Multifunctional Receptor-Directed Drugs for Disorders of the Central Nervous System

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Summary: The marked decline in FDA-approved new drug candidates in recent years suggests the possibility that the "low-hanging fruit" has been almost entirely harvested. This might be particularly applicable to drugs acting on the central nervous system. Fortunately, there are several examples extant for the utility of multifunctional drugs, compounds, or drug mixtures that act on multiple additive or synergistic targets. However, to exploit this approach may require the willingness to consider the possibility that drug targets might be addressed by molecules of rather low specificity and moderate potency. The expectation is that single target molecules with high specificity might not have access to complex interacting neural pathways, and that moderate potency could engender fewer off-target side effects. Though novel compounds might be developed by combining the active functional groups of two or more drug molecules, the approach still lends itself to high throughput screening of large chemical libraries. Multifunctional compounds might be designed with the ability to: 1) offer both palliative and disease modifying actions, 2) act on targets that produce additive or synergistic therapeutic responses, 3) simultaneously evoke a therapeutic response at the desired target and prevent an undesired response mediated by an alternate target, 4) allow one component to promote the drugable characteristics (e.g., brain penetration) of the therapeutic component, and 5) prolong the duration of effectiveness of one compound by contributing the pharmacodynamic actions of another. The author takes the liberty to include examples of the situations just mentioned from studies in his laboratory in the following discussion. **Key Words:** Drug discovery, Alzheimer's disease, cognition, working memory, attention deficit disorder, delayed responses tasks, neuroprotection.

WHY TARGET MULTIPLE SYSTEMS FOR THE TREATMENT OF CNS DISORDERS?

Decades of success in modern drug development have led to general acceptance of the premise that a promising drug candidate should exhibit, in addition to good bioavailability and pharmacokinetics, high potency and high selectivity of action. The latter two properties are important, not only in terms of limiting tablet size and reducing off-target effects, but they allowed for a systematic search for highly specific single-drug targets. The complexity of human disease often requires multiple approaches for effective treatment. This concept is well known for the treatment of cancers and metastatic disease. But even in more chronic human ailments, such as essential hypertension, often multiple medications targeting different aspects of blood pressure lowering mechanisms are required.[1] In CNS disorders, multiple treatment approaches have not been the rule, except perhaps in the treatment of Parkinson's disease.[2] Despite the known multitude of potential CNS drug targets, the "magic bullet" approach continues to lead drug discovery.[3] The premise that high selectivity and high potency are the most desirable properties for a new therapeutic agent may not be the case for many approaches designed to treat brain disorders. For example, in the treatment of schizophrenia, several subtypes of dopamine and serotonin receptors have therapeutic value as drug targets.[4] It is still not clear as to whether subtypes of adrenergic, cholinergic, and histaminergic receptors, long considered off-target sites of drug action, play some role in the therapeutic actions of the latest class of atypical or second-generation antipsychotic drugs. For neurodegenerative diseases, the requirement for multiple palliative approaches is perhaps obvious, as is the need for compounds that modify the disease process. Irrespective of which of these approaches is most successful in the coming years, it is likely that the Alzheimer's or Parkinson's patient of the future will require multiple treatment

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approaches, both palliative and disease-modifying. Sivachenko and colleagues[5] have argued that the recent downward trend in Food and Drug Administration approval rates is attributed to the "magic bullet" approach. They contend that pathway analysis allows for multiple target development that is more relevant to CNS disorders, which are complex and multigenetic in origin. They are also in favor of what they term "promiscuous" drugs, preferring to deal with off-target effects separately as a necessary "payment" for the benefit of actions of several beneficial target molecules. One example would be the use of histamine H2 antagonists and proton pump inhibitors to control gastric acid secretion as a side effect of aspirin treatment for inflammatory pain or as a regimen for cardiovascular protection.[6,7]

In certain respects this approach has been partially achieved in the treatment of Parkinson's disease. Compounds like carbidopa and entacapone inhibit peripheral dopa decarboxylase and catechol-O-methyl transferase activities, respectively. When used in conjunction with levodopa, both compounds increase the bioavailability of levodopa by inhibiting peripheral metabolism and thus facilitating the drug's passage across the blood brain barrier. Entacapone also stabilizes extracellular levels of brain dopamine resulting in better control of symptom expression. In fact, most Parkinson's disease patients are prescribed treatment regimens that could include compounds with additive therapeutic properties, such as anticholinergics and amantidine, compounds like carbidopa and entacapone, and direct-acting dopamine receptor agonists, such as lisuride or pramipexole. The precise regimen for each patient is dictated mainly by empirical observation, stage of the disease, and response to side effects.[2] Thus, in the future, there could be a concerted drug development effort aimed at treating the side effects associated with the use of the primary therapeutic agents. A current example is the development of new cannabinoid products for the treatment of the side effects associated with cancer chemotherapy.[8]

It is possible, and no doubt desirable, to develop compounds that exhibit the attributes of the therapeutic approaches just described. Multifunctional compounds might be designed with the ability to: 1) offer both palliative and disease modifying actions, 2) act on targets that produce additive or synergistic therapeutic responses, 3) simultaneously evoke a therapeutic response at the desired target and prevent an undesired response mediated by an alternate target, 4) allow one component to promote the drugable characteristics (e.g., brain penetration) of the therapeutic component, and 5) prolong the duration of effectiveness of one compound by contributing the pharmacodynamic actions of another. The primary advantage of combining drug targets in a single molecule is largely pharmacokinetic. It is much easier to predict pharmacological responses to a single molecule than to a drug mixture. In the case of levodopa/carbidopa preparations, it is fortuitous that pharmacokinetics of the two compounds are compatible. This would not be the case for every compound mixture that might be envisioned. Single molecules would be easier to package and dose, and would limit the total number of medications that individuals would have to remember to take, which is an important issue for the elderly. In the following paragraphs, I will provide examples of the treatment approaches previously mentioned (as we have studied them), and those that have relevance to the treatment of neurodegenerative diseases and schizophrenia.

RATIONAL MOLECULAR DESIGN

The design of bi-functional or multifunctional molecules is not straightforward. It is not always the simple combination of two or more active drug moities. The design of bi-molecular drugs, although inherently more difficult than for single-targeted drugs from a molecular modeling standpoint, provide perhaps no additional difficulties in this era of large combinatorial libraries and high throughput screening techniques. One laboratory that has been particularly successful in the design of bi-functional molecules is headed by Moussa Youdim (Technion-Rappaport Faculty of Medicine, Haifa, Israel). Of particular relevance to this discussion is their combination of the dopaminergic (monoamine oxidase inhibition) and cholinergic (cholinesterase inhibition) activities associated respectively with rasagiline and rivastigmine.[9] The resulting compound, ladostigil (TV3326), exhibits both monoamine oxidase inhibitory and anticholinesterase activities, and the compound mimics the established neuroprotective profile of rasagiline. Ladostigil increases striatal, hippocampal, brainstem and hypothalamic dopamine, serotonin, and noradrenaline levels in rats and mice.[10] The drug also produces a neurochemical profile suggestive of potential antidepressant activity, as was confirmed in a rat model of helplessness.[11] In a subsequent series of experiments, ladostigil was administered acutely to aged Rhesus monkeys well trained to perform versions of a delayed matching-to-sample (DMTS) task.[12] The drug was very effective in improving the ability of subjects to titrate to longer delay intervals (increasing memory load). In a measure of attention deficit, ladostigil also significantly improved task accuracy during distractor (interference) sessions. The compound was effective enough to return group performance efficiency to baseline nondistractor trial accuracies. In addition to these pharmacological properties, ladostigil is effective in stimulating the expression of the anti-apoptotic genes, *Bcl-2* and *pPKC*, while inhibiting the expression of the apoptosis-inducing genes, *Bax, Bad*, and *caspase 3*. Ladostigil also has been reported to enhance soluble amyloid precursor protein

(APP)-α secretion via the protein kinase C-MAP kinase-dependent pathway, and to decrease the levels of holo-APP (i.e., actions that favor cell viability).[13–15] Thus, ladostigil represents a new drug class that is potentially suitable for the treatment of Alzheimer's disease (AD). Patients with AD require therapies that will delay the progression of the disease, and they may suffer from impaired attention, impaired memory, extrapyramidal disorders, and depression. Another recent example of this concept is the development of benzofuran-based compounds that simultaneously inhibit acetylcholinesterase and prevent amyloid Aβ peptide aggregation.[16] This latter property is expected to prevent the formation of the most neurotoxic species of amyloid, while the inhibition of acetylcholinesterase would be expected to improve cognition. In the next section, examples will be provided of the various approaches we have used both to support the proof of concept of side-effect suppression with the use of promiscuous drugs, and the relevance of single molecules that have multiple drug targets. These examples will have the most application for disorders of cognition and neurodegeneration, such as AD.

ATTENUATION OF PERIPHERAL SIDE EFFECTS

Many drugs and other natural substances derived from a wide variety of chemical and pharmacological classes have been shown to improve memory-related task performance in animals and humans. The clinical use of acetyl cholinesterase inhibitors is likely to continue for some time into the future. This drug class is associated with only limited effectiveness, as well as a limited therapeutic dose window. The latter problem is common for most classes of cognition-enhancing drugs in clinical use or as studied in animals. Generally responsiveness to an acetyl cholinesterase inhibitor (such as donepezil) is relegated to one or two doses in a series of less than two log units.[17] The question remains as to the mechanism(s) contributing to this inverted-U dose-response relationship. At least one contributing factor is that troubling side effects can be encountered before or near the maximal therapeutic response, insofar as the therapeutic target(s) for which the drug is designed exist both in the brain and the periphery. Thus, simultaneous inhibition of peripheral cholinesterase has the strong potential to evoke side effects related to parasympathetic and somatic cholinergic over-stimulation. Alternatively, it might be possible to expand the therapeutic window of certain agents like donepezil by preventing peripheral side effects with the use of low levels of selective peripherally-acting muscarinic acetylcholine receptor-blocking drugs. Along these lines, it is somewhat perplexing as to why low doses of antagonists, such as methylatropine or glycopyrrolate have not been used in combination with cholinesterase inhibitors to help limit side effects associated with therapy.

Although we have no data to support this contention for the class of acetyl cholinesterase inhibitors, it was clear from our earliest studies (in which nicotine [structure, FIG. 1] was used to improve working memory in monkeys) that the compound was effective over a very narrow range of doses.[18–20] Early on, we studied the potential for the nonnelective nicotinic receptor antagonist mecamylamine (structure, FIG. 1), which has access to the CNS to block the positive mnemonic response to nicotine in monkeys.[18] To obtain a reproducible response to nicotine, we first evaluated a dose series of the drug in the subjects. Next an individualized best dose was determined that was based on the dose of nicotine (0.625–7.5 μg/kg) for each subject that produced the greatest improvement in DMTS accuracy. Mecamylamine (0.5 mg/kg) or its vehicle (i.e., sterile normal saline) was administered 15 min prior the administration of nicotine. DMTS testing was initiated 10 min later. As indicated in FIG. 2, mecamylamine pretreatment completely abolished the nicotine-induced increase in matching-to-sample accuracies. Next we used the quaternary nicotinic acetylcholine receptor antagonist hexamethonium (structure, FIG. 1) to limit the potential peripheral actions (mainly ganglionic activation) of nicotine. When the monkeys were pretreated with hexamethonium (2 mg/kg), the nicotine-induced improvement in average task accuracy was enhanced across all three delay intervals (FIG. 2). No attempt was made to optimize the use of the two compounds: 1) agonist and 2) antagonist. It remains to be determined as to whether simultaneous hexamethonium treatment has the ability to widen the effective dose window for nicotine. In the field of cognitive pharmacology, studies of the use of drug combinations have not been significantly explored. It might be noted that for the nicotine field, in the area of smoking cessation, the combination of nicotine and mecamylamine has shown effectiveness in clinical trials.[21] The addition of the antagonist to the regimen was considered to inhibit the rewarding effects of smoking, thus promoting extinction of the behavior. The agonist was included to prevent nicotine withdrawal and craving.

A TWO-COMPOUND REGIMEN AND THE ROLE OF PHARMACODYNAMIC ACTIONS

Noradrenergic and cholinergic neurons have been shown to play a role in different components of learning and memory. As such, the combined therapy with adrenergic agonists (e.g., clonidine) and cholinergic agonists (e.g., acetyl cholinesterase inhibitors) might result in additive or synergistic effects to improve working memory and cognition. In addition to this practical approach to combination therapy, there could be another rationale for

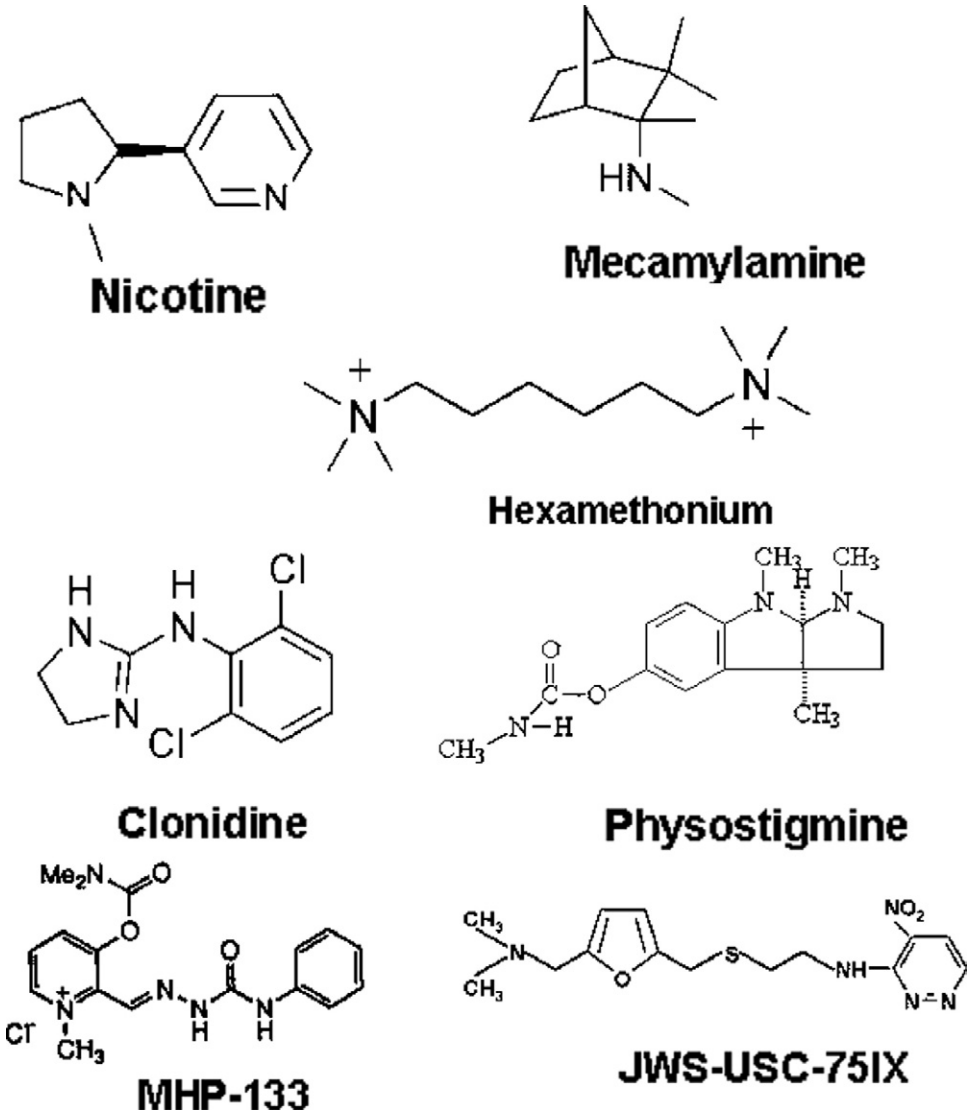

FIG. 1. Compound structures.

considering that combined therapy is superior to monotherapy. We reported that clonidine (structure, FIG. 1) is a potent inhibitor of the biosynthesis and the release of acetylcholine within specific brain regions (particularly in hypothalamic and hindbrain regions) in the rat, and that the drug can inhibit the expression of cholinergic signs of toxicity to physostigmine (structure, FIG. 1) and other cholinesterase inhibitors.[22] However, clonidine is only weakly effective in inhibiting cholinergic function within higher brain regions,[23] presumably more relevant to the cognitive enhancing actions of AChE inhibitors. We tested the possibility that combined treatment with clonidine and physostigmine could result in enhanced effects on DMTS performance accuracy by mature adult and aged macaques. One of the most obvious effects of adding 0.5 μg/kg clonidine to the physostigmine regimen was that the animals were able to tolerate much higher doses of physostigmine. The individualized best dose of physostigmine was determined for each animal as that dose which provided the greatest improvement in task accuracy averaged over the entire 96-trial session. The best doses determined for physostigmine alone ranged from 5 to 40 μg/kg (mean, 21.4 $\pm$ 4.5 μg/kg). Best doses determined for physostigmine in the presence of 0.5 μg/kg clonidine ranged from 10 to 60 μg/kg (mean, 40.0 $\pm$ 6.9 μg/kg), almost a two-fold increase. Despite the fact that doses used for physostigmine were maximal for each animal, when the two drugs were combined, a further improvement in performance was obtained (FIG. 3A).

Even beyond the potential for additivity of their individual cognition-enhancing actions, and for the suppression of each other's side effects, is the possibility of extending the duration of action of the treatment based on the pharmacodynamic actions associated with one of the compounds. For the acetyl cholinesterase inhibitors

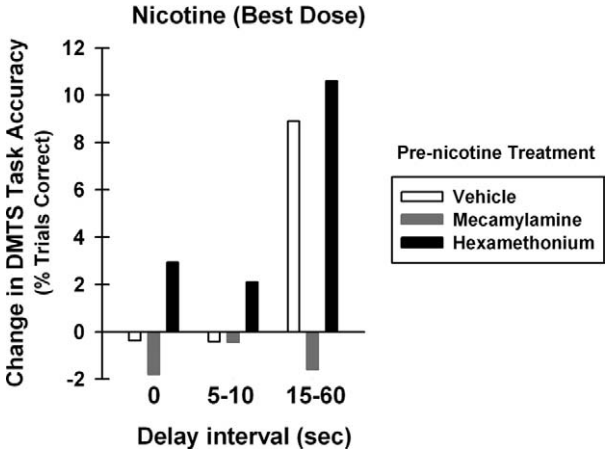

FIG. 2. The change in delayed matching-to-sample (DMTS) accuracy by adult macaques produced by nicotine alone, and by treatment with combinations of nicotine and mecamylamine (0.5 mg/kg) and nicotine and hexamethonium (2.0 mg/kg). Vehicle was sterile normal saline, and all injections were made into the thigh muscle (0.04 ml/kg). Nicotine was administered as the individual best dose (0.625–7.5 μg/kg) determined from a previous dose-response series as the dose that evoked the maximal improvement in task accuracy for each subject ($n = 5$). The antagonists were administered 15 min before nicotine, and nicotine was administered 10 min before DMTS testing. The delay interval was the time between extinguishing the sample color presentation and the presentation of the two-choice color presentations.

that have been used in the treatment of AD, the duration of action is related to the pharmacokinetics of the drug and the duration of enzyme inhibition, and there is little carryover of the positive mnemonic action to the next day after administration.[24] In contrast, clonidine, like several other cognition-enhancing drugs, has been shown to enhance task performance over a time course not predicted by its very short half-life. We first reported this phenomenon for nicotine.[25] Monkeys received single intramuscular administrations prior to DMTS testing. The increase in task accuracies measured during sessions that were run shortly after nicotine administration were largely maintained during testing on the following day. Support for this observation in the clinical setting comes from studies with the nicotinic acetylcholine receptor partial agonist ispronicline. The compound improved cognition in subjects diagnosed with age-associated memory impairment. The pro-cognitive effect carried over significantly beyond the original dosing schedule.[26] Most often, the magnitude of the pro-mnemonic action produced by cognition-enhancing drugs is greater during testing just after administration as compared with the subsequent testing on the day or days following. However, we have encountered compounds for which the opposite was the case. One of these was the partial $\alpha 7$ nicotinic acetylcholine receptor agonist GTS-21.[27] It has not been possible to predict, even knowing the pharmacological or chemical class, or knowing the profile of responsiveness in sessions run shortly after drug administration, the potential for a compound to produce a protracted improvement in cognition.[28] Thus, we routinely run sessions on the day or days after drug administration to directly assess this potential.

Clonidine possesses the pharmacological ability to enhance memory-related task performance on the days after drug administration. In fact, improvement in DMTS task accuracies by adult macaques recorded during sessions (run 24 and 48 h after clonidine administration) was substantially more impressive than the measurements recorded during the first (60 min) test session.[29] Moreover, significant increases in task accuracy were apparent for up to 6 days after a single administration. Returning to the example of combining clonidine with physostigmine, the data presented in FIG. 3B show that when animals were tested during sessions 24 hrs after

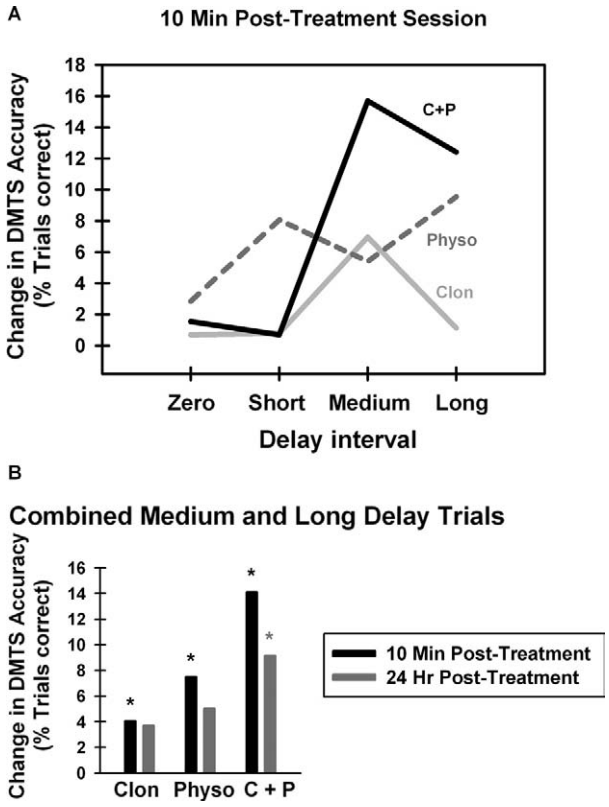

FIG. 3. **A:** The change in delayed matching-to-sample (DMTS) accuracy by adult macaques produced by clonidine (0.5 μg/kg) alone, by physostigmine (1.25–90.0 μg/kg) alone, and by the combination of clonidine and physostigmine ($n = 5$–7). The data represent the most effective (greatest increase in task accuracy) dose or dose combination for physostigmine. Vehicle was sterile normal saline, and all injections were made into the thigh muscle (0.04 mL/kg) 10 min before DMTS testing. Zero, short, medium, and long refer to the delay intervals presented randomly during 96-trial sessions. The clonidine-physostigmine combination produced a statistically greater increase in mean accuracy across medium and long delay trials than either of the constituents administered alone. **B:** The medium and long delay data from panel **A** are presented along with the results of DMTS sessions run on the day after drug treatment (24-h post-treatment) with no intervening administrations. *Significantly different ($p < 0.05$) from mean accuracies obtained after vehicle administration.

receiving the combination regimen, task improvement was maintained. Clearly this protracted mnemonic action could be attributed to the pharmacodynamic actions that are characteristic of the response to clonidine.[29]

Therefore, in this example, the following factors appear to contribute to the superior effectiveness of the combination regimen: 1) the targeting of separate neural substrates that each play a role in cognitive function; 2) a widening of the therapeutic window associated with physostigmine treatment, most likely contributing a reduction in physostigmine-associated side effects; and 3) the addition of clonidine extended the regimen's overall duration of action, possibly through a unique pharmacodynamic action. In this particular study we used a fixed dose of clonidine previously determined to be optimal when used alone. It is possible that additional improvement might be obtained with further optimization of the regimen.

MULTIPLE TARGETS IN A SINGLE MOLECULE FOR COGNITION ENHANCEMENT

MHP-133 (structure, FIG. 1) is the lead compound of a novel series of analogs designed to target multiple brain substrates expected to have synergistic actions in the treatment of human cognitive disorders such as AD.[30,31] MHP-133 was designed to target components of acetylcholine neurons that would act synergistically to enhance cholinergic function, including the stimulation of cholinergic receptors and the inhibition of acetyl cholinesterase. The strategy was to develop compounds with multiple targets relevant for enhancing cognition and memory, but avoiding the serious side effects attributed to high potency cholinergic agonists. A preliminary assessment of the neurochemical properties exhibited by MHP-133 suggested that the drug might indeed fit this profile. For example, MHP-133 was shown in ligand binding studies to interact with subtypes of cholinergic (nicotinic and muscarinic, M1 and M2) receptors and to weakly inhibit acetyl cholinesterase. Most importantly, MHP-133 enhanced the accuracies by young and aged macaques in their performance of the DMTS task. As indicated in FIG. 4A, MHP-133 significantly improved accuracy during long, delayed trials over a wide-dose range. MHP-133 also reversed distractor-induced (interference trials) performance decrements, suggesting that part of its positive mnemonic action included improved attention (FIG. 4B). No untoward effects were observed out to the highest doses tested. This synergy of therapeutic actions could underlie both the marked effectiveness of the drug on memory, as well as the lowered potential for producing side effects.

In a series of *in vitro* studies we found that MHP-133 enhanced nerve growth factor-TrkA receptor expression

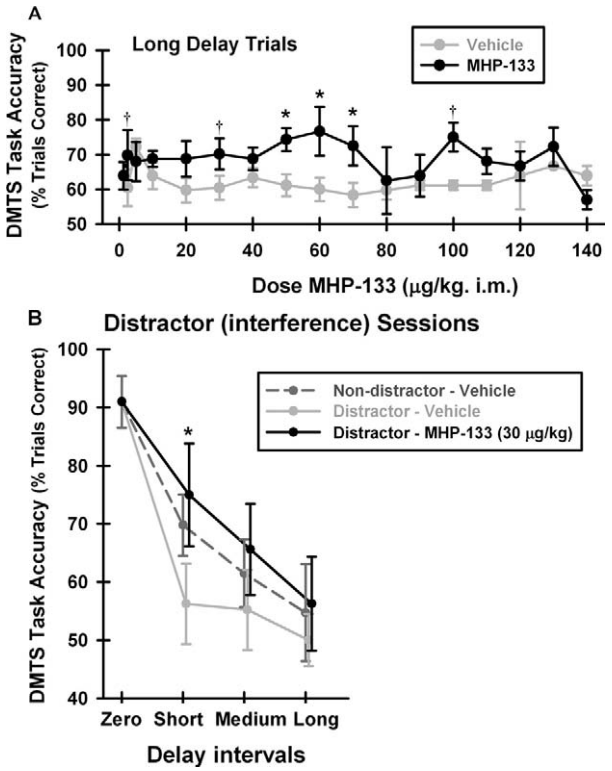

FIG. 4. **A:** The ability of MHP-133 to improve delayed matching-to-sample (DMTS) task accuracy by mature macaques ($n = 6$) over a broad dose range. MHP-133 or vehicle (sterile normal saline) was administered intramuscularly 10 min prior to DMTS testing. Zero, short, medium, and long refer to the delay intervals presented randomly during 96-trial sessions. **B:** The ability of MHP-133 (30 μg/kg) to reverse distractor (interference trials)-induced impairments in accuracy by 4 macaques in the distractor version of the DMTS task. "Non-distractor-vehicle" indicates sessions of standard DMTS task (no distractor trials) by the cohort. "Distractor-vehicle" indicates distractor trials in which the pretest administration was vehicle. *Significantly different ($p < 0.05$) from mean accuracies obtained after vehicle administration. †$p < 0.10$.

in a neuronal cell line. The compound also significantly increased the secreted form of amyloid precursor protein APP in cultured astrocytes. Both actions are consistent with MHP-133s potential as a neuroprotective agent.[32] The approximate ED50s for MHP-133 in the TrkA receptor and secreted form of APP assays just previously indicated, in cytoprotection assays, and in competition binding assays are presented in Table 1. The relative affinities of the compound for the cholinergic and serotonergic binding sites were within range of potency for the TrkA receptor and secreted form of APP assays. However, it is clear that potency of MHP-133 in the two cytoprotection assays (Table 1) and in the DMTS studies in monkeys can not be related to the rather low affinity of the compound in the receptor binding assays, nor can they all be attributed to the weak anti-cholinesterase activity of the compound. The cytoprotective actions and the cognition-enhancing effects occur at concentrations much lower than the individual binding affinities. A wide

Table 1. *Comparison of Approximate EC50 Values for the Effects of MHP-133 in Several In Vitro Assays*

Assay Identification	EC50 (μM)
Cytoprotection in differentiated PC-12 cells subjected to growth factor withdrawal	0.0044
Cytoprotection in primary hippocampal neurons subjected to medium-change toxicity	0.00046
Induce APP secretion from glial cells	0.58
Increase TrkA receptor expression in differentiated PC-12 cells	1.42
Displacement of [^{3}H]cytisine binding in synaptosomal membranes	69
Displacement of [^{3}H]methylscopolamine binding in synaptosomal membranes	174
Displacement of [^{3}H]pirenzepine binding in synaptosomal membranes	3.3
Displacement of [^{3}H]GR113808 binding in transfected non-neural membranes	1.73

APP = amyloid precursor protein.

neurotransmitter-related screen also showed no other significant interactions between MHP-133 and the target molecules.[32] Therefore, the possibility should be considered that MHP-133 exerts at least some of its pharmacological actions through partial interaction with multiple synergizing targets.

Despite the promise of MHP-133 and its analogs as potential therapeutic entities for AD and related disorders, these compounds are not yet optimized regarding the important pharmacological actions described above. Also, the concentrations required for TrkA receptor expression and altered amyloid metabolism represent *in vivo* dose-ranges greater than those used for memory enhancement. It is not known at present whether these effects of MHP-133 can be realized *in vivo* at doses relevant for cognition enhancement. Part of the challenge to developing bi-functional or multifunctional molecules is the ability to address the various targets with equivalent efficacies.

A MULTIFUNCTIONAL MOLECULE DEVELOPED FOR FUNCTIONAL AND METABOLIC SYNERGY

JWS-USC-75IX (structure, FIG. 1) is a relatively potent acetyl cholinesterse inhibitor, but it also exhibits high-affinity antagonism for the M2 subtype of the acetylcholine muscarinic cholinergic receptor.[33] As acetyl cholinesterase inhibitors have the potential of limiting their own actions through acetylcholine-induced feedback inhibition (mediated via activation of presynaptic M2 receptors), it was reasoned that M2 receptor antagonism could result both in the enhanced release of acetylcholine and mitigation of the anti-cholinesterase-induced feedback inhibition. JWS-USC-75IX improved the performance of rats in three different memory-related tasks, and in one of these, a delayed discrimination task, the drug-elicited repeatable improvements in task performance without the development of tolerance. The task allowed us to use an operant paradigm (not unlike the primate DMTS task) in rats. JWS-USC-75IX also exhibited an excellent safety profile relative to drugs acting only to inhibit AChE.[33] More recently, we examined the effects of JWS-USC-75IX in the DMTS task by adult macaques. The compound produced a rather highly subject-specific improvement in task accuracies so that a best dose was derived from the dose-response data. As indicated in FIG. 5, the best dose of JWS-USC-75IX significantly shifted the accuracy-delay curve to the right in a parallel fashion. This suggests the potential of the compound to affect multiple components of working memory, including attention.[34] Another interesting feature of the compound is its ability to maintain effectiveness on the day after testing in the absence of any further treatment. Presently, we have no pharmacokinetic data pertaining to JWS-USC-75IX, and so it is not clear as to whether the protracted mnemonic action is related to a long brain or plasma half-life, or to a pharmacodynamic action of the compound as previously discussed.

As an analog of ranitidine, JWS-USC-75IX also possesses weak-moderate ability to interact with the histamine H3 receptor (A. Terry, oral communication). This subtype of the histamine receptor also has been targeted for cognition enhancement.[35–37] Again, it is not clear as to the extent that this pharmacological property contributes to the ability of the compound to improve working memory. However, the overall pharmacological profile of JWS-USC-75IX might be compared with that described for certain of the atypical (second generation) class of antipsychotic drugs, which often have strong anti-cholinergic and anti-histaminergic actions. In fact, the H3 antagonists, ABT-239,[38] and thioperamide,[39] have been shown to produce a preclinical profile of activities predictive of clinical antipsychotic activity. In the rat, the motor response to acoustic startle can be inhibited by the presentation of a low-level acoustic prepulse presented just in advance of the high-level acoustic pulse, thereby providing a measure of sensory gating. Disruption of sensory gating can be produced by drugs like apomorphine that can induce a schizophreniform action. Under the conditions established at baseline, apomorphine treatment suppresses the ability of the prepulse to inhibit acoustic startle (FIG. 6). Most antipsychotic drugs reverse the effects of apomorphine. Rats were administered JWS-USC-75IX by oral gavage 20 min before being evaluated in the prepulse inhibition procedure. The effects of vehicle plus apomorphine (0.5 mg/kg), a reference dose (0.3 mg/kg) of the antipsychotic drug haloperidol plus apomorphine, and several doses of JWS-

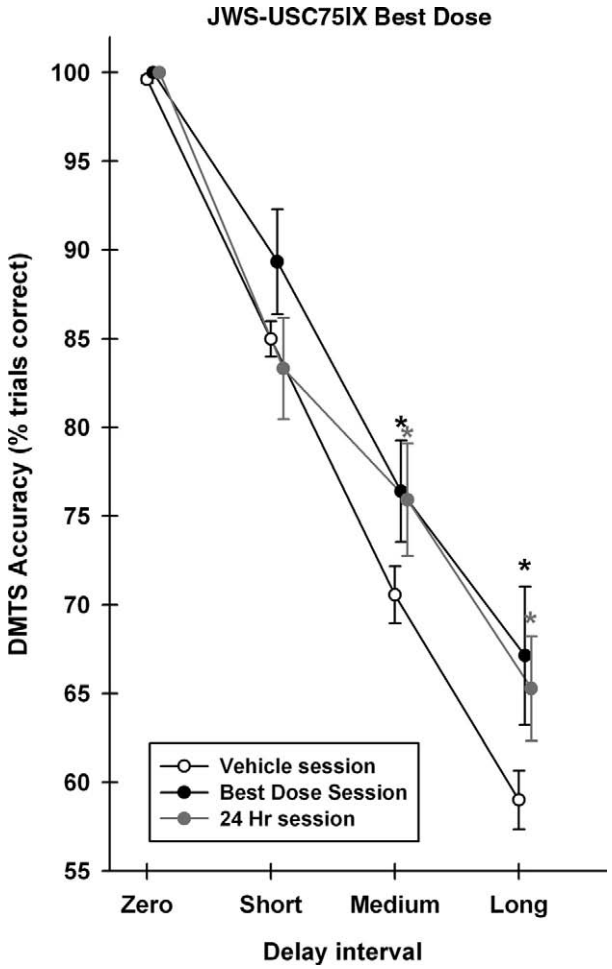

FIG. 5. The ability of a best dose of JWS-USC-75IX to improve delayed matching-to-sample (DMTS) task accuracy by mature macaques ($n = 6$) in their performance of a DMTS task. The individual best dose (0.05–1.5 mg/kg) was determined from a previous dose-response series as the dose that evoked the maximal improvement in task accuracy for each subject. JWS-USC-75IX was dissolved in 0.5 mL DMSO and this solution was added to 10 mL commercial fruit punch. DMSO/fruit punch served as the drug vehicle. All subjects voluntarily consumed the total dose delivered from a syringe and spout. JWS-USC-75IX was administered 30 min before DMTS testing. "24-h session" indicates the results of DMTS sessions run on the day after drug treatment with no intervening administrations. *Significantly different ($p < 0.05$) from mean accuracies obtained after vehicle administration.

USC-75IX on apomorphine-induced deficits in prepulse inhibition (averaged across prepulse level) are presented in FIG. 6. Although the dose-response to JWS-USC-75IX appeared to exhibit an inverse-U-profile, the 0.3 and 1.0 mg/kg doses of the compound significantly reversed the apomorphine-induced prepulse deficit. In this respect JWS-USC-75IX was similar in efficacy to haloperidol. Additional studies will be needed to confirm the antipsychotic potential for JWS-USC-75IX.

At this point it is appropriate to point out that all efforts to combine multiple actions in one molecule have not met with success. An example is the compound RS66331, which (neurochemically) exhibits the properties of a $5HT_4$ agonist and a $5HT_3$ antagonist. Both properties have been associated with enhanced release of brain acetylcholine.[40] We studied this compound in aged rhesus monkeys and compared its effectiveness with that produced by individual administration of a $5HT_4$ agonist and a $5HT_3$ antagonist, both of which were demonstrated previously to enhance task performance in the same subjects. Rather than this combination of properties providing RS66331 with augmented memory-enhancing action, the effectiveness of the drug proved to be similar to that produced by the $5HT_3$ antagonist RS56812, but it was considerably reduced in effectiveness compared with the $5HT_4$ agonist RS17017.[41] However, RS66331 was developed prior to our work with the individual compounds. There are many reasons for the failure of compounds to achieve expectations in memory paradigms; however, this may be one case wherein the information derived from the combined administration of various dose-regimens of RS56812 and RS17017 may have alerted us to the possibility that this is not a useful neural target combination, or to the possibility that different proportions of relative receptor activity were needed as compared with that inherent in RS66331.

One last example with relevance to this discussion pertains to the situation in which two compounds, at the higher end of their respective dose ranges, each exhibit

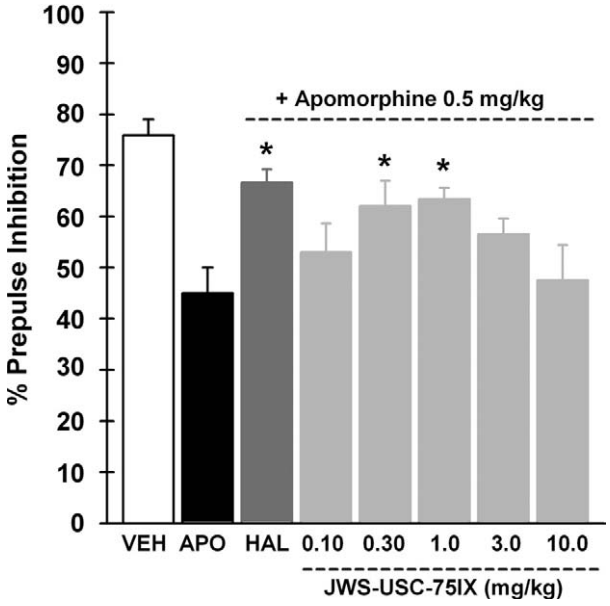

FIG. 6. The ability of JWS-USC-75IX to attenuate apomorphine-induced deficits in prepulse inhibition (PPI). Rats were administered JWS-USC-75IX by oral gavage 20 min before being evaluated. The effects of vehicle + apomorphine (0.5 mg/kg), a reference dose (0.3 mg/kg) of the antipsychotic haloperidol + apomorphine, and several doses of JWS-USC-75IX on apomorphine-induced deficits in PPI (averaged across prepulse level) are compared. Bars represent mean ± S.E.M. for each treatment ($n = 9–12$). VEH = vehicle; HAL = haloperidol; APO = apomorphine. *Significantly different ($p < 0.05$) from the vehicle.

memory-impairing effects, but when combined they tend to cancel each other's negative mnemonic action. Levin and colleagues[42] recently studied the effects of nicotine and thioperamide by using the repeated acquisition test in the radial-arm maze. They first determined a dose of nicotine (0.4 mg/kg) that impaired task acquisition, and a dose of thioperamide (10 mg/kg) that increased errors in the task. When the two regimens were combined, the working memory deficits attributed each compound were eliminated, although the regimens failed to improve task performance above control. Again, no attempt was made to optimize the combination regimen. Nevertheless, the authors suggest that the interaction between the two compounds occurs at the level of brain acetylcholine neurons that possess both H3 histaminergic hetero-receptors and nicotinic cholinergic receptors.

The examples previously cited provide only a glimpse into the potential for drug discovery when new molecules are designed to act on multiple targets. The marked decline in new drug candidates approved by the Food and Drug Administration in recent years suggests the possibility that the "low-hanging fruit" has been almost entirely harvested. But many of the available targets could be revisited as novel multi-functional compounds are developed. This approach holds the potential for new heights of drug efficacy, convenient dosing regimens, and reduced side effect profiles.

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