Drugs of the Future 2005, 30(7): 725-736

CCC: 0377-8282/2005
DOI: 10.1358/dof.2005.030.07.929862

Review Article

Phosphodiesterase type 5 (PDE5) inhibition and cognitive enhancement

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Abstract

Drug development for the treatment of dementia and age-related cognitive decline has been slow to produce clinically viable alternatives to the two existing FDA approved drug treatments, AChE inhibitors and the noncompetitive NMDA receptor antagonist, memantine. Recent human and preclinical animal studies suggest that phosphodiesterase type 5 (PDE5) inhibitors, already in clinical use for the treatment of erectile dysfunction in men, may have central cognitive enhancing effects. Recent behavioral pharmacological studies with rodents demonstrate that PDE5 inhibitors improve memory consolidation in young animals using passive avoidance and object recognition tasks. In addition, we have shown that pretraining administration of the PDE5 inhibitor sildenafil citrate can attenuate a spatial learning impairment in the 14-unit T-maze. Research findings with aged animals show that pretraining administration of sildenafil can improve the long-term retention of spatial memory 1 week following maze acquisition. We propose that the available research findings provide preclinical support for a possible alternative use of PDE5 inhibitors as cognitive enhancing agents that improve different kinds of learning and memory and protect brain systems against neurodegeneration.

Introduction

Drug development for the treatment of Alzheimer's disease (AD) has resulted in two palliative treatment strategies to date. Based on the cholinergic hypothesis of geriatric memory function (1, 2), the first approach has produced therapeutic compounds that increase acetylcholine (ACh) activity by inhibiting the enzyme that hydrolyzes ACh, acetylcholinesterase (AChE) (3, 4). Inhibition of AChE is consistent with the observed loss of cholinergic basal forebrain neurons in AD patients (5, 6) and has been found to improve learning and memory in animal models of aging (7-11).

More recently, the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, memantine, has been approved by the U.S. Food and Drug Administration for treatment of dementia in patients with moderate to severe AD (12) and has been in widespread clinical use for the treatment of neurodegenerative conditions in Europe since 1982. This drug does not appear to interact with cholinesterase inhibitors (13). Its primary pharmacologic action is the blockade of ionotropic receptors, including NMDA, serotonin (5-HT_3) and nicotinic ACh receptors. Although the mechanism of its therapeutic activity in AD is not fully understood (14), the characteristics of its action as a noncompetitive, low-affinity, open-channel blocker at NMDA receptors is considered the most important therapeutic benefit (15, 16). The reduction in NMDA receptor activation by memantine is neuroprotective during acute ischemia (17), and may also prevent β-amyloid neurotoxicity (18) and the loss of cholinergic basal forebrain neurons (19). Although it has been suggested that clinically relevant low doses of memantine may promote synaptic plasticity and preserve mnemonic function in animal models of AD (20-24), other noncompetitive NMDA receptor antagonists (*e.g.*, dizocilpine) inhibit synaptic efficacy and impair learning (25). Consistent with the latter effect, recent animal studies have shown that low doses of memantine impair spatial learning in adult rats (26, 27).

Given the limited number of treatment options and the modest utility of approved drug therapies for dementia, preclinical investigations have focused on the development of other putative “cognitive enhancers” for AD and other neurodegenerative conditions. Development of one class of compounds as a viable therapeutic for improving cognitive function has increasingly focused on inhibiting the phosphodiesterase type 5 (PDE5) isoenzyme that hydrolyzes guanosine 3´5´-cyclic monophosphate (cGMP). The main effect of PDE5 inhibitors on peripheral tissue results in relaxation of vascular smooth muscle and increased blood flow to the corpora cavernosa, hence providing its usefulness in treating erectile dysfunction in men (28).

The fact that three PDE5 inhibitors (*e.g.*, sildenafil, tadalafil and vardenafil) are already in widespread clinical use suggests that problems with acute side effects and health risks are minimal for the majority of patients (29, 30). Side effects for all PDE5 inhibitors include vasodilation, small reductions in blood pressure, headache and nasal congestion. Visual disturbances due to some minor inhibition of the PDE6 isoenzyme found in photoreceptors have been reported but are typically reversible within 24 h (31, 32). A recent study reports that sildenafil may increase the risk of blindness from nonarteritic anterior ischemic optic neuropathy (NAION) (33), although in a recent news release (34), Pfizer Inc. concludes that there is no evidence of an increased risk of blindness among sildenafil patients. Other adverse psychological effects of sildenafil have been described in case studies and anecdotal reports (35). However, in a controlled study (double-blind, balanced crossover design) of central nervous system (CNS) effects of sildenafil (36), results revealed positive effects on attention and memory function in young healthy male subjects. Although sildenafil did not produce any overt changes in behavioral performance of a spatial auditory attention task, it did enhance event-related brain potentials (ERPs), indicative of an increased ability to focus attention and select relevant target stimuli. Positive CNS effects were also reported for a visual word recognition task. These findings with human subjects are consistent with the results reported in a number of preclinical animal studies reviewed below.

Neurotransmitter system interactions

Our interest in PDE inhibition as a possible therapeutic for treating age-related cognitive decline followed from the results of previous studies indicating that central ACh and NMDA systems were both important for complex maze learning in rodents (37-40). In addition to the wealth of evidence supporting the cholinergic hypothesis, neurobiological research on the long-term potentiation (LTP) of synaptic transmission suggested that the glutamatergic NMDA system may play an important role in synaptic plasticity as a coincidence detector mechanism for learning and memory (41-43). Other evidence suggested that the same excitatory amino acid system may be responsible for some of the clinical manifestations of AD (44) which, as noted above, is the current rationale for memantine treatment (12). In one type of interaction that occurs in hippocampus and neocortex, acetylcholine modulates glutamatergic NMDA receptor activity, which is believed to coordinate states of learning new information with recall of previously acquired cognitive representations (45, 46). In AD, reduced cholinergic modulation and excessive NMDA neurotransmission could result in runaway synaptic modification (47) that interferes with attention, learning and cognitive memory.

NO/cGMP signal transduction

Nitric oxide (NO) functions as a diffusible messenger in intercellular signaling, including smooth muscle relaxation, inhibition of platelet aggregation, immune cell-mediated cytotoxicity and neuronal signaling (48). It is synthesized from the amino acid, L-arginine, by a family of NO synthases (NOS). Three isoforms have been cloned and characterized: 1) neuronal NOS (nNOS), also known as type I (NOS-1), was originally identified as a constitutive form in neuronal tissue; 2) inducible NOS (iNOS), also known as type II (NOS-2), was originally identified as a form inducible by cytokines in macrophages and hepatocytes; and 3) endothelial NOS (eNOS), also known as type III (NOS-3), was identified as a constitutive form in vascular endothelial cells (49).

NO is believed to function as a retrograde messenger in the CNS (see Fig. 1). Following postsynaptic formation by NOS, resulting from NMDA receptor activation (Fig. 1a), NO diffuses into the synaptic cleft and then into the presynaptic nerve terminal (Fig. 1b) where it stimulates soluble guanylyl cyclase (sGC) to form the second messenger cGMP (50, 51). Depolarization of the presynaptic terminal and subsequent release of glutamate is regulated by cation flux through cGMP-gated ion channels (52). Evidence suggests that cGMP-stimulated release of glutamate following retrograde signaling of NO is a presynaptic mechanism of long-term potentiation (51, 53-55). Elevated cGMP levels stimulate cGMP dependent protein kinase (PKG or cGK), which regulates neural function through a diversity of substrates, many of which have not yet been identified (56).

Several animal studies have implicated the NO-cGMP pathway in learning and memory (39, 40, 57, 58). The present review will focus on recent behavioral studies with rodents that have used PDE5 inhibitors to influence signal transduction along this pathway as a means to improve cognitive function. Table I summarizes many of the studies reported to date. In addition, we present some novel findings recently obtained in our laboratory with young and aged rodents.

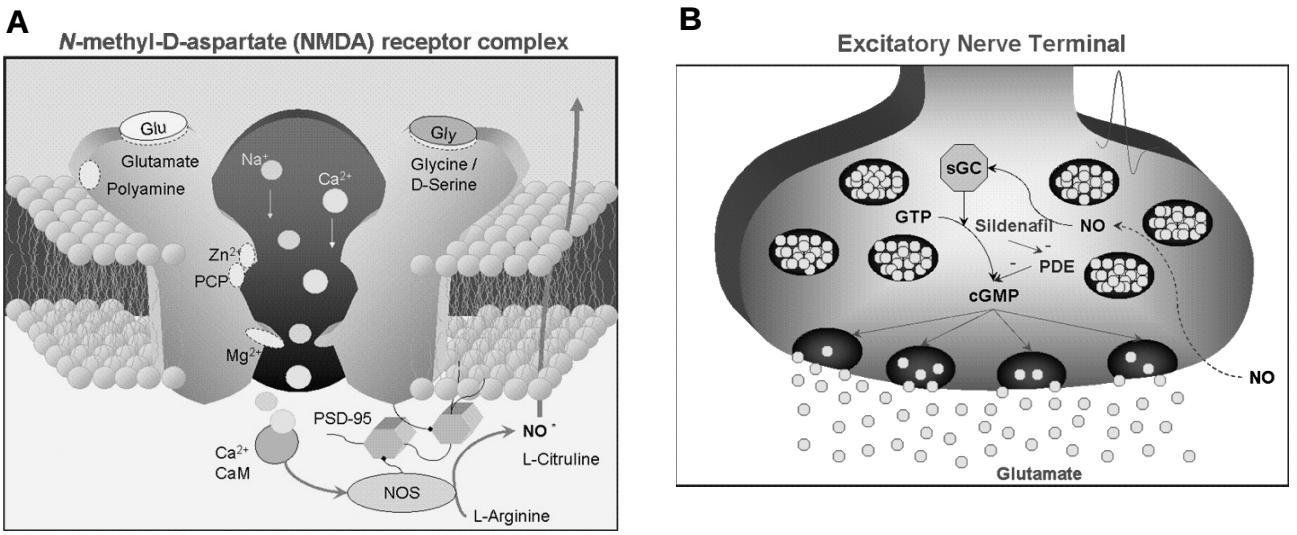

Fig 1. A. signal transduction pathway associated with activation of *N*-methyl-D-aspartate (NMDA) receptors. When glutamate and glycine/D-serine bind to their respective recognition sites on the NMDA receptor complex, the ligand-gated receptor channel opens allowing the influx of sodium and calcium in the cell. Intracellular calcium forms a complex with calmodulin (Ca^{2+}CaM), which in turn activates nitric oxide synthase (NOS) anchored to the intracellular portion of the receptor complex via the postsynaptic density 95 (PSD95). NOS activation results in the conversion of L-arginine to nitric oxide (NO) and the byproduct L-citruline. NO is a highly reactive gas that diffuses through the cell membrane into the synaptic cleft. B. NO diffuses into the excitatory presynaptic nerve terminal and stimulates soluble guanlyly cyclase (sGC), which converts guanosine triphosphate (GTP) to guanosine 3´5´-cyclic monophosphate (cGMP). cGMP promotes the release of glutamate through different mechanisms and is hydrolyzed by specific phosphodiesterases. Sildenafil is a phosphodiesterase type 5 (PDE5) inhibitor that attenuates the degradation of cGMP, resulting in elevated cGMP levels and further release of the neurotransmitter glutamate.

Cognitive enhancing effects of PDE5 inhibitors

Retention of passive (inhibitory) avoidance

Baratti and Boccia (59) investigated the effects of sildenafil (1, 3, 10 and 30 mg/kg i.p.) on retention performance of a one-trial step-through passive (inhibitory) avoidance task in male Swiss mice. Sildenafil was given immediately after training, and performance on a retention test was measured 48 h, 1 week or 1 month later. The results revealed an inverted U-shaped dose-response curve, although only the 3-mg/kg dose of sildenafil significantly improved retention performance. Response latencies of control mice not receiving footshock on the training trial indicated that the effect of the drug was not due to a nonspecific proactive effect on retention performance. Furthermore, sildenafil administered 30 min prior to the retention test did not affect retention performance, demonstrating that the cognitive improvement was time-dependent. The authors also showed that administration of sildenafil (3 mg/kg i.p.) 30 min before training enhanced performance of male mice and that immediate, but not delayed (3 h), posttraining injections of the same dose of sidenafil enhanced the retention performance of female mice, suggesting that the improvement was not dependent on sex. Based on these results, the authors suggested that sildenfil has long-lasting effects on cognitive function by modulating time-dependent mechanisms involved in memory storage.

Object recognition memory

Prickaerts, Blokland and coworkers have used an object recognition task (60) with rats and mice (61) to study the effects of different PDE inhibitors (57, 62-65). The task involves a single acquisition trial (T1) in which an animal is exposed to two identical objects in an open arena. Following a specific intertrial interval (ranging from 1 min to 24 h), the animal is placed back in the arena with one of the familiar objects and a novel object. The time spent exploring the novel object is taken as evidence that the animal remembers the familiar object. With short intertrial intervals (*e.g.*, 1 min to 1 h) control animals typically spend more time exploring the novel object (60, 63); however, with longer intervals (4 to 24 h) controls spend an approximately equal amount of time exploring both objects (63, 64). The failure to discriminate among the objects with longer intertrial intervals is interpreted as evidence that the controls do not remember well the familiar object; hence, the procedure can be used to measure improvements in memory because there is no ceiling effect that might obscure the results. In other words, longer intertrial intervals are sensitive to improved recognition performance because they exceed the limit of normal forgetting in control animals.

As with the passive avoidance task, drugs may influence acquisition, consolidation or retention processes depending on the specific drug administration protocol used with the object recognition task. Drugs administered

Table I: Summary of studies that have used PDE5 inhibitors to improve learning and memory on rodent behavioral tests of cognitive function.

Task/behavior Drug (ref)	Effective dose(s) (mg/kg route)	Retention interval	Drug admin. time	Processing phase
Passive (inhibitory) avoidance in mice				
Sildenafil (59)	3, i.p. 3, i.p.	48 h	Immed post T1 30 min before T1	Consolidation Acquisition
Object recogntion in rats				
Zaprinast (63)	3 and 10, i.p.	4 h	Immed post T1	Consolidation
Sildenafil (64)	3 and 10, p.o.	24 h	Immed post T1	Consolidation
Vardenafil (64)	0.3, 1 and 3, p.o.	24 h	Immed post T1	
Sildenafil (65)	10, p.o.	24 h	30 min before T1	Acquisition
Vardenafil (57)	1, (?)	(?) h	Immed post T1 but not 3 h delayed	Consolidation
Object recogntion in mice				
Sildenafil [cited in (57)]	1, p.o.	24 h	Immed post T1	Consolidation
Morris water maze in rats (allocentric spatial learning)				
Zaprinast [presented in (57)]	No effect of 10, i.p.	Probe trial	Immed post T2 each day	Consolidation
Y maze one trial learning in rats (spatial recognition memory)				
Vardenafil [presented in (57)]	No effect of 3, i.p.	24 h	Immed post T2	Consolidation
14-unit T-maze in rats (egocentric spatial learning and retention)				
Sildenafil (70)	3, i.p.	—	15 min before T1	Acquisition
Scopolamine	0.75, i.p.	—	30 min before T1	
Sildenafil (71)	1.5, i.p.	—	15 min before T1	Acquisition
L-NAME	60, i.p.	—	30 min before T1	
Sildenafil				
Young animals (71)	No effect		15 min before T1	Acquisition
Aged animals (in prep)	3, i.p.	7 days	15 min before T1	Consolidation

before T1 can influence acquisition and possibly also consolidation, depending on drug kinetics, while immediate posttraining administration (*i.e*., after T1) is selective for the consolidation period, and administration of drugs just prior to T2 influences retention performance. The strongest support for the claim that a drug or other intervention has selectively influenced a time-dependent memory consolidation process, as opposed to acquisition or retrieval performance, is provided by the posttraining paradigm (66).

Using the object recognition task described above, Prickaerts *et al*. (63) investigated the effects of a putative selective inhibitor of nNOS, 7-nitroindazole (10 and 30 mg/kg), and the PDE5 inhibitor, zaprinast (3 and 10 mg/kg), administered i.p. immediately after T1. Following an intertrial interval of 1 h, control rats spent more time exploring the new object during T2; however, rats receiving 7-nitroindazole immediately after T1 showed impaired discrimination performance on T2. With a 4-h intertrial interval, control rats failed to discriminate between the objects. In contrast, rats given the highest dose of zaprinast were able to discriminate, thus demonstrating an improvement of object recognition performance. Moreover, the same dose of zaprinast (10 mg/kg) reversed the recognition memory impairment induced by 7-nitroindazole (10 mg/kg) using the 1-h delay interval. Zaprinast (10 mg/kg) increased mean arterial blood pressure 4 h (but not 1 h) after systemic administration, while 7-nitroindazole (30 mg/kg) slightly increased blood pressure 1 h after administration (suggesting that it is not a selective inhibitor of nNOS). Consequently, the authors point out that similar cardiovascular effects of the drugs

cannot account for the different effects on object recognition memory. These results suggest that NO/cGMP signal transduction is involved in object recognition memory independently of its role in cardiovascular function.

In another study, Prickaerts *et al*. (64) investigated the effects of two selective PDE5 inhibitors, sildenafil (1, 3 and 10 mg/kg) and vardenafil (0.1, 0.3, 1 and 3 mg/kg), on performance of rats in the object recognition task. The drugs were given p.o. immediately after T1, followed by a 24-h intertrial interval and T2. Vehicle-treated control rats failed to discriminate between the familiar and novel objects during T2; however, rats given 3 and 10 mg/kg sildenafil or 0.3, 0.1 and 3 mg/kg vardenafil achieved a high level of discrimination performance. Additional studies of rat hippocampal slices incubated with the compounds *in vitro* showed that vardenafil (100 μM), but not different concentrations of sildenafil, increased cGMP levels when combined with sodium nitroprusside (0.1 mM); whereas, neither compound affected cAMP levels. Immunohistochemistry revealed that incubation with vardenafil (in the presence of sodium nitroprusside) produced a concentration-dependent staining of cGMP, predominantly of neuronal fibers in the CA2/CA3 region of the hippocampus. Incubation with sildenafil also revealed cGMP-positive fibers at a higher concentration. The authors interpreted the combined findings as evidence that the improved performance of object recognition following posttraining administration of sildenafil or vardenafil might be explained by the increase in hippocampal cGMP. This interpretation is supported by another finding in which the cGMP analog, 8-Br-cGMP, but not the cAMP analog 8-Br-cAMP, improved object recognition performance when infused directly into the hippocampus immediately after T1 using a 24-h intertrial interval prior to T2 (67).

In a recent study of object recognition in rats (65), the cognitive enhancing effects of AChE inhibitors, metrifonate (30 mg/kg) and donepezil (0.1, 0.3 and 1 mg/kg), were compared to the PDE5 inhibitor, sildenafil (1, 3 and 10 mg/kg). Evidence of a time-dependent dissociation of drug effects on acquisition *versus* consolidation processes was presented. In accordance with the dissociation, drugs were administered orally either 30 min before or immediately after T1 with a 24-h intertrial interval between trials. Posttraining sildenafil improved the discrimination performance on T2, resulting in an inverted U-shaped dose-response curve with a peak effective dose of 3 mg/kg. Sildenafil also improved performance when given before T1; however, a higher dose (10 mg/kg) was required for the effect. The authors suggested that the findings represent a shift right in the dose-response curve based on the metabolic clearance of the high dose of sildenafil. In comparison, the AChE inhibitors had no effect on performance at the doses tested when administered after T1. However, when administered before T1, an increasing dose-response curve was found for donepezil with a maximum effective dose of 1 mg/kg. A similar pattern was found for the single dose of metrifonate, suggesting that AChE inhibitors improve acquisition whereas PDE5 inhibitors improve consolidation of object information.

Spatial learning in the water maze and Y-maze

The Morris (68) water maze is one of the most widely used behavioral paradigms in neuroscience research (69). In the standard version of the task that assesses place learning, rats use extramaze distal room cues to find a hidden platform submerged just below the water surface in a large circular pool. On a probe test that is typically conducted at the end of training, the platform is removed from the pool and the spatial distribution of the animals' search behavior is recorded via an automated tracking system during a brief test period (30-120 sec). If an animal has acquired a spatial representation of the environment, then they will show a spatial bias on the probe test as they search near the former goal location. One advantage of the place task compared to other traditional maze paradigms is that because there are no local cues available to solve the task, animals must rely on a cognitive map-like spatial representation of the environment to learn the hidden goal location.

Prickaerts *et al.* (57) presented water maze data showing that zaprinast (10 mg/kg i.p.) given immediately after the second trial of each training day failed to improve escape acquisition or performance on multiple probe tests conducted at different times during place learning. These investigators concluded that the lack of a drug effect may be attributed to the difference in the type of information that animals have to learn and remember. That is, PDE5 inhibition has no effect on spatial learning compared to object recognition. Prickaerts et al. also presented similar findings using a more traditional spatial Y-maze task where the controls failed to demonstrate spatial discrimination between goal arms following a 24-h intertrial interval. Their data showed that 3 mg/kg of vardenafil given immediately after training had no effect on spatial recognition memory. In contrast, the results described above showed that the same treatment did enhance object recognition.

Spatial learning and retention using the 14-unit T-maze

Although the above findings suggest that the cognitive enhancing effects of PDE5 inhibitors may be limited to passive avoidance and object recognition memory in rodents, some recent studies using the 14-unit T-maze task provide evidence of enhanced spatial learning (70, 71). The 14-unit T-maze is a spatial learning paradigm frequently used to model age-related cognitive decline in rodents (72-74) and is sensitive to learning impairments induced by central and systemic administration of several drugs, including antagonists of cholinergic- and NMDA receptors, and specific or nonspecific inhibition of NOS isoenzymes (7, 25, 40, 72-82). As shown in Figure 2., the maze is composed of a complex series of choice-points in which the correct pathway leads to a goal box. Animals have to learn a series of turning responses over successive trials to avoid mild footshock. Thus, while the water maze is considered an allocentric spatial task

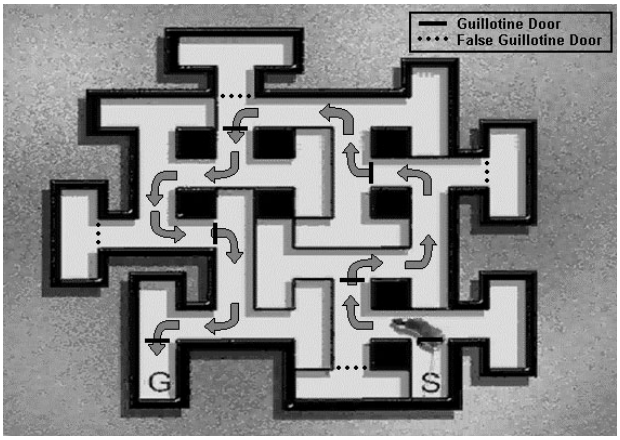

Fig. 2. A top view of the 14-unit T-maze apparatus used in rodent studies of spatial learning. Solid bars indicate guillotine doors that the animal must pass through to avoid/escape from mild footshock while dashed lines indicate false/dummy guillotine doors that are blocked. To reach the goal (G) box from the start (S) chamber, animals must traverse the correct path shown by the arrows. Animals are given 10 sec to advance through each segment of the maze before the footshock is activated. Control animals acquire this task very rapidly, typically requiring a few trials to completely avoid the footshock.

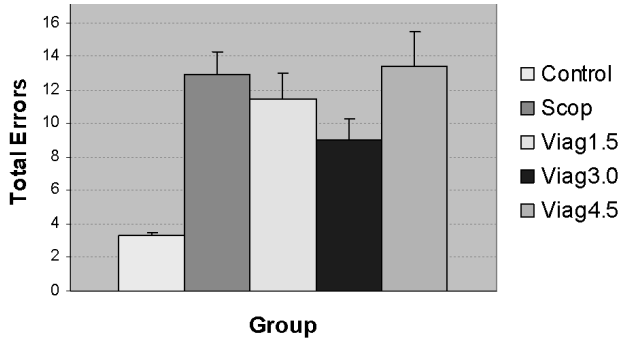

Fig. 3. Results of the study reported by Devan *et al*. (70). The graph shows the mean total errors for each group collapsed across 15 trials. Animals received systemic injections 30 and 15 min, respectively, prior to the start of the first trial. Control animals (saline/vehicle) averaged less than 4 errors, while the group receiving scopolamine plus vehicle (Scop; 0.75 mg/kg) performed very poorly (mean > 12 errors). The group that received scopolamine plus 3.0 mg/kg sildenafil (Viag3.0) performed better than the Scop alone group (average approx. 9 errors), whereas groups receiving the 1.5- or 4.5-mg/kg dose of sildenafil combined with scopolamine also performed poorly.

because it is believed that animals use the relations among distal room cues to form a cognitive map-like representation of the environment, the 14-unit T-maze may be considered more of an egocentric spatial task because performance is based on turning responses centered on the body axis. It should be noted, however, that both tasks may involve a combination of each type of information processing, but nevertheless may differ in the degree to which each type of information contributes to optimal performance.

Using the 14-unit T-maze task, we have shown that systemic administration of sildenafil (3 mg/kg) 15 min prior to T1 has cognitive enhancing effects in attenuating the maze learning impairment induced by cholinergic receptor blockade with scopolamine (0.75 mg/kg) administered 30 min prior to T1 (see Fig. 3) (70). Consistent with this finding, in a recent water maze study, the novel nitrate ester GT-715 (2,3-dinitrooxy-(2,3-bis-nitrooxy-propyldisulfanyl)-propane), which activates sGC and increases cGMP levels in the brain, was effective at improving the acquisition performance of rats pretreated with scopolamine (83). Another novel nitrate compound that serves as an NO mimetic, GT-1061, was also effective at attenuating a scopolamine-induced spatial learning impairment in the water maze (84) and also reversed a visual recognition memory impairment induced by neocortical and hippocampal cholinergic depletion (85). In a recent study with the 14-unit T-maze, we have demonstrated that pretraining administration of sildenafil (1.5 mg/kg) reversed the spatial learning impairment induced with the nonspecific NOS inhibitor N^{ω}-L-nitro-arginine methyl ester (L-NAME), administered systemically (60 mg/kg i.p.) (71) or infused directly into the lateral ventricles of the brain (*i.e*, 48 mcg bilateral i.c.v.; manuscript in preparation).

There are several factors that may contribute to the apparent discrepancy regarding whether PDE5 inhibitors specifically enhance object recognition memory or whether they may also improve spatial learning and memory. First, it is possible that the lack of improvement of a relatively high dose of zaprinast is due to an inherent ceiling effect using the standard water maze paradigm. Controls performing the place task rapidly acquire a spatial representation and consequently there may not be much room for improvement of escape acquisition. Also, in addition to measuring spatial retention, the probe test is an extinction trial and significant improvement may be masked by new learning that the platform is not always available to support escape (86, 87). Hence, it is possible that a more detailed analysis of the temporal dynamics of place learning using a bin analysis of probe test performance (87, 88) could reveal a cognitive enhancement effect that is restricted to the early part of the probe test, when place learning is prominent and new learning about the absence of the platform is limited. Another factor concerns the current lack of a dose-response assessment. Although a relatively high dose of zaprinast would seem to have the best chance of improvement, the frequency of U-shaped dose-response curves in several behavioral studies suggests the somewhat counterintuitive possibility that a lower dose may be more effective. In addition to testing a range of doses for zaprinast, other more selective PDE5 inhibitors should be tested using the standard water maze task as well as alternative versions that assess working memory and competing response tendencies (69). Finally, our findings using the 14-unit T-

maze employed a pretraining drug administration protocol and demonstrated an attenuation of the impairing effects of scopolamine or L-NAME. Perhaps PDE inhibitors may be particularly effective for improving impaired learning and retention only when given before training, without producing improvements above a normal level of performance (*i.e.*, a ceiling effect for optimal performance).

To test this possibility, we recently conducted a dose-response study with sildenafil in unimpaired control rats tested on the 14-unit T-maze (71). Different doses of sildenafil (1.5, 3 and 4.5 mg/kg i.p.) administered 15 min before the first trial did not improve the overall mean errors scores across 15 trials of learning the 14-unit T-maze task. The lack of effect of sildenafil in young animals, together with our positive results using the impairment models, suggests that the cognitive enhancement of spatial tasks may be restricted to animals that are cognitively impaired due to a deficiency in normal neurotransmission leading to spatial learning.

Our primary goal of modeling impaired learning and memory with drugs that antagonize or inhibit different neurotransmitter systems is to identify compounds that may improve cognitive function in aged individuals. Accordingly, we are currently testing whether sildenafil will improve the performance of aged rats relative to young controls. Although different doses of sildenafil (1.5, 3 and 4.5 mg/kg i.p.) did not influence acquisition performance, there was a significant improvement of retention performance in aged rats when the animals were retested 1 week after the original learning experience (manuscript in preparation). This finding suggests that the enhancing effect of PDE5 inhibition may be useful for treating age-related cognitive deficits. Although sildenafil was given 20 min prior to T1, the fact that it was able to improve retention performance a week later suggests that long-term consolidation was influenced by the pretraining drug treatment. This finding highlights the interaction between learning and memory processes. By targeting an early stage of acquisition, consolidation processes leading to the long-term retention of cognitive information can also be enhanced.

cGMP/PDE localization in brain

Systems level localization of the PDE5 isoenzyme shows that it is most abundant in Purkinje cells of the cerebellum (89-92). Strong PDE5 mRNA expression was also detected in scattered cells in all cortical layers, hippocampal subfields and in the caudate-putamen complex, with minimal detection in olfactory bulb, cortical layers and in the hippocampus (90). *In vitro* localization of cGMP after incubation of rat hippocampal slices with different PDE inhibitors revealed cGMP immunostaining in fibers of the CA3/CA2 region, with some positive fiber staining found in the CA1 region and dentate gyrus (93). The origin of these fibers and colocalization of neurotransmitters have not yet been determined. Interestingly, incubation with the cAMP-specific PDE4 inhibitor, rolipram, revealed a small increase in cGMP levels. Immunocytochemical localization of the cGMP response to rolipram revealed that cGMP-positive cells were astrocytes, a finding consistent with the results of a more recent study (94). Overall, these findings suggest an interaction between cGMP and cAMP signaling pathways in the hippocampus.

The role of NO as a retrograde messenger has been questioned, primarily on the basis of the localization of the cGMP response to PDE5 inhibitors (57). The concept of NO as a retrograde messenger predicts that sGC and cGMP will be localized presynaptically in glutamatergic nerve terminals. Prickaerts *et al*. note that although co-localization of cGMP immunoreactivity with the presynaptic marker, synaptophysin, has been observed, cGMP immunoreactivity is primarily found in postsynaptic structures (94). Further doubt that the retrograde messenger function of NO is related to memory formation stems from the fact that sGC immunostaining was found in pyramidal cell bodies and astrocytes, whereas cGMP immunoreactivity was found in varicose fibers and astrocytes but not in pyramidal cells (57). Finally, Prickaerts *et al.* state that they have only occasionally colocalized cGMP immunoreactivity with the neuronal glutamate or acetylcholine transporter molecules in the hippocampus, whereas cGMP immunoreactivity was colocalized with the acetylcholine transporter in cortex and caudate-putamen complex. Clearly, other mechanisms of action may contribute to the cognitive enhancing effects of PDE5 inhibitors.

NO/cGMP involvement in synaptic plasticity

In addition to LTP, the other major form of activity-dependent synaptic plasticity in the brain is long-term depression (LTD) (95), which has also been proposed as a celluluar/molecular mechanism for certain kinds of learning and memory (96, 97). LTD of excitatory synaptic transmission has been best characterized in the cerebellum where its induction requires co-activation of alpha-amino-3-hydroxy-5-methyl-4-isoxalepropionate (AMPA) receptors, metabotropic glutamate receptors (mGluRs) and L-type voltage-dependent Ca^{2+} channels. In addition, NO, cGMP, PKG, and protein kinase C (PKC) are important messengers in the signal transduction following induction of LTD (92, 96, 98, 99).

Different forms of LTD have been described in the hippocampus (100-103), some of which are dependent on NMDA/NO/sGC signal transduction (104, 105). In addition, high-frequency stimulation of corticostriatal glutamatergic fibers induces LTD of excitatory synaptic potentials recorded from spiny neurons in the caudate-putamen complex (106). Zaprinast can mimic this form of striatal LTD, which is calmodulin-dependent and sensitive to cGMP PDE activity; and intracellular cGMP, activating PKG, also induces striatal LTD that is blocked by NOS inhibitors, including L-NAME.

Systems level analysis of cognitive enhancement

Evidence suggests that the cerebellum and striatum also make important contributions to cognitive aspects of spatial learning (107-119). Because NO/sGC/cGMP signal transduction within these regions can contribute to synaptic plasticity and spatial learning, systemically administered PDE inhibitors may enhance cognitive processes within these structures in addition to the hippocampus and related cortical regions. Future studies should include a wider focus of the CNS to determine the therapeutic efficacy of PDE inhibitors within multiple neural systems.

Neuroprotective effects of PDE5 inhibition

NO-dependent signal transduction may contribute to neuroprotection (120). For example, NO activation of a cGMP-dependent pathway controls mitochondrial biogenesis and energy balance (121). Studies of cultured motor and non-motor neurons have shown that PDE inhibitors, particularly PDE5 inhibitors, protect against reactive oxygen species (ROS)- and chronic glutamate-induced neurotoxicity, whereas selective inhibitors of PDE1-4 offer little or no protection (122). Treatment with sildenafil (2 or 5 mg/kg p.o.) for 7 days following embolic stroke has been shown to induce neurogenesis and promote functional recovery in young rats (123). Aged rats subjected to embolic stroke have also shown improvements in functional recovery with a high dose of sildenafil (10 mg/kg s.c. for 7 days post-stroke), but not with the 2-mg/kg low dose (123, 124). Thus, repeated administration of PDE5 inhibitors may improve cognitive function via a second longer term action compared to the single-dose effects reported in many of the behavioral studies reviewed above.

Conclusions

Preclinical studies have identified PDE5 inhibitors as viable drugs that can improve different forms of learning and memory in rodents, but considerably more preclinical and clinical research is needed to establish the efficacy of such compounds. Most of the studies to date have only used acute systemic treatments; therefore, it is not known whether chronic treatment protocols would increase drug efficacy, particularly in aged animals that show evidence of cognitive impairment. Because PDE5 inhibitors increase glutamatergic neurotransmission, it is possible that prolonged treatment could have deleterious effects via excitotoxicity; however, the studies on neuroprotection reviewed above suggest that this may not be the case.

Further research should be conducted to determine the extent of the cognitive enhancing effects of PDE5 inhibitors, the precise mechanism(s) of action within different neural systems responsible for the behavioral-cognitive effects, and the potential role of these drugs in abating the neurodegeneration that accompanies aging. Given that multiple cofactors may contribute to age-related cognitive decline (125), combination therapies may have greater efficacy than single drug treatments. Therefore, studies should assess the therapeutic potential of combining PDE5 inhibitors with other cognitive enhancing agents. These basic research efforts may complement programs of drug design to produce newer, highly selective compounds that have the potential to improve the quality of life of a growing proportion of older individuals in our society.

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