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Subchronic treatment increases the duration of the cognitive enhancement induced by metrifonate

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Abstract

The study compared the efficacy of acute versus chronic metrifonate treatment to improve initial and reversal learning of the water maze spatial navigation task in medial septal-lesioned rats. Acute oral administration of 30 mg/kg metrifonate at 30 min, but not at 150 or 360 min, before training improved the initial acquisition of the water maze task. In contrast, improvement of initial learning performance of medial septal-lesioned rats pretreated for 21 days with metrifonate was observed irrespective of the timing of metrifonate treatment relative to behavioral testing. Reversal learning was assessed after a four-day wash-out period. No drug treatment was administered during this part of the study. All the medial septal-lesioned rats that had received only acute treatment with metrifonate during the initial learning stage were now as impaired as vehicle treated medial septal-lesioned rats. However, the group subchronically pretreated with metrifonate performed better than the vehicle-treated medial septal-lesioned controls. These results indicate that both acute and subchronic treatment with metrifonate can facilitate spatial learning in medial septal-lesioned rats and the transient nature of this beneficial effect after single acute administration is transformed into a long-lasting improvement by subchronic treatment. © 1997 Elsevier Science B.V.

Keywords: Metrifonate; Spatial navigation; Cholinesterase inhibition; Alzheimer's disease; Medial septal-lesion

1. Introduction

Cholinesterase inhibitors are currently the most extensively investigated drugs available against the cognitive decline associated with Alzheimer's disease, a disorder which is characterized by a severe loss of presynaptic cholinergic function (Bierer et al., 1995). Theoretically, long-lasting cholinesterase inhibition should be accompanied by long-lasting clinical benefits. However, it is also possible that sustained stimulation of the cholinergic system in the brain leads to compensatory changes such as receptor down-regulation or desensitization and thereby diminishes the behavioral benefit of cholinesterase inhibitors.

We now addressed this issue by measuring the duration of the cognitive stimulation attained with metrifonate in rats after acute and subchronic administration. Metrifonate is a well-tolerated, long-acting cholinesterase inhibitor (Schmidt et al., 1996) which is currently in phase III of clinical testing as an Alzheimer therapeutic agent (Cummings et al., 1996). It differs from other cholinesterase inhibitors for Alzheimer therapy by its unique mechanism of action, acting as a prodrug of an active metabolite which in turn covalently phosphorylates the catalytic site of the enzyme (for review see Schmidt et al., 1996). Interestingly, a substantial degree of cholinesterase inhibition is maintained for longer periods (Reiner and Plestina, 1979) and after 15–20 single doses, the long-lasting component of cholinesterase inhibition reaches a steady state which is comparable to the levels of peak inhibition seen after a single acute administration (Hinz et al., 1997; Kronforst-Collins et al., 1997a).

This difference in cholinesterase inhibition kinetics after acute and repeated administration of metrifonate, makes the compound particularly suitable for evaluating the relationship between the duration of cholinesterase inhibition on one hand and cognition enhancement on the other. Thus, we tested the effects of metrifonate on cognitive performance of medial septal-lesioned rats at various time

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periods following acute administration (transient cholinesterase inhibition; Hinz et al., 1996a,b) and after a three week pretreatment regime with metrifonate (stable cholinesterase inhibition; Hinz et al., 1997). The medial septal-lesioned rat was selected as a model as its sensitivity to the cognition-enhancing effects of metrifonate has been shown (Riekkinen et al., 1996, 1997a,b; Jäkälä et al., 1996).

2. Materials and methods

2.1. Animals

Young (3 month-old, $n = 8$/group) male Han:Wistar rats were used. Study groups and training schedule are detailed in Table 1 and Figs. 1 and 2. The rats were housed 3 per cage in a controlled environment (20 ± 2°C, humidity at 50–60%, light period 07.00–09.00). Food and water were available ad libitum. The local ethical committee gave permission to carry out this study.

2.2. Drugs

Metrifonate (30 mg/kg, p.o.) (a donation from Bayer AG) was dissolved in 5% sodium citrate (pH 5.5, buffered with citric acid). In our previous studies, acute administration of metrifonate at 30 mg/kg improved water maze spatial navigation in medial septal-lesioned rats (Riekkinen et al., 1996, 1997a,b).

Two separate experiments were carried out, experiments I and II (Table 1). In both of these studies, some of the medial septal-lesioned rats received two daily injections of metrifonate 30 mg/kg for three weeks before the water maze testing (Pretreatment). The medial septal-lesioned animals and all the sham-lesioned rats received appropriate vehicle injections during the pretreatment period. During the first stage of behavioral testing initiated on the day after the pretreatment period was finished, metrifonate and vehicle were administered at different times relative to testing (Initial learning). Metrifonate was only given once per day (experiment I: 30 or 150 min before testing; experiment II: 360 min before or immediately after daily testing) during this part of the behavioral testing. After the three-day 'initial learning' experiment, drug treatment was discontinued for the remaining part of the study (four-day recovery, one-day 'reversal learning'). The treatment conditions are detailed in Table 1.

2.3. Surgery

Medial septal (A: 0.0 mm, M: 0.0 mm, D: − 7.0 mm relative to the bregma) lesioning was done by passing an anodal dc current (2 mA, 5 s) via stainless-steel electrodes. Controls were treated identically, but no current was applied. The rats were deeply anesthetized with equithesin during surgery.

2.4. Water maze

The circular water maze pool and computerized video-tracking system have been described in detail previously (Riekkinen et al., 1991; Riekkinen et al., 1996). The computer calculated and stored the total distance swum (in cm). The starting locations, labelled north, south, east and west, were located arbitrarily on the pool rim. To start the test, rats were placed in the water at one of the starting points in a random manner with their nose pointing towards the wall. Testing was carried out over four days of testing. During the 'initial learning' stage five platform trials of 70 s were assessed per day during the first three training days. The platform location was kept constant (in the southwest quadrant) during this period of training. When the 'initial learning' stage of training was accom-

Table 1
Design of experiments I and II

	Subchronic pretreatment	Acute treatment during day 1–3 testing
Experiment I		
Sham-lesioned	vehicle	vehicle 30 and 150 min before test
Septal-lesioned	vehicle	vehicle 30 and 150 min before test
Septal-lesioned	vehicle	metrifonate 30 and vehicle 150 min before test
Septal-lesioned	vehicle	vehicle 30 and metrifonate 150 min before test
Septal-lesioned	metrifonate	metrifonate 30 and vehicle 150 min before test
Septal-lesioned	metrifonate	vehicle 30 and metrifonate 150 min before test
Experiment II		
Sham-lesioned	vehicle	vehicle 360 min before and immediately after testing
Septal-lesioned	vehicle	vehicle 360 min before and immediately after testing
Septal-lesioned	vehicle	metrifonate 360 min before and vehicle immediately after testing
Septal-lesioned	metrifonate	metrifonate 360 min before and vehicle immediately after testing
Septal-lesioned	metrifonate	vehicle 360 min before and metrifonate immediately after testing

During the 21-day subchronic treatment period, metrifonate or vehicle was given orally twice daily. Next, during the initial learning stage (day 1–3 testing), the combination of metrifonate and vehicle was given 30, 150 or 360 min before or immediately after daily training, as detailed below. After a four-day washout period, reversal learning was tested without additional treatment of the animals.

plished, a recovery of four days was allowed. Then, the one-day 'reversal learning' experiment was performed. The location of the escape platform was changed to the northeast quadrant and six trials of 50 s were assessed. In each trial, during both 'initial' and 'reversal' learning, the rats were allowed to stay on the platform for 10 s. If the animals failed to find the platform during the maximum duration of the trial, the experimenter placed them on it for 10 s. A 30 s interval was maintained between training trials.

2.5. Statistics

The one-way-analysis of variance followed by Duncan's post-hoc multiple group comparison, was used to analyze group differences between escape distances.

3. Results

3.1. Experiment I

Escape distance values measured during the 'initial learning' stage of training showed that medial septal-lesioned rats were impaired compared with the sham-lesioned rats (overall effect: $F(5,42) > 4.7$, $P < 0.001$; for all daily comparisons; $P < 0.05$ versus sham-operated).

During the first two training days, none of the medial septal-lesioned groups treated with acute or chronic metrifonate performed significantly better than the vehicle treated medial septal-lesioned rats ($P > 0.05$) (Fig. 1A and B).

On the third day of testing, the medial septal-lesioned rats that were treated for 21 days with metrifonate 30 mg/kg and/or only acutely with metrifonate 30 min before training performed better than the vehicle-treated medial septal-lesioned rats ($P < 0.05$) (Fig. 1A). These two groups of medial septal-lesioned rats did not differ in their abilities to find the platform ($P > 0.05$). During the 'reversal learning' stage on day 4, the escape performance of the previous vehicle treated group and of those rats that had received only acutely metrifonate 30 min before behavioral training was equally impaired ($P < 0.05$ versus sham-lesioned, for all comparisons) (Fig. 1A). In contrast, the escape performance of the group of medial septal-lesioned rats that had also received the subchronic pretreatment with metrifonate for 21 days was significantly better

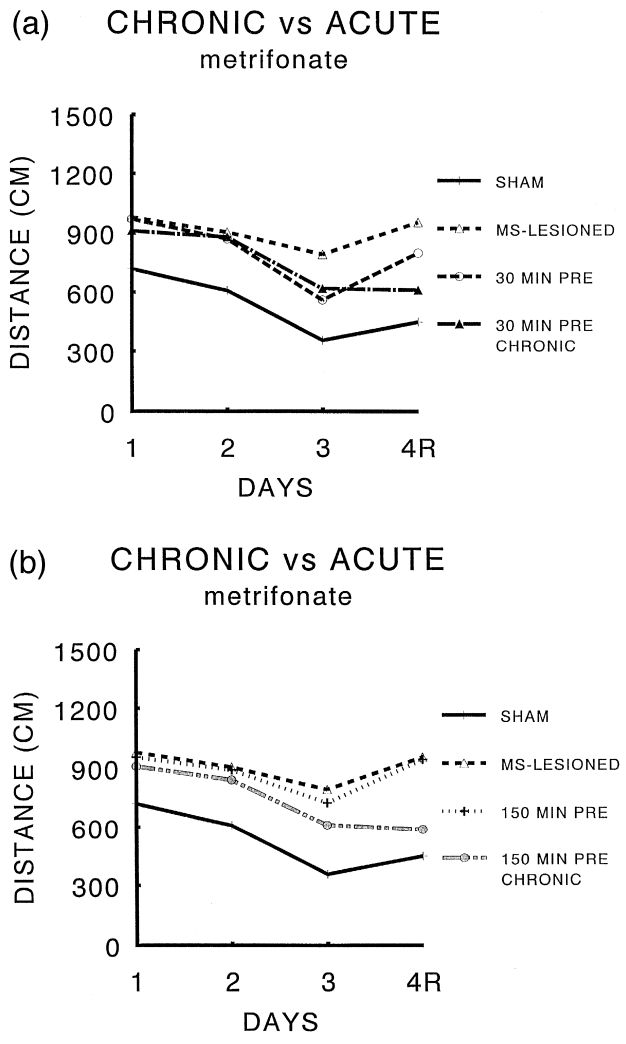

Fig. 1. The effects of acute and chronic treatment with metrifonate on water maze spatial navigation performance. The *X*-axis shows the training days. The *Y*-axis shows the daily group mean escape distance values (cm). Metrifonate 30 mg/kg or vehicle was given twice daily for 21 days. During the initial learning phase (days 1–3) all the rats received daily p.o. injections of vehicle and metrifonate 30 mg/kg before testing. Next, a four-day wash out period was allowed and no drug treatments were given during the reversal learning (day 4R). (A) The improving effect of metrifonate 30 mg/kg administered 30 min before initial learning on days 1–3 was not affected by the pretreatment for 21 days. Next, a four-day wash out period was allowed and no drug treatments were given during the reversal learning (day 4R). During the reversal learning period the medial septal-lesioned rats that had received the pretreatment for 21 days with metrifonate 30 mg/kg performed better than the group that had received metrifonate only on days 1–3. (B) Metrifonate 30 mg/kg administered only acutely 150 min before initial learning on days 1–3 was not effective to stimulate water maze navigation performance in medial septal-lesioned rats. In contrast, the group of medial septal-lesioned rats pretreated with metrifonate 30 mg/kg twice daily for 21 days and metrifonate 30 mg/kg 150 min before the initial learning on days 1–3 performed significantly better than the control-treated medial septal-lesioned rats. Next, a four-day wash out period was allowed and no drug treatments were given during the reversal learning phase (day 4R). During the reversal learning period, medial septal-lesioned rats that had received 21 days of pretreatment with metrifonate 30 mg/kg performed better than the group that had received metrifonate only on days 1–3. Group symbols: sham = sham-lesioned vehicle 30 and 150 min before testing, MS-lesioned = medial septal-lesioned vehicle 30 and 150 min before testing, 30 min pre = medial septal-lesioned metrifonate 30 and vehicle 150 min before testing, 150 min pre = medial septal-lesioned vehicle 30 and metrifonate 150 min before testing, 30 min pre chronic = medial septal-lesioned metrifonate 30 and vehicle 150 min before testing + 21-day twice daily pretreatment with metrifonate, 150 min pre chronic = medial septal-lesioned vehicle 30 and metrifonate 150 min before testing + 21-day twice daily pretreatment with metrifonate. These treatment descriptions refer to subchronic pretreatment period and to the initial learning stage.

than that of the control medial septal-lesioned group ($P < 0.05$) (Fig. 1A).

On the third day of testing, the medial septal-lesioned rats which had been treated for 21 days with metrifonate 30 mg/kg and acutely with metrifonate 150 min before training performed better than the vehicle treated medial septal-lesioned rats ($P < 0.05$) (Fig. 1B). In contrast, the medial septal-lesioned rats that had been treated with metrifonate 30 mg/kg only acutely 150 min before the daily behavioral testing on days 1–3 were just as impaired as the control treated medial septal-lesioned rats ($P > 0.05$) (Fig. 1B). On the third day of testing, metrifonate was given to subchronically pretreated rats 30 min or 150 before behavioral testing was as effective as an acute treatment given 30 min before to drug-naive animals ($P > 0.05$) (Fig. 1A and B).

During the 'reversal learning' stage, the escape performance of the previous vehicle treated group and of those rats that received metrifonate only acutely 150 min before behavioral training during the initial acquisition stage was equally impaired ($P < 0.05$ versus sham-lesioned, for all comparisons) (Fig. 1A and B). In contrast, the group of medial septal-lesioned rats that received subchronic pretreatment with metrifonate performed significantly better than the two other medial septal-lesioned groups ($P < 0.05$ for all comparisons) (Fig. 1B).

3.2. Experiment II

Performance of all the medial septal-lesioned rats was impaired during the 'initial learning' stages of training compared with that of the sham-lesioned rats (overall effect: $F(4,35) > 4.5$, $P < 0.004$; for all daily comparisons; $P < 0.05$ versus sham-operated) (Fig. 2). Furthermore, during the first two training days, none of the medial septal-lesioned groups treated with acute or chronic metrifonate found the platform better than did the vehicle-treated medial septal-lesioned controls ($P > 0.05$) (Fig. 2). However, on the third day of testing the medial septal-lesioned rats which had been treated subchronically with metrifonate before the initiation of the 'initial learning' stage then received metrifonate during this stage of training also performed better than vehicle-treated medial septal-lesioned control rats ($P < 0.05$) (Fig. 2). This was observed for the group tested 360 min after administration and for the group treated immediately after testing during the preceding training sessions ($P < 0.05$, for both comparisons). However, the performance of these two groups was still impaired with respect to that of the sham-lesioned rats ($P < 0.05$, for both comparisons). In agreement with the results from experiment I, the medial septal-lesioned rats that had received metrifonate only acutely 360 min before daily testing did not perform better than the vehicle treated medial-septal-lesioned controls ($P > 0.05$).

During the 'reversal' learning stage, the escape performance of the previous vehicle treated group and that of rats that had received only acute metrifonate 360 min before behavioral training was equally poor ($P < 0.05$ versus sham-lesioned, for both comparisons). In contrast, the two groups of medial septal-lesioned rats that had received subchronic pretreatment with metrifonate before the 'Initial learning' stage of training performed better than the above two groups ($P < 0.05$ for all comparisons).

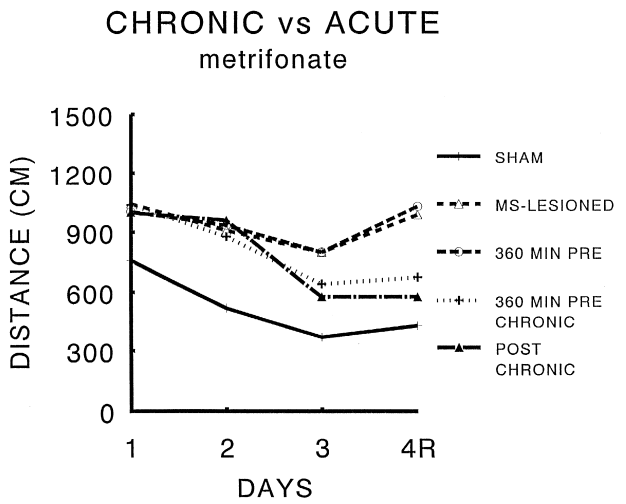

Fig. 2. The effects of acute and chronic treatment with metrifonate on water maze spatial navigation performance. The *X*-axis shows the training days. The *Y*-axis shows the daily group mean escape distance values (cm). Metrifonate 30 mg/kg or vehicle were given twice daily for 21 days. During the initial learning testing (day 1–3) all the rats received daily p.o. injections of vehicle and metrifonate 30 mg/kg 360 min before or immediately after testing. Next, a four-day wash out period was allowed and no drug treatments were given during the reversal learning (day 4R). Group symbols: sham = sham-lesioned vehicle 360 min before and immediately after testing, MS-lesioned = medial septal-lesioned vehicle 360 min before and immediately after testing, 360 min pre = medial septal-lesioned metrifonate 360 min before and vehicle immediately after testing, 360 min pre chronic = medial septal-lesioned metrifonate 360 min before and vehicle immediately after testing + 21-day twice daily pretreatment with metrifonate, post chronic = medial septal-lesioned vehicle 360 min before testing and metrifonate immediately after testing + 21-day twice daily pretreatment with metrifonate. These treatment descriptions refer to subchronic pretreatment period and to the initial learning stage.

4. Discussion

The present results confirmed the cognition-enhancing effect of metrifonate and showed that the improving effect on spatial reversal learning is transformed into a long-lasting phenomenon by subchronic pretreatment.

The beneficial effect of metrifonate on cognition in rats has also been observed in a number of other short-term studies using aged, scopolamine-treated and basal forebrain-lesioned rats trained in passive avoidance, the object recognition test and the spatial water escape task (Riekkinen et al., 1996; Van der Staay et al., 1996a,b; Scali et al., 1997; for review see Schmidt et al., 1997). The present study was not designed to investigate the nature of the spatial memory process(es) improved by metrifonate, though we did observe that treatment enhanced the accu-

racy of spatial navigation at the end of the reference memory training and also during the platform reversal trials. A previous study of Riekkinen et al. (1997a,b) involving various conditions led to the conclusion that metrifonate (30 mg/kg p.o.) specifically enhances the speed and accuracy of development and durability of spatial reference memory engrams in a water maze. Furthermore, Riekkinen et al. (1997a,b) trained rats to perform well in a reference memory test in the water maze and induced medial septal lesions only after adequate learning had been attained. It was found that retrieval of spatial memories learned before induction of the lesion was unaffected, but that reversal learning was impaired (Riekkinen et al., 1997a,b). Finally, in the same study (Riekkinen et al., 1997a,b), metrifonate treatment alleviated the defective reversal learning in medial septal-lesioned rats. Therefore, the previous data (Riekkinen et al., 1997a,b) suggest that spatial reference and reversal learning can be enhanced by metrifonate treatment in medial septal-lesioned rats.

The present and previous (Kronforst-Collins et al., 1997a) results indicate that repeated administration of metrifonate is able to produce a long-lasting improvement of learning and memory performance in medial septal-lesioned rats and aged rabbits. First, the present results clearly indicated that metrifonate is as effective in this respect in rats which had received twice daily pretreatment with metrifonate for three weeks before the start of the behavioral training as in drug-naive medial septal-lesioned rats. Second, the cognition-enhancing effect of metrifonate becomes long-lasting upon repeated administration and thus independent of the timing of drug administration with respect to its assessment. Metrifonate given even 360 min before or immediately after daily training, i.e., 24 h before the next training session, enhanced the acquisition of the task. Furthermore, following a 4-days wash-out period, subchronically metrifonate-pretreated rats still had an improved water maze performance.

Previous biochemical and behavioral studies showed that the effect of acute treatment with metrifonate on cholinesterase inhibition and cognitive functions are closely correlated. It is noteworthy that, the present behavioral and previous biochemical (Hinz et al., 1997) studies indicated that this close association is maintained after repeated treatment with metrifonate. Repeated daily administration of metrifonate for 2–3 weeks produces stable and long-lasting cholinesterase inhibition in rat brain and blood, as measured 18 h after the last drug treatment (Soininen et al., 1990; Hinz et al., 1997; Kronforst-Collins et al., 1997b). After 15–20 single doses, cholinesterase inhibition reaches dose-dependent steady state levels which are maintained on continuation of the treatment (Hinz et al., 1997; Kronforst-Collins et al., 1997a,b). This inhibition recovers slowly after discontinuation of treatment and approaches the control levels of enzyme activity only after a period of four weeks (Hinz et al., 1997; Kronforst-Collins et al., 1997b). Chronic metrifonate treatment does not decrease either nicotinic and quinuclidinylbenzilate (muscarinic) binding or activity of the enzyme which synthetizes acetylcholine, suggesting that, in rats, the increase in cholinergic activity may be long-lasting. In contrast, cholinesterase activity after acute administration of metrifonate is only transiently decreased and rapidly returns to baseline levels after a peak of inhibition which occurs 30–60 min post-administration (Hinz et al., 1996a,b; Hallak and Giacobini, 1987). These data, suggests that no major cholinesterase inhibition would be expected to persist 150 or 360 min after a single acute dose (Hinz et al., 1996a). The differential effects of acute and repeated administration of metrifonate are explained by the fact that a small portion of the inhibition achieved (peak inhibition) is long-lasting, and that this gradually accumulates during repeated administration.

One might argue that subchronic treatment with metrifonate could result in accumulation of the drug and/or its active metabolite e.g. in fat tissue depots, from where they are slowly released after treatment discontinuation and mediate the long-lasting behavioral and neurochemical effects. This possibility is, however, rather unlikely because of the following reasons. First, metrifonate is rapidly and completely eliminated from the body with a half-life of 2.3 to 3.3 h (Nordgren and Holmstedt, 1988; Villén et al., 1990). Second, the active metabolite of metrifonate is eliminated even more rapidly by renal excretion and degradation by plasma enzymes with a half-life of only about 13.5 min (Blair et al., 1975; Villén et al., 1990). Third, if metrifonate or the active metabolite were released from some hypothetical storage deposit, plasma butyrylcholinesterase and erythrocyte acetylcholinesterase would be expected to show the same recovery kinetics upon discontinuation of subchronic metrifonate administration, since metrifonate is also an inhibitor of these enzymes (Hinz et al., 1996a,b; Pacheco et al., 1995). However, this is not the case. Plasma cholinesterase recovers within a maximum of 6 days after cessation of treatment (Hinz et al., 1997), whereas erythrocyte and brain cholinesterase activity requires more than 4 weeks to fully return to control values (Hinz et al., 1997; Kronforst-Collins et al., 1997b).

In conclusion, the present data indicate that, following subchronic treatment, there is a stable cholinesterase inhibition and amelioration of the water maze failure caused by medial septal lesion. These data may be relevant for the treatment of cognitive deficits associated with Alzheimer's disease, as they suggest that, during chronic treatment, the therapeutic effect of metrifonate is independent of the time of daily drug administration and may be insensitive to short-lasting interruptions in medication.

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