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ORIGINAL INVESTIGATION

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Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation

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Abstract *Rationale:* Phosphodiesterase enzyme type 5 (PDE5) inhibitors and acetylcholinesterase (AChE) inhibitors have cognition-enhancing properties. However, it is not known whether these drug classes affect the same memory processes. *Objective:* We investigated the memory-enhancing effects of the PDE5 inhibitor sildenafil and AChE inhibitors metrifonate and donepezil in the object recognition task to find out whether acquisition or consolidation processes were affected by these drugs. *Methods:* The object recognition task measures whether rats remembered an object they have explored in a previous learning trial. All drugs were given orally 30 min before or immediately after learning to study acquisition and consolidation, respectively. *Results:* Sildenafil given immediately after the first trial improved the memory performance after 24 h and resulted in an inverted U-shaped dose–effect curve with the peak dose at 3 mg/kg. When given before the first trial, sildenafil also improved the memory performance. However, the dose needed for the best performance under this condition was 10 mg/kg, suggesting that the dose–effect curve shifted to the right. This can be explained by the metabolic clearance of the high dose of sildenafil. Donepezil had no memory improving effect when given after the first trial. However, when given before the first trial, a gradually increasing dose–effect curve was found which had its maximum effect at the highest dose tested (1 mg/kg). Likewise, only when metrifonate (30 mg/kg) was given before the first trial did rats show an improved memory performance. *Conclusion:* Our data strongly suggest that PDE5 inhibitors improve processes of consolidation of object information, whereas AChE inhibitors improve processes of acquisition of object information.

Keywords Acetylcholinesterase · Phosphodiesterase · Acquisition · Consolidation · Memory · Object

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Introduction

It has been shown that administration of zaprinast, sildenafil or vardenafil, which are inhibitors of the phosphodiesterase enzyme type 5 (PDE5) that selectively breaks down cyclic GMP (cGMP), improves the performance of adult rats in an object recognition task (Prickaerts et al. 1997, 2002b). This suggests that PDE5 inhibitors may have cognition-enhancing properties.

The object recognition task is a one-trial learning task that measures in a test trial whether rats remember an object that has been presented in one previous learning trial. One trial learning task like the object recognition task allow to investigate different processes of memory, depending on the time of drug treatment (Izquierdo 1989; Riekkinen et al. 1998; Abel and Lattal 2001; Prickaerts et al. 2004). Thus, administration of a drug before the learning trial should have an effect on the acquisition of information, while administration of a drug immediately after the learning trial should have an effect on the consolidation of information. Of note, administration of a drug before the learning trial may also have an effect on consolidation and even on retention, depending on its pharmacokinetic properties. Since all PDE5 inhibitors in previous studies were always administered

immediately after the learning trial, it was concluded that processes of early consolidation of object information are influenced (Prickaerts et al. 1997, 2002b).

Well-known compounds having cognition-enhancing properties are acetylcholinesterase (AChE) inhibitors such as metrifonate and donepezil. Cognition-enhancing properties of both compounds were intensively investigated and found in various spatial learning tasks with the use of deficiency models, i.e. animals with lesions in cholinergic structures or animals treated with anticholinergic drugs (e.g. Riekkinen et al. 1996; Itoh et al. 1997; Ogura et al. 2000; Chen et al. 2002). Both compounds have also thoroughly been investigated in one-trial passive avoidance learning tasks in combination with deficiency models and were found to ameliorate the impaired avoidance performance (e.g. Riekkinen et al. 1991; Itoh et al. 1997; Kojima et al. 1997; Bejar et al. 1999; Ogura et al. 2000; Chopin et al. 2002; Tokita et al. 2002). The passive avoidance task is a one-trial learning paradigm similar to the object recognition task. However, in this task subjects receive an electric foot shock in the acquisition trial and have to remember this aversive stimulus in the retention trial.

The effects of metrifonate on one-trial learning in intact healthy adult rats have been investigated twice in the passive avoidance task (Schmidt and De Jonge 1991; Riekkinen et al. 1996) and once in the object recognition task (Scali et al. 1997a). In these studies, metrifonate was administered 30 min before the acquisition trial. It is likely that the compound affected acquisition processes, because under this condition, the compound had a beneficial effect on the retention performance, 24 h after the acquisition trial (Schmidt and De Jonge 1991). To our knowledge, the pro-cognitive potential of donepezil has never been tested using a one-trial learning paradigm in healthy adult rats.

The more cognitive processes are improved by a compound, the more likely it is that it will be useful for the treatment of memory deficits in patients. In the present study, we investigated in healthy adult rats the effects of two classes of drugs with cognition-enhancing potential on cognitive processes which have not been tested before; we assessed the effects of PDE5 inhibitors on acquisition, and of AChE inhibitors on consolidation processes. To complete the picture, the PDE5 inhibitor sildenafil or the AChE inhibitor metrifonate or donepezil were administered 30 min before or immediately after the learning trial of the object recognition task. Thus, the effect of each compound on both acquisition and consolidation of object information was investigated.

Materials and methods

Animals

All experimental procedures were approved by the local ethical committee of the Maastricht University for animal experiments according to governmental guidelines. Male Wistar rats (Charles River, The Netherlands) were used. Twelve 5-month-old rats with mean body weights of 428 ±7 (SEM) g were used for the metrifonate experiment. Twenty-four 4-month-old rats weighing on average 341 ±3 (SEM) g were used for the sildenafil and donepezil experiments. The animals were housed individually in standard type 3 Makrolon cages on sawdust bedding in an air-conditioned room (about 20°C). They were kept under a reversed 12/12-h light/dark cycle (lights on from 1800 to 0600 hours) and had free access to food and water.

Treatments

Each compound was freshly dissolved or suspended in its vehicle on every experimental day. Metrifonate and donepezil were dissolved in 0.1 M sodium citrate buffer (pH 5.5) and administered PO in an injection volume of 1 ml/kg. A dose of 30 mg/kg was used for metrifonate and doses of 0.1, 0.3 and 1 mg/kg were used for donepezil. Sildenafil, as citrate, was suspended in 1% tylose (methylcellulose) and was given PO in an injection volume of 2 ml/kg. Doses of 1, 3 and 10 mg/kg were used. Metrifonate, donepezil and sildenafil were administered both immediately after and 30 min before the first trial in the object recognition task. Metrifonate, donepezil and sildenafil were kindly donated by BAYER AG (Wuppertal, Germany).

Rats were not housed in the same room as where the animals were tested. A radio, which was playing softly, provided background noise in all rooms. All testing was done between 0900 and 1700 hours. In the following object recognition experiments each rat served as its own control. Metrifonate was tested using 12 rats. For the sildenafil and donepezil study 24 rats were randomly assigned to two experimental groups of 12 animals each. One group was used for testing the effects of sildenafil administration immediately after the first trial in the object recognition test. This experiment was part of another study (see Prickaerts et al. 2002b). Subsequently, all 24 rats were used for the sildenafil treatment 30 min before the first trial. Thereafter, the rats of the experimental group that initially had also been treated with sildenafil after the first trial were now used for testing the effects of donepezil treatment 30 min before the first trial. At the same time, the other group was used for the donepezil treatment immediately after the first trial.

Object recognition task

Metrifonate, donepezil and sildenafil were tested in the object recognition test which was performed as described elsewhere (Ennaceur and Delacour 1988). The apparatus consisted of a circular arena, 83 cm in diameter. Half of the 40 cm high wall was made of gray polyvinyl chloride, the other half of transparent polyvinyl chloride. The light intensity (20 lux) was equal in the different parts of the apparatus. Two objects were placed in a symmetrical position about 10 cm away from the gray wall. We used

four different sets of objects. The different objects were: (1) a cone consisting of a gray polyvinyl chloride base (maximal diameter 18 cm) with a collar on top made of brass (total height 16 cm); (2) a standard 1 l transparent glass bottle (diameter 10 cm, height 22 cm) filled with sand; (3) a massive metal cube (10.0×5.0×7.5 cm) with two holes (diameter 1.9 cm), and (4) a massive aluminum cube with a tapering top (13.0×8.0×8.0 cm). The objects could not be displaced by a rat.

In the first week, the animals were handled daily and were adapted to the procedure in 2 days, i.e. they were allowed to explore the apparatus (without any objects) twice for 3 min each day. In the 2 following weeks, the rats were adapted to the testing and oral administration procedure by a saline injection (0.4 ml) immediately after the first trial until they showed a stable discrimination performance, i.e. a good object discrimination at a 1-h interval. Subsequently, testing of the drugs began.

A testing session comprised two trials. The duration of each trial was 3 min. During the first trial (T1), the apparatus contained two identical objects (samples). A rat was always placed in the apparatus facing the wall in the center of the transparent front segment. After the first exploration period, the rat was put back in its home cage. Subsequently, after a delay interval, the rat was put back in the apparatus for the second trial (T2), but now with two dissimilar objects, a familiar one (the sample) and a new one. The times spent exploring each object during T1 and T2 were recorded manually with a personal computer.

Exploration was defined as follows: directing the nose to the object at a distance of no more than 2 cm and/or touching the object with the nose. Sitting on the object was not considered as exploratory behavior. In order to avoid the presence of olfactory trails, the objects were always thoroughly cleaned. Moreover, each object was available in triplicate so none of the two objects from the first trial had to be used as the familiar object in the second trial. In addition, all combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects.

Since we expected the drug treatments to improve memory performance, we needed a delay interval at which no more discrimination between the objects occurs. Therefore, we selected a delay interval of 24 h, since there is virtually no discrimination between the two objects after this interval (Prickaerts et al. 2002a,b). Each week, two testing sessions were given, one set on Monday (T1) and Tuesday (T2) and the other set on Thursday (T1) and Friday (T2). In the sildenafil and donepezil experiments, we tested the different doses of each drug in random order.

Statistical analysis

The basic measures in the object recognition task were the times spent by rats exploring an object during T1 and T2 (see Table 1). $e1$ and $e2$ are measures of the total exploration time of both objects during T1 and T2, respectively. $h1$ was considered as an index measure of global habituation of exploratory behavior from T1 to T2. $d2$ was considered as an index measure of discrimination between the new and the familiar objects. In fact, $d2$ is a relative measure of discrimination which corrects the absolute measure of discrimination for exploration activity (see Table 1). Thus, there should be no differences in $d2$ indices between experiments with similar treatments at similar intervals. However, this need not to be the case for the absolute discrimination measure because of possible differences in exploration activity. In the present study, the absolute discrimination measure showed the same results as the relative discrimination index $d2$ (data not shown).

In previous studies, we found that a group size of 24 is sufficient for reliable statistical evaluation (Prickaerts et al. 2002a,b). Thus, the effects of sildenafil administration 30 before T1 were tested in 24 rats in one session only. Sildenafil administration after T1 and metrifonate and donepezil administration both before and after T1 were always tested in 12 rats only, yet in two sessions and the results of sessions testing the same treatment (dose/vehicle) condition were averaged.

One-sample t-statistics were performed in order to assess per treatment condition whether $h1$ differed from zero. However, comparison of the value of $d2$ with the value zero with no variance may not be the most suitable way for analyzing recognition (increased chance of making a type I error). Therefore, we created a virtual group that shows no discrimination ($d2$=0) and an SEM that corresponds to the average SEM (0.065) of seven independent samples of 24 animals which received vehicle in previous studies. The number of observations of the virtual group was always the same as the experimental group being compared with. The $d2$ value of zero is based on the observation that untreated and vehicle-treated Wistar rats do not discriminate between the objects after 24 h (Prickaerts et al. 2002a,b), and that the average $d2$ value of the vehicle sessions after a 24-h delay will approximate zero. On basis of these considerations, we assume that the comparison by using a t-test of the individual doses with this virtual group is the most valid comparison to test the effectiveness of a drug (see also Şık et al. 2003). Of note, the $h1$ measure can differ from zero 24 h after a specific vehicle treatment. Consequently, it is not possible to create the value of $h1$ of a general virtual group. Yet it is possible to create the $h1$ of a virtual group

Table 1 Measures involved in the object recognition test. $e1$ is the measure of the time spent in exploring both identical objects ($a1$ and $a2$) in the first trial, and $e2$ is the measure of the time spent in exploring both the familiar (a) and new object (b) in the second trial; $h1$ is the measure of global habituation from trial 1 to trial 2; $d2$ is the relative measure of discrimination between the new and familiar objects which corrects the absolute discrimination ($b-a$) for exploration activity ($e2$)

Exploration	Habituation	Discrimination
$e1=a1+a2$ $e2=a+b$	$h1=e1-e2$	$d2=(b-a)/e2$

for a specific vehicle treatment if one has enough observations from previous studies. However, this is not the case for the present study.

Effects between the different doses of each drug were analyzed with repeated measures ANOVA over dose. In the case of a statistically reliable dose effect, comparisons between means of the different doses were analyzed in more detail using post hoc Sidak's *t*-tests ($P< 0.05$).

Two rats were removed from the metrifonate experiment: one rat since in one session it showed no exploratory behavior in T1 (i.e. $e1=0$), and one rat because of its lack of exploratory behavior in T2 (i.e. less than 10% of mean $e2$). One rat was excluded from the experiment in which sildenafil was administered immediately after T1 because of its lack in exploratory behavior in T1 (i.e. less than 10% of mean $e1$). In the experiment with donepezil treatment 30 min before T1, one rat was excluded because of its location preference (i.e. only exploring one and the same object location in both T1 and T2). Therefore, the final number of rats per experiment used for analysis were: metrifonate before and after T1, $n=10$; sildenafil after T1, $n=11$; sildenafil before T1, $n=24$; donepezil after T1, $n=12$; donepezil before T1, $n=11$.

Results

Metrifonate

Table 2 summarizes the results of treatments with 30 mg/kg (PO) metrifonate on the activity measures and on the relative discrimination index $d2$. Saline treatment after T1, which is part of the adaptation procedure in the 2 weeks preceding the drug treatments, showed that these rats did not discriminate between the objects after a delay interval of 24 h (index of discrimination $d2$ was not different from zero, i.e. the virtual group, after saline treatment; see Table 2c). Twenty-four hours after treatment with metrifonate immediately after T1 the rats did not recognize the familiar object (see Table 2c).

Subsequently, we also investigated the effects of metrifonate administration 30 min before T1. Using this treatment, it was found that the index measure of habituation of exploratory behavior $h1$ was different from zero (see Table 2b). This indicates that the exploration time in T1 was lower than that in T2. $d2$ was found to be higher than zero when metrifonate was given 30 min before T1, thus indicating that 24 h after T1 the metrifonate-treated rats discriminated between the objects (see Table 2c).

Sildenafil—treatment after trial 1

Table 3 summarizes results of the sildenafil treatments immediately after T1 on the activity measures. Figure 1a illustrates the effects of these sildenafil treatments on the index of discrimination $d2$. This experiment was part of another study (Prickaerts et al. 2002b). No differences were found between the different doses in the total level of exploration in T1 [$e1$; $F(3,30)=1.08$, n.s.]. Neither were there differences between the doses in the total exploration time in T2 [$e2$; $F(3,30)=1.72$, n.s.]. At the medium dose (3 mg/kg), the index measure of habituation of exploratory behavior $h1$ differed from zero (see Table 3b). This indicates that the exploration time was increased from T1 to T2. However, the $h1$ indices at all treatment conditions (vehicle/doses) were not different from each other [$F(3,30)=0.67$, n.s.].

The index of discrimination $d2$ showed that 24 h after T1, the vehicle-treated rats did not discriminate between the objects ($d2$ was not different from zero; see Fig. 1a). After treatment with the medium (3 mg/kg) and high dose (10 mg/kg) of sildenafil, the rats discriminated between the objects ($d2$ values were different from zero; see Fig. 1a). Comparing between treatment conditions it was found that the $d2$ indices differed from each other [$F(3,30)=3.88$, $P<0.05$]. Post hoc analysis showed that the $d2$ index after treatment with the medium dose was higher than that after vehicle and low dose treatment.

Sildenafil—treatment 30 min before trial 1

In this experiment, the low dose of sildenafil (1 mg/kg) was not tested, since it was assumed to have no effects, based on the results from the previous sildenafil experiment. The results of the sildenafil treatments 30 min

Table 2 Results of treatment with metrifonate on the activity measures of the object recognition test. Rats ($n=10$) received a PO injection of metrifonate at a dose of 30 mg/kg immediately after or 30 min before the first trial (*T1*). Saline was given PO after the first trial only. For each treatment, the averaged data of two sessions are presented. The delay interval between the first and second trial was 24 h. $h1$ measures different from zero are depicted with an *asterisk* (one-sample *t*-test, $*P<0.05$). Likewise, $d2$ measures different from the virtual group (mean: 0, SEM: 0.065) are depicted with an *asterisk* (*t*-test, $P<0.05$)

	Saline after T1	Metrifonate after T1	Metrifonate before T1
A. Mean values (±SEM) of total exploration time (*s*) during the first ($e1$) and second trial ($e2$)			
$e1$	11.53 (1.50)	18.55 (1.55)	15.25 (1.35)
$e2$	14.78 (2.09)	19.22 (2.31)	19.61 (1.21)
B. Mean values (±SEM) of the global index of habituation ($h1$) from the first to the second trial			
$h1$	−3.25 (1.60)	−0.67 (2.26)	−4.36 (1.41)*
C. Mean values (±SEM) of the index of discrimination ($d2$) between the new and familiar objects			
$d2$	−0.11 (0.08)	0.10 (0.08)	0.26 (0.10)*

Table 3 Results of treatment with sildenafil immediately after the first trial on the activity measures of the object recognition test. Rats (n=11) received a PO injection of vehicle (1% tylose) or sildenafil at a dose of 1, 3 or 10 mg/kg after the first trial. For each treatment, the averaged data of two sessions are presented. The delay interval between the first and second trial was 24 h. This experiment was also part of another study (see Prickaerts et al. 2002b). $h1$ measures different from zero are depicted with *asterisks* (one-sample t-test, **P<0.01)

	Vehicle	1 mg/kg	3 mg/kg	10 mg/kg
A. Mean values (±SEM) of total exploration time (s) during the first ($e1$) and second trial ($e2$)				
$e1$	27.20 (2.05)	23.35 (1.94)	24.88 (2.13)	23.98 (1.97)
$e2$	28.32 (1.89)	26.40 (2.41)	29.68 (1.63)	25.58 (2.02)
B. Mean values (±SEM) of the global index of habituation ($h1$) from the first to the second trial				
$h1$	−1.12 (2.16)	−3.04 (2.34)	−4.80 (1.23)**	−1.60 (1.48)

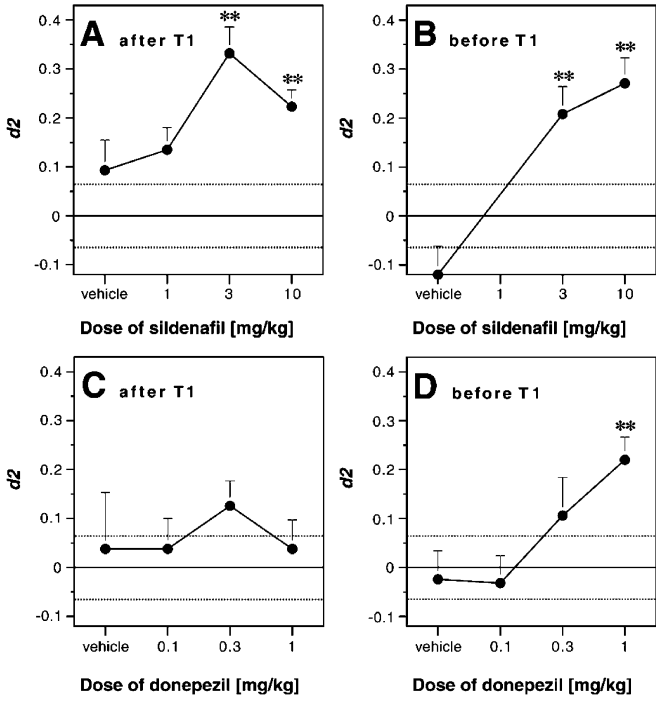

Fig. 1 Effects of different doses of **a**, **b** sildenafil and **c**, **d** donepezil on the index of discrimination $d2$ in the object recognition task (mean values+SEM). Drugs were given both immediately after (**a**, **c**) the first trial or 30 min before (**b**, **d**) the first trial ($T1$). In the vehicle sessions of the sildenafil and donepezil experiments, rats were treated (PO) with 1% tylose or 0.1 M sodium citrate buffer (pH 5.5), respectively. *Area between the dotted lines* indicates the SEM range of the virtual group (mean: 0, SEM: 0.065). **Different from virtual group (t-test, P<0.01)

before the first trial on the activity measures are summarized in Table 4. The effects of these sildenafil treatments on the index of discrimination $d2$ are shown in Fig. 1b. The levels of exploration in T1 ($e1$) and T2 ($e2$) were not different between the treatment conditions (vehicle/doses) (both Fs<2.91, n.s.). The index measures $h1$ were not different from zero, indicating that the exploration time did not change from T1 to T2 after any treatment (see Table 4b). Between the treatment conditions there was also no difference in the $h1$ indices [F(2.46) =0.45, n.s.].

The index of discrimination $d2$ showed that 24 h after T1, the rats did not discriminate between the objects after vehicle treatment (see Fig. 1b). After treatment with the medium (3 mg/kg) and high (10 mg/kg) doses of sildenafil, the rats positively discriminated between the objects, indicating recognition of the familiar object. Comparisons between treatment conditions showed a difference in $d2$ values [F(2,46)=13.00, P<0.01]. Post-hoc analysis showed that $d2$ after treatment with the medium and high dose was higher than that after vehicle treatment, while there was no difference in $d2$ between the two dose conditions.

Donepezil—treatment after trial 1

The results of the donepezil treatments immediately after T1 on the activity measures are summarized in Table 5. Figure 1c illustrates the effects of these donepezil treatments on the index of discrimination $d2$. Neither in T1 nor in T2 was there a difference between the treatment conditions (vehicle/doses) in the level of exploration (both Fs<1.76, n.s.). At the highest dose (1 mg/kg) the $h1$ index differed from zero (see Table 5b), indicating that the exploration activity increased from T1 to T2. However, the

Table 4 Results of treatment with sildenafil 30 min before the first trial on the activity measures of the object recognition test. Rats (n=24) received a PO injection of vehicle (1% tylose) or sildenafil at a dose of 3 or 10 mg/kg 30 min before the first trial. The delay interval between the first and second trial was 24 h. None of the $h1$ measures was different from zero

	Vehicle	3 mg/kg	10 mg/kg
A. Mean values (±SEM) of total exploration time (s) during the first ($e1$) and second trial ($e2$)			
$e1$	25.92 (1.97)	24.94 (2.40)	23.15 (1.45)
$e2$	28.47 (1.94)	25.94 (1.59)	22.95 (1.97)
B. Mean values (±SEM) of the global index of habituation ($h1$) from the first to the second trial			
$h1$	−2.55 (1.94)	−1.00 (2.31)	0.21 (2.01)

*h*1 indices were not different between treatment conditions [$F(3,33)=1.98$, n.s.].

The index of discrimination *d*2 showed that 24 h after T1, the rats did not discriminate between the objects, since *d*2 was not different from zero after vehicle treatment (see Fig. 1c). Treatment with different doses of donepezil had no effect on discrimination performance, as *d*2 values were not different from zero (see Fig. 1c). Between the treatment conditions, vehicle included, there was also no difference in the *d*2 indices [$F(3.33)=0.31$, n.s.].

Donepezil—treatment 30 min before trial 1

The results of the donepezil treatments 30 min before the first trial on the activity measures are summarized in Table 6. The effects of these donepezil treatments on the index of discrimination *d*2 are illustrated in Fig. 1d. The levels of exploration in T1 (*e*1) and T2 (*e*2) were not different between the treatment (vehicle/dose) conditions (both Fs<1.52, n.s.). In the low dose (0.1 mg/kg) condition the *h*1 index was different from zero, which indicated that the exploration time in T1 was lower than that in T2 (see Table 6b). However, between the treatment conditions there was no difference in the *h*1 indices [$F(3.30)=2.03$, n.s.].

The index of discrimination *d*2 showed that 24 h after T1, the rats did not discriminate between the objects after vehicle treatment (see Fig. 1d). *d*2 was higher than zero only after the high dose treatment indicating discrimination between the objects at this dose (see Fig. 1d). Comparisons between treatment conditions showed a difference in *d*2 values [$F(3.30)=3.44$, $P<0.05$]. Post-hoc analysis showed that the *d*2 index of the high dose treatment was different from the vehicle and low dose treatments.

Discussion

PDE5 inhibition and object recognition memory

Sildenafil given immediately after the first trial clearly improved the memory performance in the object recognition task. This sildenafil treatment resulted in an inverted U-shaped dose–effect curve with the highest *d*2 value at a dose of 3 mg/kg. When given 30 min before the first trial sildenafil also clearly improved the memory performance. However, the dose–effect curve was linear in the dose-range tested, with the best performance at the highest dose of 10 mg/kg.

Comparison of the two dose–effect curves of the different sildenafil treatments suggests that administration of sildenafil before the first trial instead of after the first trial shifted the dose–effect curve to the right. This is reflected in the finding that the most effective dose shifted from 3 mg/kg to 10 mg/kg when changing the administration of sildenafil from immediately after to 30 min before the first trial. This implies that sildenafil has a positive effect on consolidation of object information since the high dose of sildenafil given after the first trial had only an intermediate effect. However, when administered 30 min before the first trial the 10 mg/kg dose resulted in a maximum effect. It is likely that, as a result of its metabolic clearance by the liver the high dose (10 mg/kg) of sildenafil decreased to a similar plasma level as that produced by the medium (3 mg/kg) dose that was administered immediately after the first trial. Consequently, the drug appears to maximally improves object memory performance by influencing consolidation processes. This notion is supported by the short elimination half-life of sildenafil of about 0.4 h in male rats (Walker et al. 1999). However, 3 mg/kg sildenafil treatment before the first trial improved object memory, while 1 mg/kg sildenafil given after the first trial failed to improve memory. This apparent discrepancy merely indicates that when giving sildenafil before instead of after the fist trial, the actual shift of the dose–effect curve to the right was such that 30 min after administration of 10 mg/kg or 3 mg/kg, sildenafil levels were somewhat higher than 3 mg/kg and 1 mg/kg, respectively. Thus, in the latter case it was high enough to be effective. However, it cannot be completely ruled out that sildenafil also influences processes of acquisition.

Sildenafil has been tested in one-trial learning of mice using the passive avoidance task (Baratti and Boccia 1999). When mice were treated (1–30 mg/kg, IP) immediately after the learning trial, it was found that sildenafil improved the memory performance 48 h later. There was an inverted dose–response U-shaped curve. However, only a dose of 3 mg/kg sildenafil was effective. Only this dose of sildenafil was also given 30 min prior to the first trial and was found to improve the memory performance to the same magnitude as that produced by

Table 5 Results of treatment with donepezil immediately after the first trial on the activity measures of the object recognition test. Rats (n=12) received a PO injection of vehicle (0.1 M sodium citrate buffer, pH 5.5) or donepezil at a dose of 0.1, 0.3 or 1 mg/kg after the first trial. For each treatment, the averaged data of two sessions are presented. The delay interval between the first and second trial was 24 h. *h*1 measures different from zero are depicted with an *asterisk* (one-sample *t*-test, *$P<0.05$)

	Vehicle	0.1 mg/kg	0.3 mg/kg	1 mg/kg
A. Mean values (±SEM) of total exploration time (*s*) during the first (*e*1) and second trial (*e*2)				
*e*1	22.33 (2.69)	19.97 (2.95)	17.87 (1.20)	17.82 (1.48)
*e*2	20.12 (1.81)	19.50 (2.06)	19.50 (1.61)	21.26 (1.55)
B. Mean values (±SEM) of the global index of habituation (*h*1) from the first to the second trial				
*h*1	2.20 (1.90)	0.47 (2.64)	−1.64 (1.11)	−3.44 (1.38)*

Table 6 Results of treatment with donepezil 30 min before the first trial on the activity measures of the object recognition test. Rats (n=11) received a PO injection of vehicle (0.1 M sodium citrate buffer, pH 5.5) or donepezil at a dose of 0.1, 0.3 or 1 mg/kg 30 min in the first trial. For each treatment the averaged data of two sessions are presented. The delay interval between the first and second trial was 24 h. $h1$ measures different from zero are depicted with an *asterisk* (one-sample t-test, *P<0.05)

	Vehicle	0.1 mg/kg	0.3 mg/kg	1 mg/kg
A. Mean values (±SEM) of total exploration time (s) during the first ($e1$) and second trial ($e2$)				
$e1$	23.44 (1.91)	20.48 (1.79)	23.05 (2.41)	24.44 (2.41)
$e2$	27.33 (3.06)	27.39 (2.78)	25.79 (3.62)	23.30 (2.70)
B. Mean values (±SEM) of the global index of habituation ($h1$) from the first to the second trial				
$h1$	−3.89 (2.70)	−6.90 (2.91)*	−2.74 (2.66)	1.14 (2.11)

administration of sildenafil immediately after learning. Based on our findings, it could be expected that the effect of this dose of sildenafil should have been weaker. However, this is not necessarily the case, since the measurements of the memory improvement in the passive avoidance task are influenced by many different factors, such as species-dependent differences in sensitivity to drug treatment. Nevertheless, evidence is accumulating that PDE5 inhibition and cGMP are involved in processes of early consolidation of electric shock and object information (see also Bernabeu et al. 1996, 1997; Prickaerts et al. 1997, 2002a,b).

AChE inhibition and object recognition memory

When metrifonate was given 30 min before the first trial, it improved the performance of the rats. By injecting donepezil 30 min before the first trial, a gradually increasing dose–effect curve was found. On the basis of these data, we argue that the cognition-enhancing effects of metrifonate and donepezil emerge when the drugs are active in the learning situation, i.e. these drugs have a positive effect on acquiring object information. Since metrifonate and donepezil had no effects when they were injected immediately after the acquisition trial, they apparently do not affect the consolidation of object information. However, it should be noted that the maximal effect on AChE inhibition by metrifonate and donepezil can be observed between 30 min and 60 min after drug administration (Hinz et al. 1996a,b; Van der Staay et al. 1996), which could be a time window that exceeds the effective consolidation phase. In addition, when metrifonate or donepezil were injected 30 min before the first trial, they were active not only during the acquisition phase but also during consolidation. Therefore, although the present study mainly indicates that the drugs have an effect on the acquisition of object information, it cannot be completely ruled out that the drugs have an effect on consolidation (see also below).

Metrifonate has also been tested in one-trial passive avoidance learning of intact adult rats. It was found that metrifonate (30 mg/kg) administered orally 30 min before the learning trial improved the performance of the rats in the retention trial (Schmidt and De Jonge 1991). This "shock" finding corroborates our "object" finding that metrifonate and donepezil have beneficial effects on the acquisition of information. One trial learning of adult rats has also been investigated with the AChE inhibitor tacrine. In the object recognition task, administration of tacrine before the learning trial has recently been found to improve the memory performance (Moser et al. 2002). The same tacrine treatment was also effective in the passive avoidance task (Camacho et al. 1996), while administration immediately after the learning trial had no effect on the retention performance (Smith et al. 1996). These results of tacrine are in agreement with our metrifonate and donepezil data. However, there are also conflicting data. Administration of metrifonate, tacrine or another AChE inhibitor, physostigmine, before the learning trial had no effect on the performance of adult rats in a number of studies using one-trial learning paradigms (passive avoidance task: Riekkinen et al. 1991, 1996; Riekkinen and Riekkinen 1994; object recognition task: Ennaceur and Meliani 1992; Scali et al. 1997a,b). This lack of an effect may have been due to a ceiling effect. For instance, in the object recognition studies a short inter-test interval of 60 min was used at which the rats still remembered the familiar object. Thus, the performance of the control groups was already optimal, so that the metrifonate-treated and tacrine-treated groups could not outscore this performance level. In addition, possible adverse cholinergic side effects might have influenced the behavioral performance in the object recognition task (see Yoshida and Suzuki 1993). However, treatment with physostigmine after learning even improved the rats' performance in the passive avoidance task (e.g. Santucci et al. 1989).

It is difficult to tell whether AChE inhibitors including metrifonate, donepezil, tacrine and physostigmine have a positive effect on memory processes of acquisition and/or consolidation in healthy adult rats. When the same drugs were tested in the passive avoidance tasks but now using animal models with experimentally induced deficits, i.e. animals with lesions in cholinergic structures or animals treated with for instance scopolamine, then these drugs in general did mediate cognitive enhancement (e.g. Riekkinen et al. 1991, 1996; Yamazaki et al. 1991; Riekkinen and Riekkinen 1994; Kojima et al. 1997; Ogura et al. 2000; Chopin et al. 2002). However, it should be noted that in the cholinergic deficiency models, the effects of metrifonate, donepezil, tacrine and physostigmine on passive avoidance learning were found not only on acquisition but also on consolidation (e.g. Ogasawara et al. 1996; Bejar et al. 1999; Tokita et al. 2002).

Metrifonate has also been tested on passive avoidance learning of healthy adult mice, but appeared to be ineffective (Ikonen et al. 1999). However, in a cholinergic (medial septum) lesion model, metrifonate improved the acquisition of passive avoidance learning (Ikonen et al. 1999). Recently, tacrine has been tested in the object recognition task with mice, and it was found to improve consolidation (Chopin et al. 2002). Physostigmine has only been tested in the passive avoidance task and showed a positive effect on acquisition (e.g. Zarrindast et al. 1998), but also on consolidation (e.g. Baratti and Kopf 1996). Similar results were found in a cholinergic (scopolamine) deficit model, as acquisition as well as consolidation of passive avoidance learning were improved by physostigmine and donepezil (Suzuki et al. 1995). Taken together, although a possible effect on consolidation after AChE treatment cannot be completely ruled out, AChE inhibitors seem to have a positive effect on the acquisition of object and electrical shock information in rodents.

Effects of both drug classes on exploratory activity

We observed an incidental increase in exploratory activity 24 h after treatment with the medium dose (3 mg/kg) of sildenafil and highest dose (1 mg/kg) of donepezil when administered immediately after the first trial. Close examination of the changes in activity revealed that they could not be attributed to sildenafil or donepezil, since the change in activity after the specific doses of sildenafil or donepezil was still within the range of the non-significant changes after the other treatments, vehicle included. For donepezil an incidental decrease in exploratory activity was found 30 min after administration of the low dose (0.1 mg/kg). This was also observed 30 min after the administration of metrifonate (30 mg/kg). Again, the effect on exploration of donepezil was still within the range of the non-significant changes after the other treatments. Therefore, it can be argued that exploration is not influenced at both short (30 min) and long (24.5 h) intervals after treatment with sildenafil, donepezil or metrifonate.

Possible site and mechanism of action of PDE5 inhibition

The PDE5 inhibitors sildenafil, zaprinast and vardenafil increase cGMP accumulation in the dorsal hippocampus as assessed in vitro with radioimmunoassay and immunocytochemistry (De Vente et al. 1996; Van Staveren et al. 2001; Prickaerts et al. 2000b). In addition, it has been found that injections of 8-bromo-cGMP into the hippocampus immediately after the first trial improved the memory performance in both the object recognition (Prickaerts et al. 2002a) and passive avoidance task (Bernabeu et al. 1996). Sildenafil has been demonstrated to penetrate the brain (FDA 1998). All these findings support the notion that increased hippocampal cGMP levels may be responsible for the improved memory performance in object recognition after treatment with PDE5 inhibitors.

Several mechanisms of action of cGMP have been suggested to explain how cGMP exerts its action in processes of memory. For example, cGMP is thought to act through regulation of cGMP-gated ion channels, regulation of cAMP-selective PDEs or activation of cGMP-dependent protein kinases (Schmidt et al. 1993; Wei et al. 1998). Despite these findings regarding the possible mechanisms of cGMP action, it remains unclear how cGMP produces altered signal transduction, thereby improving memory performance (for an extensive discussion see Prickaerts et al. 2004). In addition, questions can be asked about the hippocampus as the site of action of PDE5 inhibition and thus cGMP, since the role of the hippocampus in object recognition is a matter of debate (for review, see Mumby 2001). It has been reported that object recognition memory does not depend on the hippocampus but is dependent on the rhinal cortex instead (Ennaceur and Aggleton 1997; Bussey et al. 1999; Mumby et al. 2002). Of note, using in situ hybridization, it has recently been demonstrated that PDE5 mRNA is expressed in both hippocampus (granule cells, pyramidal cell and some dispersed cells in the layers) and rhinal cortex (cells throughout the different layers) (Van Staveren et al. 2003).

The memory improving effects of sildenafil may also, or alternatively, be related to an increased blood flow and, consequently, an increased glucose metabolism. PDE5 inhibitors are known to result in vasodilatation, probably via cGMP (e.g. Dundore et al. 1993). A decreased blood pressure is also indicative of vasodilatation. It has been demonstrated that oral administration of 10 mg/kg sildenafil decreases the systolic arterial blood pressure in conscious rats (Rehse et al. 1999). This effect lasted for at least 6 h. Administration of 5 mg/kg sildenafil had no effect on blood pressure. In a recent study using anesthetized rats, oral administration of 2 mg/kg sildenafil already increased localized cerebral blood flow during 70 min after administration of sildenafil (Zhang et al. 2002). In the present study, the most effective dose of sildenafil was 3 mg/kg PO. Thus, it could be argued that peripheral administration of sildenafil increased central blood flow. However, the same doses of sildenafil had different effects on memory processes of acquisition and consolidation of object information. This suggests that it is not likely that the improved object recognition memory after treatment with sildenafil can be simply explained as a consequence of changes in blood flow or blood pressure.

Possible site and mechanism of action of AChE inhibition

The beneficial effects the AChE inhibitors metrifonate and donezepil on the acquisition of object information in the adult rat may be mediated via the hippocampus. Assuming that AChE inhibition is the mechanism of action, it should

be kept in mind that acetylcholine is also involved in attentional processes, which may influence/explain the memory performance (e.g. Blokland 1996). Moreover, there are arguments in favor of the notion that inhibition of AChE may not be, or not exclusively be, the mechanism of action. Although metrifonate and donepezil have long half-lives, AChE is not chronically inhibited in the present study, since the highest effective doses tested cause very weak inhibition of AChE in the rat brain: about 20% after oral administration of 30 mg/kg metrifonate (Hinz et al. 1996a; Van der Staay et al. 1996) and about 30% after oral administration of 1 mg/kg donepezil (Van der Staay et al. 1996). In addition, there are indications that local inhibition of AChE in, for instance, the hippocampus and cortex may even be less than 10% using such "low" oral doses of metrifonate and donepezil (Cheng and Tang 1998). Further, these levels of AChE inhibition are achieved within 30–60 min in the rat brain and appear to be fully reversible within 24 h (e.g. Hinz et al. 1996a). These findings therefore suggest that a mechanism of action other than AChE inhibition or an additional, not yet identified one is responsible for the beneficial effect of donepezil and metrifonate on the object information processing, as has been suggested earlier for spatial learning (Van der Staay et al. 1996; Itoh et al. 1997).

Conclusion

Taken together, both PDE5 and AChE inhibitors improved the performance of rats in the object recognition task. Our data strongly suggest that PDE5 inhibitors improve processes of consolidation of object information, whereas AChE inhibitors improve processes of acquisition, although consolidation cannot be ruled out completely, of object information.

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