CNS Drug Reviews
Vol. 8, No.1, pp. 31–52

S 17092: A Prolyl Endopeptidase Inhibitor as a Potential Therapeutic Drug for Memory Impairment. Preclinical and Clinical Studies

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Key Words: Memory enhancer—Prolyl endopeptidase inhibitor—S 17092—Cognitive disorders—Neuropeptides—Neuroprotection—Cerebral aging.

ABSTRACT

Any treatment that could positively modulate central neuropeptides levels would provide a promising therapeutic approach to the treatment of cognitive deficits associated with aging and/or neurodegenerative diseases. Therefore, based on the activity in rodents, S 17092 (2S,3aS,7aS)-1{[(R,R)-2-phenylcyclopropyl]carbonyl}-2-[(thiazolidin-3-yl)carbonyl]octahydro-*1H*-indole) has been selected as a potent inhibitor of cerebral prolyl-endopeptidase (PEP). By retarding the degradation of neuroactive peptides, S 17092 was successfully used in a variety of memory tasks. These tasks explored short-term, long-term, reference and working memory in aged mice, as well as in rodents and monkeys with chemically induced amnesia or spontaneous memory deficits. S 17092 has also been safely administered to humans, and showed a clear peripheral expression of its mechanism of action through its inhibitory effect upon PEP activity in plasma. S 17092 exhibited central effects, as evidenced by EEG recording in healthy volunteers, and could improve a delayed verbal memory task. Collectively, the preclinical and clinical effects of S 17092 have suggested a promising role for this compound as an agent for the treatment of cognitive disorders associated with cerebral aging.

INTRODUCTION

Many hypotheses suggest that pharmacological interventions targeted at neuropeptides might be effective treatments for age-related cognitive decline. Besides acetylcholine and biogenic amines, some neuropeptides have been described to have potent biological ef-

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fects and, more specifically, memory enhancing properties. Among them, substance P (SP) has been shown to improve learning and recall performances of rodents in a variety of memory tasks (17). Other neuropeptides such as arginine vasopressin or thyrotropin releasing hormone (TRH) (3,7,13) as well as SP (11,46) were described as cognition enhancers with positive modulatory effects on cerebral cholinergic activity. It has also been demonstrated that several neuropeptides can promote processes related to functional recovery following central nervous system (CNS) damage (8,25,29,39) and display neurotrophic actions *in vitro* (18,51). The brain levels of certain neuropeptides are known to be reduced in aged animals and humans (4,9,50). For example, deficiencies in SP and vasopressin have been reported in *post-mortem* studies of cerebral tissue derived from patients with neurodegenerative diseases (2,10,16,42). Moreover, decreases in SP levels were reported in both cortical (Alzheimer's disease, AD) and subcortical [Parkinson's (PD) and Huntington's (HD)] neurodegenerative diseases (19,30,41).

Based on these observations, it has been hypothesized that peptidergic alterations can play a significant role in age-related cognitive decline. Therefore, any treatment with a compound that could positively modulate the brain levels of these neuropeptides would appear as a promising therapeutic approach to the treatment of cognitive deficits associated with aging and/or neurodegenerative diseases. This suggestion is further supported by evidence for a beneficial effect on mnemonic performance of SP in aged rats (15). A similar facilitation has also been seen in patients with Alzheimer's disease following TRH treatment (32,33,34) and in volunteer subjects treated with scopolamine (34,35). However, the effectiveness of systemic neuropeptidergic treatments is generally viewed with scepticism because of the relative impermeability of the blood brain barrier for these peptides. This is further complicated by the possibility that not one, but several neuropeptides might be involved in the mnemonic decline associated with aging.

A common feature to the above-mentioned promnesic neuropeptides is their sensitivity to a specific prolyl-targeted protease. Such an enzyme termed prolyl-endopeptidase (PEP, EC 3.4.21.26), or post-proline cleaving enzyme, was subsequently identified (21). PEP is a member of the serine-protease family that hydrolyzes peptide bonds at the L-proline carboxy terminal. PEP plays an important role in the catabolism of proline-containing peptides such as substance P, arginine-vasopressin, TRH, bradykinin, angiotensin, neurotensin, and oxytocin (52). Consequently, a PEP inhibitor by attenuating the catabolism of neuropeptides could represent a novel therapeutic approach to the treatment of mnemo-cognitive deficits, associated with aging and certain neurodegenerative disorders. In this context, S 17092 has been found to be a potent inhibitor of cerebral PEP activity in rodent brain tissue *in vitro* as well as and *in vivo* (24). S 17092 was selected from a new chemical series as a potent and specific prolyl endopeptidase inhibitor (40). Hence, S 17092 is expected to retard the degradation of a wide range of neuroactive peptides.

This article describes preclinical studies that demonstrate the successful use of S 17092 in ameliorating learning and memory deficits in various animal models. At doses that inhibit PEP, S 17092 is a potent cognitive enhancer. S 17092 was shown to inhibit chemically induced amnesia as well as spontaneous memory deficits. The promnesic effects of S 17092 were observed using a variety of memory tasks. These tasks permitted exploration of different types of mnesic functions, namely short-term or long-term, reference and working memory. Clinical studies demonstrated that this compound can be safely administered to humans. At doses, which inhibited PEP in healthy volunteers, S 17092 had central effects, as evidenced by EEG recording. Finally, considering residual improve-

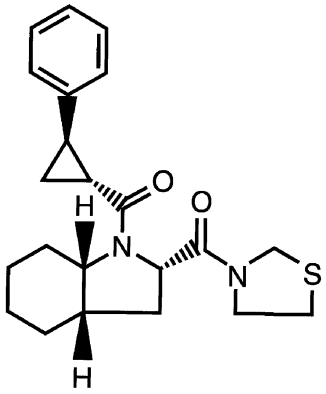

Fig. 1. The chemical structure of S 17092, (2S,3aS,7aS)-1-{[(R,R)-2-phenylcyclopropyl]carbonyl}-2-[(thiazolidin-3-yl)carbonyl]octahydro-*1H*-indole.

ments in delayed verbal memory task, S 17092 could have a favorable effect on memory and be useful in the treatment of cognitive disorders associated with cerebral aging.

CHEMISTRY

S 17092, (2S,3aS,7aS)-1{[(R,R)-2-phenylcyclopropyl]carbonyl}-2-[(thiazolidin-3-yl)-carbonyl]octahydro-*1H*-indole (Fig. 1), has been synthesized at the Institut de Recherches Servier (Suresnes, France). There are five asymmetric carbon atoms in the molecule of S 17092; three are of prime importance for a potent *in vivo* inhibition of PEP: R,R configuration on the side chain and S configuration for C_2 on the perhydroindole ring. The influence of stereochemistry at the ring junction carbon atoms 3a and 7a has not been evaluated. The CO-thiazolidine amide moiety is the chemical entity that reacts with the serine-544 of the enzyme. The S 17092 molecular formula is $C_{22}H_{28}N_2O_2S$ and its formula weight is 384.5. It has a melting point of 135°C; it is a white powder that is stable in a closed flask for 3 years.

ANIMAL PHARMACOKINETICS

Absorption

In rats or dogs the peak plasma concentration of S 17092 is reached soon after oral administration (first t_{max} in rats between 0.5 and 0.75 h and in dogs 0.4 h). In rats, the second peak was observed between 2 and 3 h (for 10, 50, and 200 mg/kg), and at 3 h (for 1000 mg/kg). The third peak was observed at 6 h (for 1000 mg/kg). These findings indicate a slow rate of absorption at high doses. Based on radioactive data ([^{14}C]-S 17092), the absorbed fraction in rats was at least 9 and 19% (for 10 and 200 mg/kg, respectively) and 28 and 17% in dogs after oral administration of the drug at 10 or 300 mg/kg, respectively. The absolute bioavailability of oral S 17092 was 15% in rats (for 10 and 50 mg/kg) and 4% in dogs (for 3 mg/kg).

Distribution

The volume of distribution of S 17092 at steady-state was 2.6 to 5.4 L/kg in rats (for 3 and 0.3 mg/kg i.v., respectively) and 3.4 L/kg in dogs (for 3 mg/kg). In rats *in vitro*, the plasma to blood concentration ratio for S 17092 was concentration-dependent: 0.62 at 50 ng/g and 1.0 at 500 ng/g, suggesting saturation of erythrocyte binding. *In vivo*, in dogs, the blood plasma concentration ratio of radioactivity ranged from 0.8 and 1.2 (i.v. and p.o. dosing). The protein binding of S 17092 was found to be dependent on drug concentration and higher in rats and dogs (≈15%) than in humans (≈7%).

Dose-Effect

Absolute bioavailability of oral S 17092 in dogs tended to increase with the increase in doses (7.2% at 300 mg/kg). This finding suggested some saturation of the first-pass metabolism in this species. After repeated oral dosing of S 17092 for 4 weeks, the dose-exposure relationship for S 17092 was proportional in rats (for 60 to 1500 mg/kg/d) and supra-proportional in dogs (48 to 60 fold factor for AUC at 20–500 mg/kg/d).

Time and Gender Effects

In rats (60 to 1500 mg/kg/d for 4 weeks), there was no relevant drug accumulation and exposure in females was consistently higher than in males. In the dog (20 to 500 mg/kg/d for 4 weeks), a decrease in exposure to S 17092 occurred only at the highest dose probably due to an induction process. There was no difference between genders in respect to drug exposure.

INHIBITORY ACTION ON PROLYL-ENDOPEPTIDASE (PEP) ACTIVITY

In vitro S 17092 produced a concentration-dependent inhibition of PEP activity in the cerebral cortex of Wistar rats; the minimal effective drug concentration was 0.1 nM. The 50% inhibitory concentration (IC_{50}) of S 17092 for PEP was 1.2 nM (24).

Following oral administration, S 17092 demonstrated a reproducible and potent dose-dependent inhibitory effect in the cerebral cortex of rodents. Indeed, at one hour after oral treatment, the 50% inhibitory dose (ID_{50}) for cortical PEP activity was 7.4 and 13.4 mg/kg in Wistar rat and NMRI mouse, respectively.

The PEP inhibitory effect of S 17092 (10 mg/kg p.o.) was of similar magnitude in young (3 months), elderly (20 months) and old (24 months) C57BL/6 mice. Studies on the time course of PEP inhibition induced by S 17092 (10 mg/kg p.o.) have shown that S 17092 was a long acting PEP inhibitor ($t_{1/2} > 9$ h). The PEP inhibitory effect of S 17092 in the cerebral cortex was of similar magnitude after chronic (15 days) and acute oral treatment of Wistar rats.

When assessed in cortex, striatum, hippocampus and brain stem of Wistar rats, the basal activity of PEP did not significantly differ between the four brain regions. Sixty minutes after acute oral administration of S 17092, the PEP activity was reduced in all

brain regions. At the lowest tested dose (1 mg/kg p.o.), a significant decrease was recorded only in the striatum (22.7%). At the highest tested dose (100 mg/kg p.o.), PEP inhibition was lower in the striatum (68.4%) and hippocampus (73.8%) than in brain stem (83.5%) or cortex (87%). The resulting ID_{50} were 7.3, 8.2, 4.8, and 4.4 mg/kg for cortex, hippocampus, striatum and brain stem, respectively.

Barelli et al. (1) conducted tests in *post-mortem* human cerebral tissues from persons who died without any history of neurological or psychiatric illness (1 male, 3 females; 65–82 years old; 7–59 h *post mortem*). S 17092 inhibited PEP activity in the following areas: putamen, caudate, external pallidum, internal pallidum, frontal cortex, temporal cortex, substantia nigra *pars compacta* and *pars reticulata* (Table 1). By contrast, S 17092 was unable to affect a series of other peptidases including aminopeptidases B and M, dipeptidylaminopeptidase IV, endopeptidases 3.4.24.11, 3.4.24.15, 3.4.24.16, calpaïns and angiotensin-converting enzyme. These results demonstrate selectivity of S 17092 for prolyl endopeptidase 3.4.21.26 (Table 2). Furthermore, we showed that the human embryonic kidney 293 cell line (HEK 293) displayed an intracellular PEP-like activity that

TABLE 1. Inhibitory concentrations (IC_{50}) of S 17092 for the enzymatic cleaving activity in various human brain regions

Brain Regions	IC_{50} ($\times 10^{-10}$ M)
Putamen	2.4
Caudate	1.9
Temporal cortex	2.5
Frontal cortex	5.2
Internal globus pallidus	2.3
External globus pallidus	2.5
Substantia nigra *pars reticulata*	3.0
Substantia nigra *pars compacta*	3.8

Adapted from Ref. 1.

TABLE 2. Inhibition of various peptidases by their specific inhibitor and S 17092

Peptidases	Specific inhibitor		S 17092 (1 µM)
Aminopeptidase B	arphamedine B, 0.5 µM	38.7 ± 0.9	104.0 ± 3.7
Aminopeptidase M	amastatin, 10 µM	30.2 ± 0.7	86.5 ± 3.0
Dipeptidylaminopeptidase IV	diprotin A, 0.1 mM	72.3 ± 5.6	97.1 ± 0.8
EC 3.4.24.15 (human)	CppAAYpAB, 1 µM	29.8 ± 5.9	112.7 ± 2.6
EC 3.4.24.15 (rat)	CppAAYpAB, 1 µM	26.9 ± 2.3	95.4 ± 1.1
EC 3.4.24.16 (human)	Pro-Ile, 5 mM	19.1 ± 2.6	107.7 ± 2.4
EC 3.4.24.16 (rat)	Pro-Ile, 5 mM	27.0 ± 2	117.2 ± 12.2
ACE	captopril, 1 µM	3.9 ± 0.1	93.9 ± 0.1
Calpaïn(s)	–	–	93.3 ± 5.6

Values (percent of control) are means for 3 determinations ± S.E.M.
Adapted from Ref. 1.

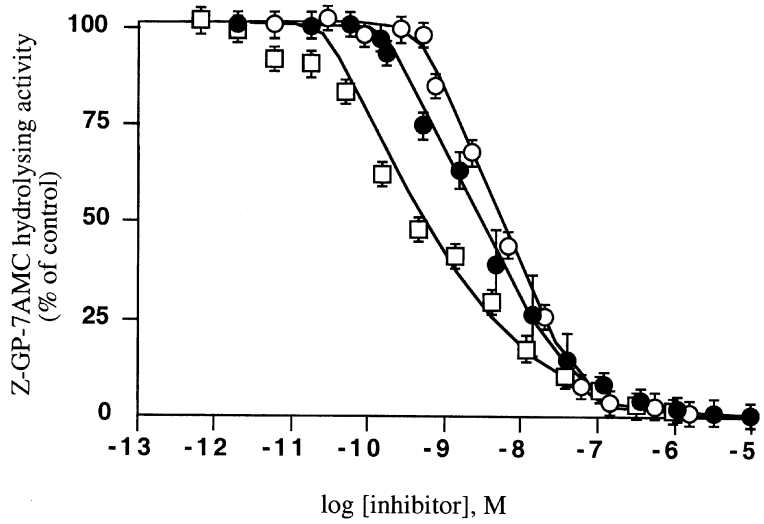

Fig. 2. Inhibitory effect of S 17092 and other endopeptidase inhibitors on purified human brain proline endopeptidase. Z-Gly-Pro-7AMC was incubated at 37°C in the absence (control) or in the presence of increasing concentrations of Z-Pro-Prolinal (○), S 17092 (●) and Fmoc-Ala-Pro-CN (□). Values are expressed as the percent of control and are means ± S.E.M. of 3 to 4 determinations. Reproduced from Ref. 1 with permission.

was blocked by S 17092. This finding indicates that this inhibitor penetrated HEK 293 cells and could affect intracellular human PEP (Fig. 2). Of course, given the abundance of known peptidases, it cannot be ruled out that S 17092 may affect other peptidases as well.

INTERACTION OF S 17092 WITH SUBSTANCE P (SP)

Studies were designed to determine whether S 17092 could interact with brain SP after oral administration of PEP inhibitory doses in rodents. The effects of acute or chronic treatments with S 17092 on striatal SP-like immunoreactivity (SSPLI) levels in the Wistar rat were examined using a standard RIA kit (Peninsula Laboratories). Rats were treated orally with S 17092 (1, 3, 10, or 30 mg/kg) acutely or over a 7 day period (once a day). Acute oral treatment with S 17092 (1 to 30 mg/kg p.o.) tends to increase striatal SSPLI levels (+19%, +32%, and +43% at 1, 3, and 10 mg/kg, respectively) compared with control values. At the highest tested dose of S 17092 (30 mg/kg) a significant increase of striatal SP level (+80%; $P < 0.01$) was observed. Chronic oral treatment with S 17092 also increased striatal SP immunoreactivity levels. This effect was not dose-dependent since a significant effect was observed only at 10 and 30 mg/kg (+56%, $P < 0.01$ and +55%, $P < 0.01$, respectively).

Behavioral interactions of S 17092 with SP were also studied using a model of grooming behavior in NMRI mouse, specifically mediated by the striatonigral neurokininergic pathway (20,47,49). S 17092 was administered orally at 3, 10, 30, or 100 mg/kg 60 min prior to the administration of SP (0.5, 1, 2, 4, 8 µg/mouse) and the duration of grooming

behavior was measured over a 300 sec period. By i.c.v. administration SP induced a dose related increase in the duration of grooming behavior (17 ± 6; 90 ± 17; 106 ± 14; 131 ± 17; and 167 ± 23 sec respectively, $P < 0.001$ for the last four doses) *versus* the control group (1.1 sec). At 10 mg/kg S 17092 induced a potentiation of +240% ($P < 0.05$), while at 30 and 100 mg/kg, potentiations of +396% and +388% ($P < 0.001$), were observed (26). Thus, S 17092 potentiated SP-induced grooming behavior in NMRI mice immediately after the injection of SP into the right ventricle.

At PEP inhibitory doses S 17092 increased the SSPLI. The increase of SSPLI probably reflected an increase of SP levels in the striatum, the most SP-enriched brain area in neurokininergic neurons. Moreover, our behavioral data on SP-induced grooming in mouse clearly indicated that S 17092, at PEP inhibitory doses, could enhance the SP neuromodulation in subcortical regions such as the striatonigral pathway. This is likely due to the fact that SP-induced grooming in rodents have been shown to be specifically mediated by this neuronal pathway (20,47,49). Collectively, these results suggest that the promnesic activities of S 17092 could be partially related to a facilitatory effect on brain SP neuromodulation via PEP inhibition. However, there is no clear evidence indicating that SP modulation is more or less important than that of any other peptides affected by S 17092 to explain its promnesic effects.

MEMORY ENHANCING EFFECTS

Effects on Drug-Induced Learning and Memory Impairment in Rodents

One-trial passive avoidance is a test of long-term memory in rodents. Rats have to learn and recall the location of an inescapable electric foot-shock (0.6 mA, 400 V) in a two-compartment box. Two paradigms were used, one with S 17092 treatment 60 min before acquisition (session 1) and test (session 2) and the other by pretreatment with S 17092 120 min before session 1 and session 2. Rats were rendered amnesic by cholinergic neurotransmission blockade with scopolamine 0.3 mg/kg s.c., 30 min prior to the first session. When treated rats received S 17092 (0.01–30 mg/kg i.p.) 60 min before session 1 then 24 h later 60 min before session 2, the drug dose-dependently increased the retention time, alleviating scopolamine-induced amnesia. The lowest dose demonstrating a robust effect, i.e. 73% inhibition of scopolamine amnesia ($P < 0.001$), was 3 mg/kg. The effect was maximal (93% inhibition, $P < 0.001$) with 10 mg/kg, while at 30 mg/kg, 91% inhibition ($P < 0.001$) was noted. Under these experimental conditions, the range of active doses was the same as for PEP inhibition.

In a second experiment rats received S 17092, at 0.3, 1, and 3 mg/kg i.p., 120 min before session 1 and then 24 h later 120 min before session 2. Controls received vehicle under identical conditions. S 17092, at 1 and 3 mg/kg i.p., significantly increased retention time: scopolamine amnesia was inhibited by 79% ($P < 0.01$) and 94% ($P < 0.01$), respectively. The maximum effect using 10 mg/kg with the 60 min pretest dosing interval was achieved using 3 mg/kg in the 120 min tests, suggesting that brain neuropeptide turnover may have been increased by increasing the time interval between the administration of S 17092 and the test.

A spatial discrimination task was chosen in order to explore spatial reference memory in the C57BL/6 mouse using a model based on scopolamine-induced amnesia (26). Young

(3–5 months old) male C57BL/6 mice were used in the spatial discrimination test that was conducted by using an elevated opened Y-maze (3 consecutive daily sessions in an open Y-maze, 10 trials *per* day). Beginning from a variable start area animals had to learn and remember which arm of the maze contained food. First, a learning trial was conducted as follows: after a mouse was placed on the start area of an unbaited arm, the 3 doors of the Y-maze were opened in order to allow the animals to enter the goal area of one of the two opposing arms. The doors were closed and the mouse stayed in the chosen arm for 20 sec. Then, by a rotation of the maze, the animal returned to a start area for a 40 sec inter-trial interval until the next trial. Entry into the baited arm followed by reward eating was noted as a correct response for the trial. The number of correct choices over 10 trials *per* session was calculated for each animal. In order to explore the effect of orally administered S 17092 (10 mg/kg) on scopolamine-induced amnesia, the compound or vehicle were administered twice daily over a 7 day period before the spatial memory experiment, then 60 min prior to the training sessions. An amnesic group was treated with scopolamine (0.3 mg/kg i.p., 30 min before each training session) and received the vehicle instead of S 17092. A treated group received S 17092 by oral administration and received scopolamine under the conditions mentioned above.

Scopolamine (0.3 mg/kg s.c., 30 min before each test session) induced a learning deficit: 50, 63, and 78% correct responses in sessions 1, 2, and 3, respectively, in vehicle controls *vs* 44, 43, and 56%, respectively, in the scopolamine-treated group ($P < 0.001$). Repeated oral pretreatment with S 17092 (10 mg/kg for 7 days b.i.d.; then once daily 60 min before testing) alleviated the scopolamine-induced memory deficit: correct responses in the S 17092 group were 55, 58, and 74% in sessions 1, 2, and 3, respectively. Performance in S 17092-treated animals was similar to that in non-amnesic controls. Thus, S 17092 improved learning and memory performances in young amnesic C57BL/6 mice. Interestingly, the promnesic effect of S 17092 was observed at 10 mg/kg p.o. and at this dose the drug clearly inhibited brain PEP activity by more than 50% (24). S 17092 facilitated learning function related to reference (spatial discrimination) memory. These results supported the evidence that the cognitive properties of S 17092 are intimately related to the inhibition of PEP activity in the brain. Furthermore, experimental studies have shown that spatial memory processing could depend on cholinergic neurotransmission originating, notably, from basal brain nuclei (53). SP positively modulates the cholinergic activity of both the basalo-cortical and septo-hippocampal pathways (11,46) and impairment of neurokininergic pathways by specific subcortical lesions could induce memory deficits (12). All these results indicate that acetylcholine and SP are associated with cognitive functions, and it could be of interest to investigate the impact of S 17092 upon cholinergic neurotransmission.

Effects on Drug-Induced Learning and Memory Impairments in Monkeys

Non-demented Parkinson's disease (PD) patients exhibit a number of neuropsychological deficits (23,27,37), some of which consist of problems in spatial working memory (38). The relationship between the dopaminergic defect and cognitive deficits in PD is not clear (6,14,22). Cognitive deficits may arise from dysfunction of several cortical and subcortical neurotransmitter systems, including neuropeptides. Deficits in levels of various neuropeptides may contribute to Parkinsonian-like symptoms (30). For example, the

levels of substance P, which has been implicated in learning and memory (17) are also depleted in the striatum of monkeys made Parkinsonian by exposure to the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In particular, substance P binding and SP-like immunoreactivity (SP-LI) have been reported to be significantly reduced in the parietal cortex and *nucleus basalis* of Meynert. The levels are also reduced in the *substantia nigra* and the internal *globus pallidus* in PD brain (48).

It can, therefore, be assumed that one therapeutic approach to the cognitive deficits associated with Parkinsonism is to enhance endogenous neuropeptide levels. Given the proline endopeptidase inhibitor properties of S 17092 and the fact that this compound can reverse memory deficits and increase SSPLI in rodents, a study was aimed to assess the extent to which S 17092 might improve cognition in chronic low dose MPTP-treated monkeys (43). MPTP-treated monkeys provide a model of early-stage Parkinsonism previously shown to exhibit cognitive deficits similar to those detected in PD patients (44,45).

In this study, four adult male *Macaca fascicularis* monkeys were trained to perform variable delayed response (VDR), delayed alternation (DA), delayed matching-to-sample (DMS) and visual discrimination tasks. MPTP was administered intravenously two to three times *per* week to each animal (0.075 mg/kg) until stable baseline task performance was achieved. By the time drug testing commenced, animals had received cumulative MPTP doses ranging between 0.38 and 4.8 mg over periods of 27 to 63 days. Pharmacological testing commenced after animals consistently showed at least a 15% performance deficit on each task for approximately 2 months. S 17092 was administered for 7 days prior to formal testing on cognitive tasks. On the day of testing, cognitive performance was assessed 30 min after the last administration of S 17092. The following range of doses, 1.0, 3.0, and 10.0 mg/kg, was used, with at least 2 weeks of washout prior to starting 7 day dosing with a new dose of S 17092.

Prior to initiation of MPTP exposure, the animals had a mean baseline performance of 81.2% correct responses on the VDR task. In normal animals performance at shorter duration delays (2, 5, and 10 sec) differed significantly ($P < 0.0001$) from performance at longer duration delays (20, 30, 45, and 60 sec, Fig. 3A). Normal performance declined with increasingly longer duration of delays. After chronic MPTP exposure, overall performance on the VDR task deteriorated to 60.9% correct responses. In contrast to the normal performance of this task, monkeys exhibited a delay-independent performance deficit after chronic exposure to MPTP. Monkeys were now almost as likely to perform poorly on shorter as on longer delay trials. The effect of MPTP exposure on performance at different delays was significant [$F(6,132) = 3.5$, $P < 0.005$]. Pairwise post-hoc comparisons showed that performance changed significantly after MPTP exposure at 2, 5, 10 ($P < 0.01$), and 20 sec ($P < 0.05$) delays, while performance at longer delays was unaffected by the MPTP exposure.

Figure 3A shows that S 17092 caused a dose-dependent improvement in performance of the VDR task. When the results from each animal were analyzed together, S 17092, 3 mg/kg, significantly improved VDR performance (76.9% correct responses, $P < 0.01$ vs. non-drug post-MPTP performance) but not at 1 or 10 mg/kg doses. After administration of S 17092 at 3 mg/kg, VDR performance tended to revert back to a normal delay-dependent pattern of response. At the 3mg/kg dose, performance significantly improved on shorter ($P < 0.01$ for the 2, 5, and 10 sec delay) but not on longer delay trials. Improved performance on shorter delay trials was not seen with either the 1 or 10 mg/kg doses.

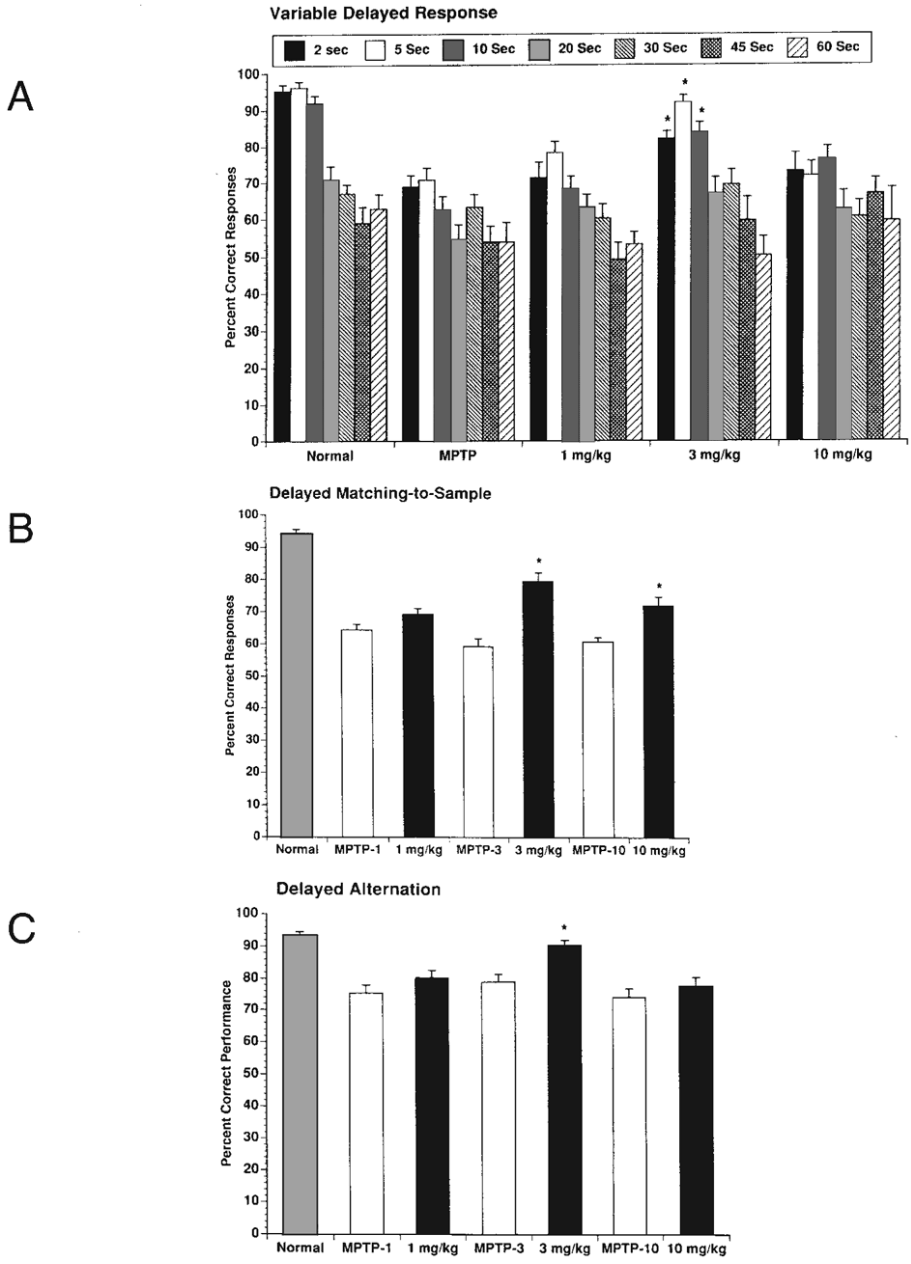

Fig. 3. Effects of S 17092 on cognition in MPTP-treated monkeys. In control group (normal), there was a delay-dependent decrease in performance on the variable delayed response task. After MPTP exposure, trials at all delays were performed poorly. The 3 mg/kg dose of S 17092, after a 7 day exposure to the drug, caused significant improvement in performance of 2, 5, and 10 sec delay trials (**A**). Performance of the delayed matching-to sample task (**B**) and delayed alternation task (**C**) were significantly impaired after MPTP exposure. After a 7 day exposure to S 17092, the drug significantly improved the delayed matching to sample and delayed alternation performance at the 3 mg/kg dose, and only the delayed matching-to sample at 10 mg/kg. *$P < 0.01$ vs. non-drug control trials. MPTP-1, MPTP-3, MPTP-10: control trials (including washout) associated with testing 1, 3, and 10 mg/kg S 17092, respectively.

While the DMS task was performed at a level of 94.4% correct responses at baseline, performance significantly deteriorated to 62.2% correct responses ($P < 0.01$) after chronic MPTP exposure (Fig. 3B). Significant improvements in DMS performance were observed after S 17092 administration at either 3 or 10 mg/kg doses ($P < 0.01$). While no significant effect was observed at the lowest dose, performance improved from 59.3 to 79.7% at 3 mg/kg dose, and from 60.9 to 72.2% at 10 mg/kg dose of S 17092.

The DA task was performed at a level of 93.5% correct responses prior to MPTP administration, whereas performance significantly deteriorated to 76.2% correct responses ($P < 0.01$) in MPTP-treated monkeys (Fig. 3C). Here again, S 17092 administration significantly improved DA performance at the 3 mg/kg dose ($P < 0.01$), from 79% at baseline to 90.6% after 7-days or longer treatments.

Monkeys, treated chronically with MPTP showed significant cognitive impairments; in these animals oral S 17092 improved, at least partially, the performance of cognitive tasks (VDR, DMS, DA) in a dose-dependent manner. The most efficacious dose of S 17092 was 3 mg/kg. The 1 mg/kg dose was ineffective in all tests whereas the 10 mg/kg dose significantly improved only DMS performance.

S 17092 did not significantly improve performance on long duration delay trials (20 sec) in the VDR task. Interestingly, the normal limit of working memory in these monkeys was 10 sec. That is, under normal conditions, task performance significantly deteriorated with delays of 20 sec or longer. Thus, in these animals S 17092 appeared to improve memory within its normal limits. It may have also enhanced attention, as suggested by the improved performance at short duration delays. This study was not, however, designed to separate out attentional from memory effects on task performance and additional work is necessary to determine whether S 17092 preferentially affects attention or memory functions.

Chronic oral pretreatment with S 17092 (7 days prior to administration of the test dose of drug and cognitive testing) was necessary in order to observe cognition-enhancing effects. Thus, during the first 3 days of pre-treatment period, S 17092 did not improve any of the cognitive functions measured (data not shown). These data support findings that by chronic oral administration S 17092 is more effective than by acute administration in increasing striatal substance P-like immunoreactivity (see previous section). They also strengthen the hypothesis that the cognition enhancing properties of S 17092 are related to enhanced neuropeptide activities in the brain. However, the neurochemical deficits underlying the cognitive dysfunction in chronic MPTP-treated monkeys and the status of brain neuropeptides in these animals are unknown. Proline endopeptidase inhibitors may improve cognitive function in MPTP treated monkeys by indirectly promoting function of a variety of neurotransmitter systems (including norepinephrine and acetylcholine) and/or by direct neuropeptide effects in the basal ganglia and other subcortical and cortical regions.

While there is no evidence of motor deficit improvement with S 17092, its relative effectiveness as a cognition enhancing agent in PD animal model makes it a potential candidate for the treatment of mnesic deficit associated with PD.

Effect on Social Memory

Social recognition in the rat is a form of short-term memory and can be inferred by measuring the amount of time spent in investigating a juvenile. The difference in the duration of social investigation of the same juvenile, presented at different time intervals, is one index of the rat's ability to recognize this particular juvenile. With a 120 min long re-

tention interval, duration of investigation did not differ significantly between first and second exposure; control rats did not remember the juvenile.

By acute administration S 17092 (1, 3, or 10 mg/kg p.o.) had no effect when given post-learning (i.e. after the first exposure), nor when given at 60 min before the first exposure. On the other hand, repeated dosing with S 17092 (1, 3, 10, 30 mg/kg p.o. for 7 days), at 60 min prior to first exposure, decreased juvenile investigation time. This effect was significant ($P < 0.05$) at 10 mg/kg (–24.1 sec ± 5.7 *versus* 5.73 sec ± 7.03 for the vehicle), but not at 30 mg/kg (–18.5 ± 6.8 sec).

Effects on Learning and Memory in Aged Mice

The cognition enhancing properties of S 17092 were also determined with age-associated memory deficits in the C57BL/6 mouse by using the delayed alternation task, an expression of spatial working memory in rodents. In this test, mice were trained to find a food pellet in a Y-maze in the arm alternate to that previously visited. Young animals performed well and were successful in 60 to 70% of the trials. Aged animals (21–22 months) showed working memory deficits and a success rate approximating 50% (chance).

The spatial discrimination test was conducted during 4 consecutive days and 2 complementary daily sessions (with 2 days of training interruption between the 4th and 5th session) during which mice were submitted to 11 successive trials. During the learning session mice were placed on the start area, the 3 doors of the Y-maze were opened in order to allow the animals to enter the goal area of one of the 2 opposite arms and collect the food reward. Then, by a rotation of the maze, the animal returned to the start area for a 20 sec inter-trial interval. As the food pellet was always to be found in the arm opposite to the one previously visited, the animals had to alternate the arm choices in order to be rewarded. The percentage alternation over 10 trials *per* session was calculated.

Oral pretreatment with S 17092 (10 mg/kg for 7 days) before learning, and again 1 h before each daily memory test, inhibited age-associated learning impairment. The effect became apparent when the number of sessions increased. The cognition enhancing effect of S 17092 was robust. A similar value of alternation was obtained after two days of training interruption, with correct responses of 45 and 70% in control and treated animals, respectively, in session 5 and 46 and 65%, respectively, in session 6 (see Fig. 4). The results indicate that S 17092 improves learning and working memory performances in aged C57BL/6 mice, an effect that was still observed for 2 days following discontinuation of the drug (26).

Memory enhancing properties of S 17092 have also been studied in mice with age-associated deficits using a new model of selective alteration of declarative memory observed in aged humans. Animals were separately assigned to six adjacent arms; out of them, three served as positive (baited) arms, and the remaining three as negative (not baited) arms. These six arms could be grouped into three pairs of adjacent arms with opposing valence. The experiment consisted of a two-stage paradigm. The only parameter that was changed from stage one to stage two was the way in which the arms were presented. In Stage 1, the mice learned to discriminate between the valence of three positive and three negative arms in the radial maze with each of the arms presented one at a time, i.e. successive go-no-go discrimination. Each daily session consisted of 24 trials with four presentations of each of the six arms. In Stage 2, the discrimination problems were between the same six arms as those in Stage 1 and the reward valence of each arm also remained unchanged. However,

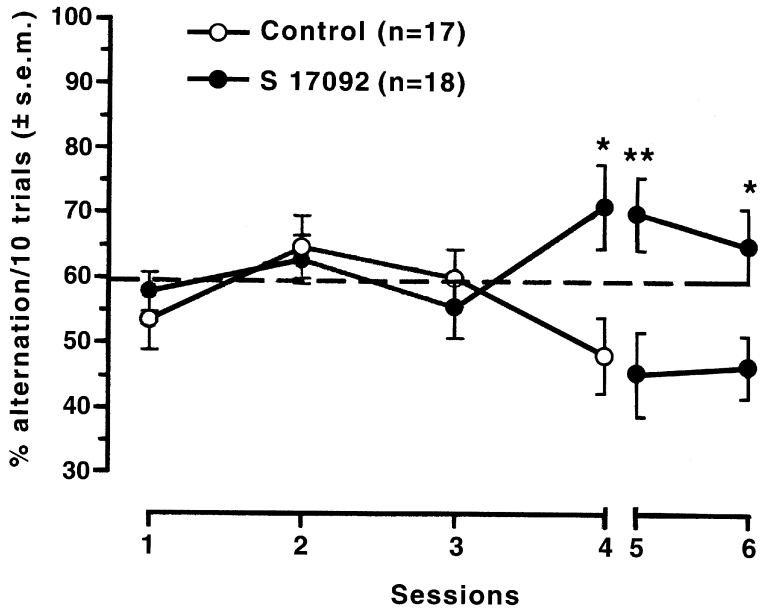

Fig. 4. Effect of S 17092 (10 mg/kg, p.o.) on age-associated memory deficit in sequential reinforced alternation task in 21 to 22 months old C57b1 mice. Animals were tested (inter-trial interval: 20 sec) during four consecutive sessions then after 72 h interruption during 2 consecutive daily sessions. Chronic oral pretreatment for 7 days with vehicle or S 17092 then, once daily until the end of the experiment (one hour before training during the memory test). $*P < 0.05$, $**P < 0.01$, S 17092 *vs*. vehicle.

their presentation was modified, namely, the six arms were now grouped into three pairs. In each trial, the subject was confronted with access to two adjacent arms with opposing valence. Each daily session consisted of 20 consecutive trials comprising of alternating presentations of pairs. In such experimental conditions, Marighetto et al. (28) have demonstrated that aged, but not young, mice were unable to translate their preference for the positive arm shown in Stage 1 into a correct choice in Stage 2. Such inflexibility of mnemonic expression represents a specific alteration in the ability to compare and contrast information originating from separate sources and this ability has been considered as a cardinal characteristic of human declarative memory (5).

Aged (25 months old) mice received orally, once daily either 10 mg/kg of S 17092 or vehicle for one week before the beginning of behavioral training and then one hour before each training session. Results showed that the performance of the vehicle and S 17092 groups were not different in Stage 1. Conversely, the performance between the two groups was significantly different in Stage 2. Chronic treatment of S 17092 significantly enhanced choice accuracy (percent correct analysis) relative to controls in Stage 2. The vehicle mice behaved as if they were naive (54% of correct choices), whereas the performance of S 17092 animals was clearly above chance (69%, Fig. 5). S 17092 had no effect on the acquisition speed of go-no-go discrimination as reflected on the no-go/go ratio of arm-entry latencies in Stage 1. S 17092-1 also failed to affect the run-time differential in both Stage 1 and Stage 2.

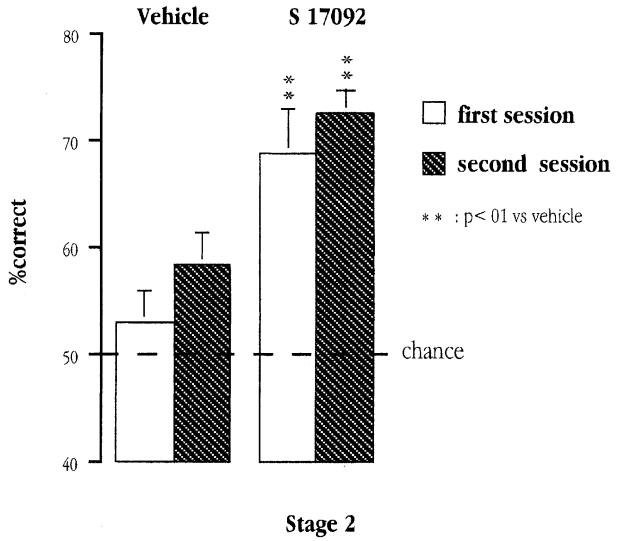

Fig. 5. Effects of S 17092, 10 mg/kg/day p.o., for 7 days (last treatment at 1 h before training session) on choice accuracy of aged mice. Mean percent correct choices on each of the two sessions of Stage 2 for groups of mice treated with a vehicle or S 17092.

This study demonstrated that S 17092 has a beneficial effect on the selective deficit observed in aged mice. Vehicle-treated aged mice successfully discriminate between arms of opposing valence when these are presented one by one, in a successive go-no-go procedure. However, they failed to translate their preference for the positive arms to correct choices in subsequent simultaneous discriminations (Stage 2). S 17092 also decreased response latency in the initial sessions of Stage 1 and increased the overall run time along the arms during Stage 2.

The deficit observed in control aged mice (28) was specific for one form of memory expression, acquired spatial discrimination. Successful discrimination between arms of opposing valence was obtained when these arms were presented one at a time in a successive go-no-go procedure. Yet, such demonstrable acquired knowledge failed to guide the aged mice towards the positive arm when confronted with an explicit choice between two arms. The mnemonic inflexibility seen in the aged mice could be linked to an alteration in the relational processing of incoming/stored information. Consequently, they could perform normally when the test situations encourage the storage and use of separate representations of individually experienced items (successive go-no-go discriminations of arms). However, they failed in test situations that emphasize a judgement of the relative valence between two arms (which involves a comparison between two separate representations).

S 17092 enhanced choice accuracy in Stage 2 without inducing major changes on other performance indices in Stage 1 or in Stage 2. This led to the conclusion that this drug specifically improves flexible deployment of passed experiences. Within the theoretical framework of relational memory, this flexible deployment of memories can be seen as a result of the acquisition of complex associations among separately experienced items. This inter-

pretation is further supported by the fact that the beneficial effect of S 17092 was readily visible and statistically significant in the initial trials of Stage 2.

The different expressions of 2-arm discriminations revealed by the effects of aging and S17092 are reminiscent of a dissociation of memory systems revealed through a comparative analysis among the effects of hippocampal, striatal, and amygdala lesions (31). The "relational" memory system sustains the ability to compare information that originates from separate sources. Two-choice discrimination performance is impaired by aging but enhanced by S 17092. Taken together, these results conferred to S 17092 a wide promnesic profile concordant with physiological clinical conditions observed in aged patients and/or in the course of neurodegenerative diseases. Therefore, S 17092 could be envisaged as a potential therapeutic agent for the treatment of cognitive disorders associated with aging.

PHARMACOKINETICS AND PHARMACODYNAMICS AFTER SINGLE AND REPEATED ADMINISTRATION IN HEALTHY YOUNG AND ELDERLY VOLUNTEERS

The tolerability, pharmacodynamics and pharmacokinetics of S 17092 have been assessed following single dose administration in healthy young volunteers, and following single and repeated administration to elderly healthy volunteers (36).

A phase I randomized double-blind, placebo controlled study was performed to evaluate safety, tolerance, pharmacokinetics and neuropsychological effects of 12 doses of S 17092, after a single oral administration in 36 young male healthy volunteers ($n = 9$ *per* group). Neither serious nor severe adverse events were observed. Most adverse events were of mild intensity. Five adverse events were of a moderate intensity (3 headache, a hand trauma and hematoma) and were considered to have a non-obvious relation to the treatment. Twenty-one of the 104 adverse events have a possible relation to the treatment; almost all of them were complaints related to the central nervous systems (9 headache, 3 impaired concentration, 2 weakness and 2 drowsiness, dizziness, a feeling of pressure on the eyes, increased sensitivity to light and unsteady feeling). No clinically relevant abnormalities related to the treatment were observed in the volunteers during telemetric ECG monitoring.

Pharmacokinetics data showed that plasma concentration-times profiles of S 17092-1, after single oral administration (5, 15, 50, 100, 200, 400, 600, 800, 1000, 1300, 1600, and 2000 mg) were characterized by three peaks: one within 1 h, one after about 4 h, one at 12 or 24 h. C_{max} was often observed between 0.75 and 2.5 h after treatment. Plasma concentrations of S 17092 decreased slowly and in a manner parallel with mean apparent terminal half-lives ranging from 6 to 17 h.

Pharmacodynamics data showed that S 17092 had no significant effect on simple reaction time in Cognitive Drug Reaction Tests, and, more precisely, attentional tasks. Measurements of choice reaction time were significantly shorter with S 17092 (200 and 1600 mg, $P < 0.05$) than with placebo. On the other hand, no clear effects were shown on the vigilance task.

In the preliminary study, S 17092 exerted no clear effect upon the sensitivity index for numeric working memory. For speed on this task, there was a trend for an interaction between dose and time. There was no consistent effect on the spatial working memory task, the only significant effect being a decline in sensitivity with 1300 mg ($P < 0.05$). The 1300 mg dose had an overall beneficial effect on both immediate and delayed word recall tests ($P < 0.05$). At 1600 mg S 17092 produced an overall benefit in word recognition test ($P < 0.05$). There was a significant effect on the speed of response in the word recognition task ($P < 0.05$). The 5 mg dose showed a trend for improved speed at 2 h. Significant benefits were also seen for 100, 400, and 1300 mg.

The second phase I trial was a double-blind placebo-controlled, randomized, rising dose study, with interim evaluation after each single dose and between consecutive groups. This study was performed in healthy elderly volunteers (55–70 years old) with single dose (D1) and repeated dose (D7 to D14) (36 volunteers, 9 *per* group). S 17092 was used at 100, 400, 800, or 1200 mg.

Neither serious nor severe adverse events were observed during this study. Three adverse events were considered to be possibly related to the treatment: nausea (1 subject receiving 400 mg and one receiving 1200 mg) and dizziness (1 subject receiving 1200 mg). Considering vital signs, ECG, respiration rate and oral body temperature, no clinically relevant changes were observed after any of the doses used.

Plasma concentrations of S 17092 were quantified using specific and sensitive high-performance liquid chromatography and tandem mass spectrometry (MS/MS). The pharmacokinetic parameters for S 17092 are summarized in Table 3. The pharmacokinetics data showed that 2 peaks characterized the concentration times profiles. C_{max} was observed between 0.75 and 5 h. The apparent half-life at D1 and D14 were 14 to 31 h and 15–18 h respectively. Stability of C_{min} values between the first day of repeated administration, D7, and D14 showed that steady-state has been reached.

The effect of S 17092 on the PEP activity was assessed in blood samples collected prior to treatment and at 0.5, 1, 2, 6, and 12 h after treatment on days 1 and 14. Mean values of the minimal PEP activity and of the PEP activity at 12 h were significantly lower than in subjects receiving placebo. Furthermore, the mean values showed a clear dose dependent reduction of PEP activity; this effect was more pronounced at 12 h after treatment.

After single dose administration (Day 1), minimum PEP activity and PEP activity at 12 h after S 17092 were significantly lower ($P < 0.05$) than after placebo, except for the 12 h value after a single 100 mg dose of S 17092. After multiple dose administration no significant effect was found in the 100 and 400 mg dose groups. On the other hand, for the 800 mg dose group, both the minimal PEP activity and the PEP activity at 12 h after treatment were significantly different from PEP activity in placebo treated subjects ($P < 0.05$; Fig. 6). No clear difference was observed for the mean PEP activity at various time points on Day 1 and Day 14 for the active dose levels. However, the PEP activity in plasma following placebo administration was lower on Day 14 compared to Day 1, thereby explaining the observation that statistical significance of PEP inhibition at the lower dose levels was lost at Day 14. PEP activity in plasma decreased rapidly after oral administration of S 17092 showing a mean maximal decrease in activity at 0.5 to 2 h after treatment on Day 1 as well as on Day 14.

The central effectiveness of S 17092 was assessed by means of quantitative EEG as an index of drug penetration into the human brain; this technique also provided information on onset, duration and dose dependency of S 17092 effects. The EEG recordings were

TABLE 3. Mean pharmacokinetic parameters of S 17092 after single (day 1) and repeated (day 8 to 14) oral administration of S 17092 to healthy elderly volunteers

Day	Parameters	Mean Values for Doses of S 17092			
		100 mg	400 mg	800 mg	1200 mg
1	C_{max} (ng/mL)	31.4	68.6	152	292
	t_{max} (h) median	5	1	1.5	2.75
	$AUC_{0-24\,h}$ (ng/mL · h)	534	862	2485	4510
	$t_{1/2}$ (h)	14.2	31.1	8.97	14.6
	AUC (ng/mL · h)	939	1307	3705	6424
8	C_{min} (ng/mL)	BLQ	BLQ	BLQ	BLQ
9	C_{min} (ng/mL)	17.9	30.6	96.6	159
10	C_{min} (ng/mL)	23.2	37.3	104	150
11	C_{min} (ng/mL)	20.5	52.3	93.9	131
12	C_{min} (ng/mL)	24.9	47.5	106	132
13	C_{min} (ng/mL)	25.9	47.5	81.6	151
14	C_{min} (ng/mL)	26.8	45.5	71.9	114
	C_{max} (ng/mL)	56.2	111	158	286
	t_{max} (h) median	1	0.71	0.75	0.75
	$t_{1/2}$ (h)	15.9	18.1	6.8	13
	AUC_{24} (ng/mL · h)	820	1420	2534	4043
	Rac	1.36	1.8	0.98	0.94

BLQ: below the limit of quantitation (1 ng/mL). Rac-accumulation ratio from Ref. 36 with permission from Blackwell Science.

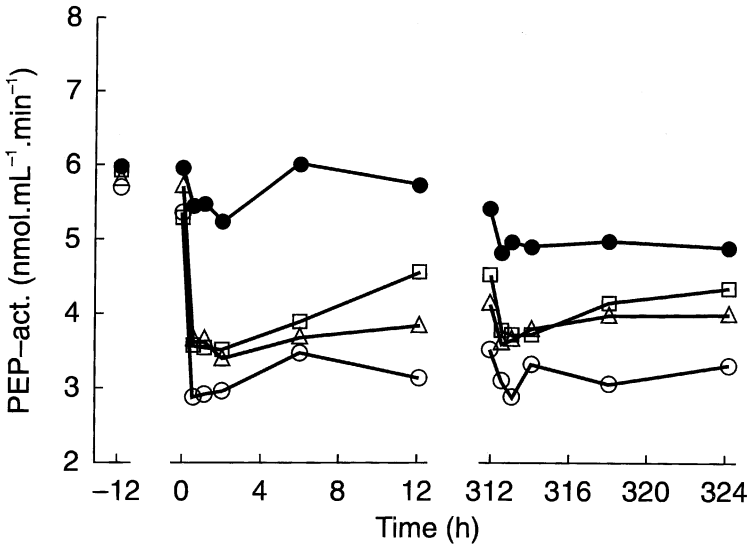

Fig. 6. Effects of S 17092 on plasma PEP in elderly healthy volunteers on day 1 and day 14 of treatment. Placebo (●), 100 (□), 400 (△), and 800 mg (○) of S 17092. From Ref. 36 with permission from Blackwell Science.

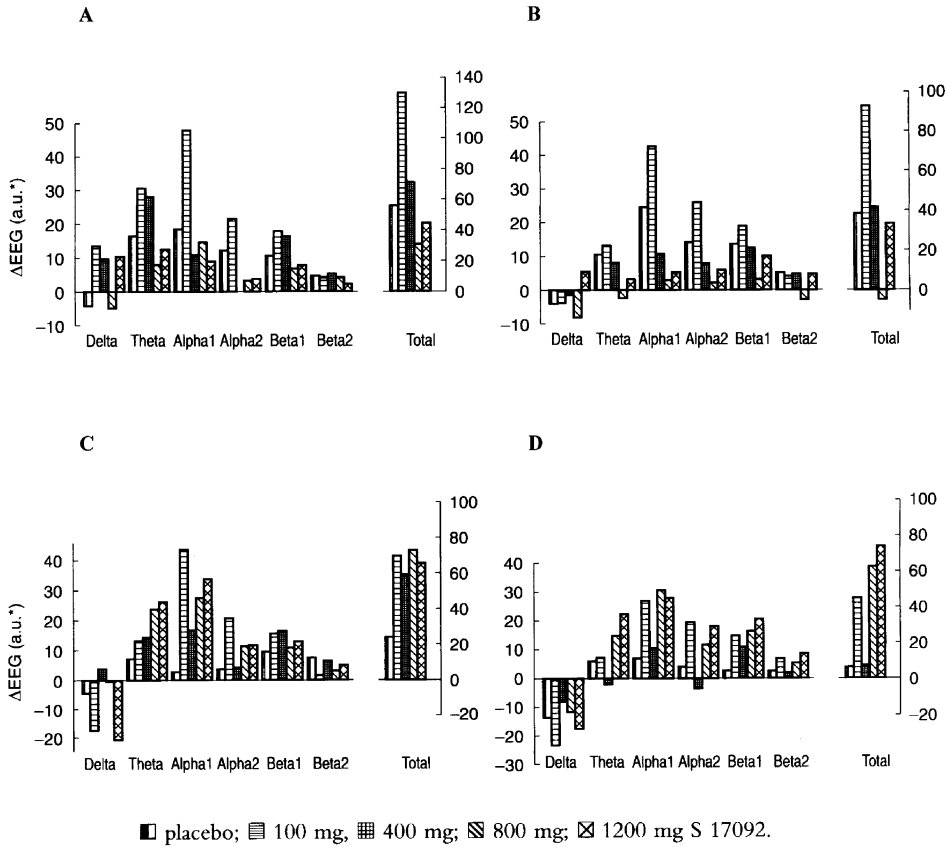

Fig. 7. Effects of S 17092 on EEG absolute power in human volunteers. The effects of single doses of S 17092 (100, 400, 800, or 1200 mg) on day 1 (**A**-**B**) and day 14 (**C**-**D**) of treatment. **A** & **C**: 4 hours after treatment; **B** & **D**: 8 hours after treatment.

carried out on Day 1 and Day 14 in each group prior to the morning dose and again at 4 and 8 h after treatment (Fig. 7). Electrical activity was processed with a Grass Neurodata Model 12 amplifier system. The EEG was recorded from four amplifiers with electrodes positioned on left and right occipital sites and on left and right frontal scalp regions using linked-earlobe electrodes for monopolar reference recordings. One electrode was placed on the mid-forehead to serve as ground and two additional electrodes were positioned over the supraorbital ridge and external canthus of one eye to monitor vertical and horizontal electro-oculographic artifacts induced by eye movements and blinks. The following spectral EEG parameters were analyzed: absolute power for delta, theta, alpha-l, alpha-2, beta-l, beta-2 and total band power; relative power for the same spectral bands expressed as a percentage of the entire total band power.

S 17092 appears to be a centrally active substance as it induces statistically significant alterations in the EEG as compared to placebo. A 100 mg dose of S 17092 was needed on Day 1 to get an acute central effect, an effect on EEG at both anterior and posterior scalp recording sites. On Day 14, central effects were limited to posterior sites. The acute

central effects of S 17092, 100 mg, were similar at 4 and 8 h after treatment. S 17092 at 100 mg exerts similar central profile changes (i.e. alpha wave increases) when administered as a single dose on either Day 1 or Day 14. At 1200 mg, however, S 17092 increased (relative to placebo) alpha/theta ratios on Day 1 but not on Day 14. No significant lasting CNS effects were produced by S 17092 when administered repeatedly over 7 days. At 100 mg S 17092 induces EEG changes which can be tentatively described as "vigilance promoting" in that alpha wave was augmented following acute dose of the drug.

Cognitive functions were assessed by the CDR computerized cognitive assessment system (Cognitive Drug Research, UK) and conducted by the subjects on study day 1, day 8 and day 13 prior to treatment and 2 and 6 h after treatment. The tests were administered in the following order: Immediate Word Recall, Picture Presentation, Simple Reaction Task, Digit Vigilance Task, Choice Reaction Task, Spatial Working Memory, Numeric Working Memory, Delayed Word Recall, Delayed Word Recognition, Delayed Picture Recognition.

When psychometric performances were analyzed on day 1, neither significant main effects of the drug, nor any interactions between dose and the repeated assessment were noted. On the other hand, when the analysis of performance is performed on day 13, there was one significant main effect of a dose for the percentage of correct detection in the digit vigilance task. Multiple comparisons showed this to reflect a lowering of detection with the 1200 mg dose compared with placebo (a drop of 5.3 compared with a rise of 0.03 in placebo, $P < 0.01$). There was a significant interaction between dose and the repeated assessment for the speed of detection in the vigilance task. Multiple comparisons showed the effect for the speed of detection in the vigilance task to reflect slowing of speed in the 100 and 1200 mg doses compared with placebo. For 100 mg, at 2 h, there was a slowing of speed by 42 ms compared to 6 ms with placebo ($P < 0.01$). For 1200 mg, there was a significant slowing at 2 h of 29 ms compared with 6 after placebo ($P < 0.01$), as well as a significant slowing at 6 h of 37 ms compared with no change under placebo ($P < 0.01$). No significant carry-over effects were noted from day 1 to day 8.

There were interactions between day and dosing condition for delayed word recall and word recognition sensitivity, providing possible evidence of residual effects on day 13. For delayed word recall, the basis for this interaction was an increase in the 1200 mg group from 18% of words recalled correctly on day 8 to 27% on day 13, whereas with placebo, the score dropped from 24 to 19% over the same period. For word recognition sensitivity, there was a similar pattern between placebo and 1200 mg dose, placebo dropping from 0.52 to 0.50 whereas at 1200 mg of S 17092 the same parameter increased from 0.31 to 0.39. However, at 100 mg there was a decrease in recognition sensitivity from 0.53 to 0.29 and at 800 mg from 0.61 to 0.36.

Taken collectively, these results provided evidence for the first time in humans that the pharmacodynamics of a PEP inhibitor can be assessed through its action on plasma PEP activity. Although the percentage of inhibition was not obviously different between doses, the duration of the inhibition showed a clear tendency to be dose-dependent. The duration of inhibition of PEP, the target enzyme of S 17092, was prolonged while the administered dose was well tolerated. The breakdown of various neuropeptides is likely to be inhibited by S 17092. Future studies will have to establish whether inhibition of PEP by S 17092, as observed in the present study, is sufficient to affect brain and plasma levels of any other neuropeptides. Although the EEG data from a rather small group of individuals showed marked variability, the exploratory analysis yielded significant drug effects. S 17092 ap-

pears to be a centrally active substance as it induces statistically significant alterations in EEG as compared with placebo. No dose-response relationship was observed as only the 100 mg dose showed an effect on the EEG. The lack of effect in EEG recordings with the 400, 800, and 1200 mg doses is difficult to interpret. One possibility is that the 100 mg dose had a maximal effect while higher doses produced a deleterious effect (inverted U-shape curve). This hypothesis should be verified through similar studies that test the effect of lower doses of S 17092. Another possibility is that a longer duration study is needed to better define a dose-effect relationship. There was no clear dose-response relationship in psychometric measurements. Upon a selection of 10 tasks from the CDR assessment system, there were beneficial effects of the 1200 mg dose on two verbal memory tasks, while there was a disruption to the vigilance task. The apparent disagreement between the effective dose in EEG recordings and the effective dose in these two verbal tests should be interpreted with caution since it is difficult to show a memory improvement during a trial of a very short duration. It will be of interest to determine the effective dose of S 17092 in memory impaired patients in trials involving multiple dosing. Then a more quantitative EEG study could be performed in order to establish the relationship between these two pharmacodynamic parameters more precisely. Concerning the relationship between the effective dose in PEP inhibition and the effective dose in psychometric tests, one hypothesis could be that memory improvement correlates more closely with the duration of PEP inhibition rather than with the levels of PEP inhibition *per se.*

SUMMARY AND CONCLUSION

The available preclinical data clearly indicate that the PEP (EC 3.4.21.26) inhibitor, S 17092 could be considered a potent cognitive enhancer acting at PEP inhibitory doses. Indeed, S 17092 is capable to inhibit both, chemically induced amnesia and spontaneous memory deficits. The promnesic effects of S 17092 were observed in preclinical studies using a variety of memory tasks permitting the exploration of different types of mnesic functions, namely inhibition of short-term, long-term, reference or working memory.

A complete characterization of peptides metabolized by PEP still remains to be elucidated. Therefore, we cannot exclude the possibility that a strategy based on PEP inhibition would lead to side effects related to the metabolism of these peptides. However, no such side effects have been observed in the preclinical studies with S 17092. Furthermore, phase I studies indicate that S 17092, administered once daily, is a well-tolerated compound with clear peripheral expression of its mechanism of action. In addition, S 17092, at PEP inhibitory doses, exhibits central effects, as evidenced by EEG. Finally, the residual improvements in the delayed verbal memory task produced by 1200 mg of S 17092 suggests that this compound will have favorable effects on memory and could be envisaged as a potential therapeutic agent for the treatment of cognitive disorders associated with aging. It is also noteworthy that using pharmacodynamic parameters, that were explored during phase I studies (EEG, PEP activity and psychometric test), the human therapeutic dose of S 17092 could not have been determined. Clinical dose-response trials will have to be undertaken as a part of Phase II studies.

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