipram that entered phase II clinical trials (Dwivedi and Pandey 2008). Unfortunately, due to its lack of PDE4 subtype specificity, rolipram resulted in unpleasant side effects such as nausea and emesis, which hindered it from reaching the market (Shaywitz and Greenberg 1999). Researchers are currently exploring the utility of more selective PDE4 subtype inhibitors that do not appear to cause emesis (Yamada et al. 2003).

Meanwhile, with the continued development of novel, more selective PDE inhibitors, researchers have been able to investigate the potential antidepressant-like properties of other PDEs. Among these, PDE2 has received particular interest, not only because of its ability to hydrolyze both cyclic nucleotides, but also because of its high levels expression in regions of the brain known to be involved with depression (Dwivedi and Pandey 2008).

As mentioned previously, the cAMP pathway is one of the major secondary messaging systems activated by numerous neurotransmitter families and is responsible for relaying ligand-receptor signals within cells, such as initiation of signaling to the nucleus for alterations in gene transcription. In the context of depression, it is thought that, following elevation of neurotransmitter levels by typical antidepressants, cAMP signaling leads to increased transcription of genes for various receptors and neurotrophic factors that enhance and stabilize neuronal outgrowth over time (O'Donnell and Zhang 2004). The slow development of clinical relief may be related to gene transcription, indicating the clinical response may be partially dependent upon the neuronal changes that happen via the cAMP signaling system specifically (H.-T. Zhang et al. 2002). For example, increased cAMP signaling activation of cAMP-dependent protein kinase A (PKA) leads to phosphorylation of a serine residue (S133) on the transcription factor cAMP response element binding protein (CREB), which, in the presence of the transcriptional co-activator CREB binding protein (CBP), then stimulates the transcription of cAMP responsive genes (Conti and Blendy 2004; Dash et al. 1991; Nestler et al. 2002; Sheng et al. 1991; Sun et al. 1994). Genes that are targeted via this pathway have been shown to be involved in several aspects of neuronal functioning, including the development of central nervous system, excitation of nerve cells, long-term synaptic plasticity and survival of neuronal cells (Alt et al. 2005; Saarelainen et al. 2003). Coincidentally, CREB mRNA levels and phosphorylated/activated CREB (pCREB) are reduced in postmortem brain samples of depressed patients (Conti et al. 2002). Antidepressants, on the other hand, are known to induce pCREB and CREB mRNA expression in the hippocampus, which is believed to be partially responsible for change of behaviors (Menniti et al. 2006). In addition, kinases that are activated by intracellular calcium,

including the calcium/calmodulin-dependent kinases (CaMKII & CaMKIV), have been reported to activate or inhibit CREB transactivation depending on the serine residues that are phosphorylated (Boess et al. 2004; Xu et al. 2013). While CaMKII phosphorylates and activates S133 on CREB, it has a second phosphorylation site at S142, which is thought to block transcriptional activation, whereas CaMKIV only phosphorylates CREB at S133, and is therefore a more potent activator of CREB (Malenka and Nicoll 1999). One major transcriptional outcome of CREB activation is thought to be the production of brain-derived neurotrophic factor (BDNF) (A Alt et al. 2005; Menniti et al. 2006). Indeed, in CREB knockout mice, antidepressants were not able to increase BDNF levels (Sheng et al. 1991). Moreover, there is a reciprocal interaction between CREB and BDNF that results in a positive feedback loop. This amplification mechanism is potentially critical for the trophic effects of antidepressants, i.e., pCREB activates BDNF production, BDNF induces phosphorylation/activation of CREB, pCREB activates BDNF production, etc. (Conti and Blendy 2004; Duman et al. 1997). Despite these findings, CREB-deficient mice show a normal behavioral response to antidepressants, indicating that the antidepressant effects are not achieved solely through the activation of CREB (Hayashi et al. 1999).

One alternative mechanism for the enhancement of the cAMP pathway includes inhibition of PDE2, which has dual substrate affinity for both cAMP and cGMP. Research has shown that the PDE2 inhibitor Bay 60-7550 is able to increase cAMP concentrations in the presence of forskolin, an activator of adenylyl cyclase, which is responsible for the synthesis of cAMP (Raison et al. 2006). In a chronic stress-induced depressive mouse model, Bay 60-7550 appears to have neuroprotective effects behaviorally (Ding et al. 2014). While the main behavioral effects seem to be conferred through cGMP/PKG signaling (discussed below), Bay 60-7550-induced increases in cAMP levels that led to changes in phosphorylation of ERKs, members of the mitogen-activated protein kinase (MAPK) family. These kinases mitigate cellular stress by interacting with nuclear targets involved in neuronal survival and plasticity, including CBP that acts as a transcriptional coactivator with several other transcription factors and components of the RNA polymerase II, as well as the neurotrophic factor BDNF. Therefore, inhibition of PDE2 appears to be a valuable drug target for enhancing cAMP signaling.

Other potential pathways affected by increases in cAMP levels include the α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor-induced signaling cascades belong to the family of ionotropic glutamate receptors. These receptors are expressed ubiquitously throughout the central nervous system and mediate the majority of rapid excitatory neurotransmission. They are not only important in regulating postsynaptic depolarization and neuronal firing, but they are also involved in a variety of other cellular responses, including the recruitment of voltage-gated ion channels and NMDA receptor activity, and the development and expression of long-term synaptic plasticity (Essayan 2001). There is also an indication that AMPA receptors are involved in the induction of neurotrophic factors including BDNF. Specifically, when stimulated by cAMP, PKA phosphorylates the GLUA1 subunit of AMPA receptors at Serine831/845 residues, activating the recep-

tor and leading to an induction of BDNF expression through both the Ca^{2+}-dependent and Ca^{2+}-independent pathway. The former is through increased AMPA receptor conductance for Ca^{2+}, which leads to activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMK)/CREB/BDNF signal transduction pathways (Gustafsson and Brunton 2002; Seybold et al. 2005). The latter is through a mitogen-activated protein kinase (MAPK) pathway mediated by Lyn, a member of the Src family of protein tyrosine kinases, which is physically associated with AMPA receptor subunits and can be activated upon AMPA receptor activation (Feil et al. 2005). While PDE2 has not been directly linked to the AMPA receptor-mediated signaling cascades, the role of these molecular pathways in the action of current or novel antidepressants has been established, and inhibition of PDE2 may be an indirect contributor via the increase in cAMP. Therefore, future research into these potential PDE2-cAMP-AMPA receptor connections is worthwhile, especially for the development of novel PDE2 inhibitors that may influence activation of these transduction pathways.

More recently, cytokines have been implicated in the pathogenesis of depression, and, coincidentally, cAMP is known to interact with cytokines. Data indicate that immune activation, as reflected by increased plasma and CSF concentrations of different cytokines including IL-1, IL-2, IL-6 and TNF-α, is associated with depression (Domek-Łopacińska and Strosznajder 2005). The mechanism of cytokine effects on depressive behavior are believed to be related in part to their ability to desensitize glucocorticoid receptors (GR) leading to glucocorticoid resistance (Lein et al. 2007). Stress exposure, a well-known inducer for depression, has been shown to activate inflammatory signaling pathways and induce glucocorticoid resistance in neuroendocrine and immune tissues in mice (Gur et al. 2007). To date, there are no direct evidence suggesting that PDE2 inhibition alleviates depressive symptoms via cytokine regulation and decreased neuro-inflammation processes. Nevertheless, it has been implicated that PDE2 is involved in certain immune responses (Corbin et al. 2000), including neuro-inflammatory processes in which cytokines such as TNF-α has been shown to induce a dramatic increase in PDE2 levels (X. Liu et al. 2013). Reciprocally, a PDE2 inhibitor EHNA is able to restore the cAMP accumulation that is previously reduced by IL-1β treatment (Snyder et al. 1999).

The cGMP signaling pathway is another intracellular nucleotide cascade that has also been implicated in various neuropsychiatric disorders. While the cGMP cascade has some overlap with cAMP signaling, the system has received far less attention in terms of its relation to MDD and antidepressant activities. The major downstream effector of cGMP is the protein kinase G (PKG), which belongs to a family of serine/threonine kinases. There are two major subtypes of PKGs, based upon their subcellular localization. PKGI, the cytosolic form, is mainly distributed in mammalian brain regions including Purkinje cells of the cerebellum, the hypothalamus, hippocampus, olfactory bulb and amygdala, most of which have high concentrations of PDE2 expression (RALL and SUTHERLAND 1958). PKGII, the membrane bound form of PKG, is also widely expressed throughout the rodent brain, including the cerebral cortex, cerebellum, brainstem, thalamus, septum, amygdala and olfactory bulb (Fujishige et al. 1999; L. Wang et al. 2005). Interestingly, the palmitoylation of certain splice variants of PDE2 primarily anchors

the enzyme to the membrane, where it functions as a component in the signaling complexes assembled in lipid rafts. This compartmentalization of PKGII and PDE2 in specific cellular microdomains provides an excellent example of the way in which the large PDE family exerts such precise control over cyclic nucleotide signaling, and offers a cellular explanation for the specific role of PDE2 in mood disorders (American Psychiatric Association Task Force On DSM-IV 2000). One function of the activated PKGs is to phosphorylate and activate PDEs that are located in these distinct macromolecular complexes, which in turn decrease the levels of cGMP, forming a negative feedback loop (Crisp et al. 2005). In cell culture, Bay 60-7550 was able to reverse corticosterone-induced neurotoxicity in different types of neuronal cells. In both cortical and hippocampal neurons, Bay 60-7550 was also found to potently increase the cGMP concentrations in the presence of guanalyl cyclase activators (Maes et al. 1993). In a mouse model of chronic stress-induced depression, Bay 60-7550 was able to reverse the behavioral abnormalities in mice, which were accompanied by significant increases in cGMP levels. These effects were blocked by administration of the PKG inhibitor KT5823, but not the PKA inhibitor H89, indicating that the antidepressant-like effects of Bay 60-7550 on behavior were mediated largely through cGMP/PKG (Bierhaus et al. 2003). Additional evidence has implicated involvement of cGMP-related pathways in neurogenesis, which is a critical marker for alleviation of depressive disorder. In an *in-vitro* study using neurospheres, activation of cGMP was shown to stimulate neurogenesis through the PI3-K/Akt/GSK-3 (phosphoinositide 3-kinase/protein kinase B (aka AKT)/glycogen synthase kinase 3) pathway (Beer et al. 1972). And Xu et al. (2015) has demonstrated that Bay 60-7550 was able to reverse the impairment of neuronal morphology in hippocampal CA1 and CA3 induced by chronic stress.

12.7.3 PDE2 and Anxiety Disorders

Anxiety disorders are a class of mood disorder characterized by excessive rumination, worrying, uneasiness and fear about future uncertainties either based on real or imagined events (Barton et al. 2004). Current psychiatric diagnostic criteria recognize a wide variety of anxiety disorders, including generalized anxiety disorder, phobic disorder, and panic disorder. Recent surveys have found that as many as 18% of Americans and 14% of Europeans may be diagnosed to have one or more of the above symptoms, indicating a great need for improved treatment options (Wang et al. 2013). Both psychological and pharmacological therapies are recognized to be effective management strategies, with a combination approach often being most successful. While benzodiazepines are frequently used to treat anxiety for the past few decades, more recent findings suggest that both selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are a safer, more tolerable first-line therapy for most of the anxiety disorders (Arranz et al. 2007), particularly for those that suffer comorbidity with depression. Selective serotonergic agents are especially preferred for certain subtypes of anxiety, such as

obsessive-compulsive disorder. Other antidepressants, such as tricyclic antidepressants (TCAs) or monoamine oxidase inhibitors (MAOIs), are generally reserved for second- and third-line strategies due to tolerability issues (Arranz et al. 2007). Anticonvulsants and atypical antipsychotics have been shown to have adjunctive beneficial effects as adjunctive treatments with antidepressants in cases of treatment resistance, while azapirones have been used effectively for generalized anxiety disorders, especially for panic disorder (Bouayed et al. 2007). Despite notable advances, many patients with anxiety disorders have a low tolerance to certain classes of drugs or fail to adequately respond to other pharmacological treatments. Researchers have focused on combined pharmacologic-psychosocial strategies and new drug targets to address treatment resistance and tolerability issues in order to increase overall success rate of remission for anxiety disorders.

Cyclic nucleotide signaling has been implicated in numerous cellular processes known to be important for proper functioning of regions of the brain specifically involved in anxiety disorders. Currently anxiolytics appear to ameliorate behavioral symptoms through either direct or indirect manipulation of the cyclic nucleotide pathways. For example: (i) potent anxiety-reducing agents are also potent inhibitors of brain PDE activities; (ii) dibutyryl cyclic adenosine monophosphate, a cell permeable cAMP analog, has the ability to reduce anxiety; (iii) the methylxanthines, semi-products of purine degradation that have great selectivity for PDEs, show significant anxiety-reducing effects (Kuloglu et al. 2002; Puzzo and Sapienza 2008).

Stress and anxiety are often closely related. Glucocorticoids and adrenal steroid hormones (i.e., corticosterone in rodents) are released in response to physical and psychological stressors, and their dysregulation is often implicated in anxiety disorders. Secretion of these hormones is the final step in the neuroendocrine cascade, known as the hypothalamic-pituitary-adrenal (HPA) axis, which is triggered following stimulation of the hypothalamus and pituitary gland. Elevated corticosterone in rodents after stress is the hallmark of the feed-forward activation of the HPA axis, which is also observed in clinically anxious patients (Gur et al. 2007; Masood et al. 2009). PDE2 is highly expressed in the rat adrenal cortex and pituitary gland (Masood et al. 2008; Raison et al. 2006). It may function as a regulator of the release of these stress hormones, specifically, aldosterone in the adrenal cortices and glucocorticoids from the pituitary gland (Masood et al. 2009). Reduction of PDE2 activity through pharmacological inhibition of this enzyme may intervene in certain stress-induced anxiety disorders via increases in intracellular cGMP concentrations (Abel and Lattal 2001). Investigation into the underlying processes involved in the protective effect of PDE2 inhibition from stress-induced changes in anxiety and depression have implicated a few different systems. Cell culture work using primary hippocampal neuronal cultures showed that corticosterone increases PDE2 expression in a dose-dependent manner, and in HT-22 cells, Bay 60-7550 was protective against corticosterone-induced cell death (Xu et al. 2013). In the same cell line, corticosterone application resulted in decreased cAMP and cGMP levels. Bay 60-7550 restored cGMP, but not cAMP levels. Additionally, a PKG inhibitor blocked the protective effects of Bay 60-7550 in the HT-22 cells, suggesting this mechanism is via the cGMP/PKG pathway (Xu et al. 2013). While CUS signifi-

cantly increases serum corticosterone levels in stressed mice, treatment with Bay 60-7550 was able to reverse these effects and return corticosterone levels to baseline. Potential anxiolytic-like effects of Bay 60-7550 was demonstrated using the elevated plus maze and marble burying test with the CUS mice (Ding et al. 2014). As a continuation of previous studies, it was further shown that pretreatment with Bay 60-7550 every day before stress effectively prevented the decrease of Cu/Zn superoxide dismutase (SOD) that could have been induced by CUS, supporting the notion that inhibition of PDE2 could protect the neurons from oxidative stress (Masood et al. 2008). Moreover, CUS-induced oxidative damage to the brain is often characterized by neuronal apoptosis, a programmed cell death that is critically controlled by the balance between pro- and anti-apoptotic proteins. Within the Bcl-2 family, Bcl-2 and Bax belong to the pro-survival and pro-apoptotic subfamily, respectively. Bax promotes the release of apoptogenic factors such as cytochrome c, which in turn stimulates initiator caspases, leading to the activation of effector caspases such as Caspase 3, a main executor of the apoptotic process. Hippocampus and amygdala in the study both expressed higher levels of Bax and Caspase 3 after CUS, while a clear regional specificity Bcl-2 expression were seen in the two brain regions (Ding et al. 2014). These changes indicative of neuronal apoptosis in the hippocampus and amygdala were prevented by pretreatment with Bay 60-7550, suggesting another mechanism of action involved with the anxiolytic effects of PDE2 inhibition. Another stress model that has been used to provide direct evidence

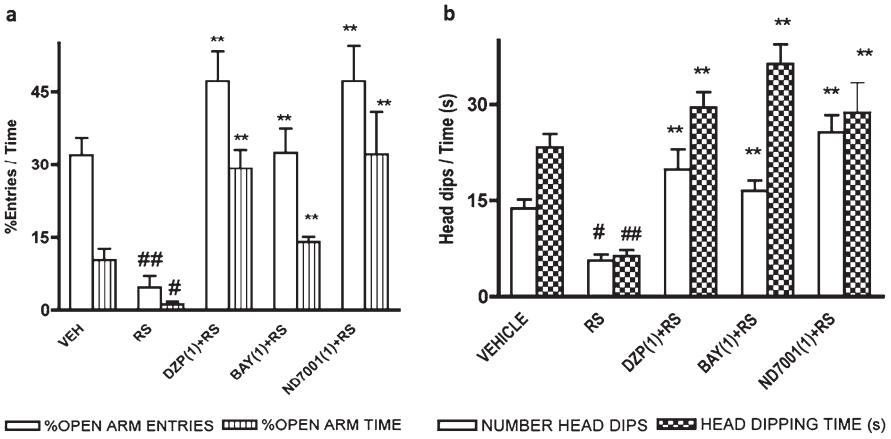

Fig. 12.5 Effects of the PDE2 inhibitors Bay 60-7550 (BAY) and ND7001 on behavior in the (**a**) elevated plus-maze and (**b**) hole board test in stressed mice. Increases in percentages open-arm entries and time indicate an anxiolytic effect, whereas decreases in these measures indicate an anxiogenic effect. Increases in head-dips and time of head-dipping indicate an anxiolytic effect, whereas decreases in these measures indicate an anxiogenic effect. Values are expressed as means ± S.E.M. (n = 6–8). #, $p < 0.05$ and ##, $p < 0.01$ compared with vehicle (VEH). ** $p < 0.01$ compared with restraint stress (RS). *DZP* diazepam. Doses shown parenthetically are milligrams per kilogram intraperitoneally. (Reprinted by permission from Macmillan Publishers Ltd.:,(Masood et al. 2009) copyright 2012)

for the benefit of PDE2 inhibition in anxiety disorder is the restraint stress (Masood et al. 2009). Increased cGMP signaling, either by administration of the PDE2 inhibitors Bay 60-7550 (0.5, 1, and 3 mg/kg) or ND7001 (1 mg/kg), or the NO donor detanonoate (0.5 mg/kg), antagonized the anxiogenic effects of restraint stress on behavior in the elevated plus-maze, hole-board, and open-field tests. These effects were antagonized by the GC inhibitor ODQ (20 mg/kg). By contrast, the NOS inhibitor L-NAME (50 mg/kg), which reduces cGMP signaling, produced anxiogenic effects similar to restraint stress, suggesting a critical role for NO/GC/cGMP signaling (Fig. 12.5).

Increasing evidence has also implicated signs of increased reactive oxygen generation and decreased antioxidant capacity in anxiety disorder (Kang et al. 2013; Rolls et al. 2013). The findings of Kuloglu et al. show that patients with anxiety disorders have higher activity levels of the antioxidant enzymes superoxide dismutase and glutathione peroxidase, an indication of high oxidative stresses, as well as higher lipid peroxidation activity compared to healthy controls (Reierson et al. 2011). Therefore, oxidative metabolism manipulation is considered a plausible pathway for the regulation of anxiety. Masood and colleagues first uncovered a direct role for the protective effects of PDE2 inhibition against oxidative stress-induced changes in behavior. Using the pharmacologic agent and inducer of oxidative stress, L-buthionine-(S,R)-sulfoximine (BSO), they induced an anxiety-like phenotype in mice. They were then able to pretreat with PDE2 inhibitor Bay 60-7550 and block the BSO-induced anxiety-like behavior (Masood et al. 2008).

Despite accumulating evidence for the anxiolytic-like effects of PDE2 inhibitors, further investigation is still required. Future research should focus on exploring the precise trafficking and subcellular localization of PDE2 splice variants, specifically in the context of potential signaling partners, in order to gain a better understanding of the crosstalk between PDE2, cyclic nucleotides, and other PDEs. In addition, researchers must continue to investigate the exact underlying working mechanisms of selective PDE2 inhibitors by using central administration paradigms, blood flow measurements, and parallel behavioral experiments. Taken together, PDE2 inhibitors offer a promising target for mood disorders.

12.8 PDE2 and Pain

Inflammatory pain is a condition of hypersensitivity in nociception that is accompanied by inflammation. One of the cardinal features of inflammation is allodynia, or a state in which normally innocuous stimuli produce pain. Chronic pain due to inflammation is often difficult to manage and pharmacologic treatment options are quite limited. Therefore, pain management has significant unmet medical need and is currently affecting a large population of patients suffering from arthritis, pancreatitis, colitis, cystitis, and other autoimmune diseases. Existing therapeutics such as NSAIDs are limited in scope, while more powerful analgesics, such as opiates, have a high rate of tolerance, addiction, and withdrawal effects, as well as narrow

therapeutic window at high doses. Therefore, it is important to identify new medicines with novel mechanisms of action for effective treatment of patients with chronic pain (Tramèr et al. 1998).

The involvement of PDE2 in the production and modulation of pain has been summarized by Plummer et al. (2013b): (1) PDE2 is highly expressed in tissues responsible for nociception, i.e., brain, spinal cord, dorsal root ganglia (DRG), as well as non-pain related tissues including adrenal cortex, heart, and platelets; (2) The non-hydrolyzable cGMP analog, 8-bromo-cGMP, when delivered intrathecal, reduces nociceptive behavior in rats at low doses in formalin-induced inflammatory pain, but causes hyperalgesia when injected at higher doses (Tegeder et al. 2002); (3) PDE2 mRNA expression is increased after formalin injection; (4) PDE2 inhibitor with higher potency (oxindole, Bay 60-7550) have effect on nociceptive behavior. In their study, series of structural modification were carried out on PDE4 inhibitors to optimize residual PDE2 activity while minimizing PDE4 activity at the same time. Modified compounds exhibited a cGMP-like binding mode to the enzyme, high selectivity to PDE2 (Plummer et al. 2013b). Among these, compound 22, pyrazolodiazepinone, was greater than 1000-fold selective for PDE2 (Plummer et al. 2013a), and had significant analgesic activity in a rat model of osteoarthritis pain.

12.9 Conclusions and Future Studies

PDE2 clearly plays an important role in both mood and cognitive functions. The research summarized above demonstrates the pro-cognitive effects of PDE2 inhibition, as well as the potential of PDE2 as a target for treatment of cognitive impairment. However, while researchers have identified critical periods when PDE2 inhibition exerts its effects, those appear to differ between mice and rats. While the reasons for this are currently unknown, replication and further elucidation of the time-course and its underlying mechanisms are essential for any hope of successful translation to human use. Additionally, PDE2 inhibitors have shown to be protective against stress-induced changes in anxiety, depression, and cognition, on a behavioral and cellular level. The potential applications for this are far reaching, especially when considering the potential use in individuals exposed high-stress/high-risk environments, such as military in combat situations. However, for such application, further investigation needs to be done, specifically with models of post-traumatic stress disorder.

While our knowledge of PDE2 in mood and cognitive disorders has greatly advanced over the past 10 years, our overall understanding of PDE2 in the CNS remains in its infancy. Researchers must not only continue to uncover the role of PDE2 in rodent behavior, but also the underlying cellular and molecular pathways responsible for mediating those changes. Nonetheless, research has confirmed the potential of PDE2 and/or members of its signaling pathway as therapeutic targets for drug discovery for the treatment of mood and cognitive disorders.

Conflict of Interest The authors declare that they have no conflicts of interest.

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